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Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments

Hema Diwan · Ishrat Khan · Altaf Ahmad · Muhammad Iqbal

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Abstract Growth performance, chromium (Cr) accumulation potential and induction of antioxidative defence system and phytochelatins (PCs) were studied in hydroponically grown *Brassica juncea* (Indian mustard) and *Vigna radiata* (mungbean) at various levels of Cr treatments (0, 50, 100, 200 μM Cr). *B. juncea* accumulated twofolds and threefolds higher Cr in root and shoot, respectively than in *V. radiata*. Compared to *B. juncea*, *V. radiata* was found to be particularly sensitive to Cr as observed by the severity and development of Cr toxicity symptoms and decreased growth. Induction of PC and enzymes of antioxidant defence system were monitored as plant's primary and secondary metal detoxifying responses, respectively. There was induction of PC and enzymes of antioxidant defence system in both the plants. PCs were induced significantly in roots and shoot of both the plants at all the levels of Cr treatments. Significantly higher activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) were observed in shoot of *B. juncea* than *V. radiata* at all the levels of Cr treatments. Induction of PCs along with antioxidant defence system in response to Cr stress suggests the cumulative role of PCs and antioxidants in conferring tolerance against accumulated Cr in *B. juncea,* and thereby signifies the suitability of this plant as one of the potential remediators of Cr.

Keywords Antioxidant defence system · *Brassica juncea* · Chromium · Phytochelatins · *Vigna radiata*

H. Diwan · I. Khan · A. Ahmad (✉) · M. Iqbal
Molecular Ecology Laboratory, Department of Botany, Faculty of Science, Hamdard University, New Delhi 110062, India
e-mail: aahmed@jamiahamdard.ac.in

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
Cr	Chromium
DTNB	5′ Dithio-bis-(2-nitrobenzoic acid)
Fw	Fresh weight
GR	Glutathione reductase
GSH	Reduced glutathione
LSD	Least significant difference
MT	Metallothioneins
MDA	Malondialdehyde
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid reactive substances

Introduction

Pollution of the biosphere with heavy metal has accelerated dramatically during the last century due to mining, smelting, amendment of agricultural soils with municipal sewage sludge, and waste disposal practices. Chromium (Cr) is one of several heavy metals (HM) that causes serious environmental contamination in soil, sediments, and groundwater (Bartlett 1991; Witmer et al. 1991; Diwan et al. 2010). Wastes coming from Cr-related industries such as electroplating, leather tanning, metal finishing, corrosion control and pigment manufacturing industries (Sklar 1980; Pawlisz et al. 1997; Shukla et al. 2007) have severely contaminated sites around the world. Among the various forms of chromium, Cr(VI) is considered as highly toxic and carcinogenic, producing cardiovascular shock affecting kidney, liver and blood-forming organs (Shanker et al.

2005, 2009). It is a very toxic, powerful epithelial irritant and an established human carcinogen by the Environmental Protection Agency (1984) and the World Health Organization (1988). One potential strategy to efficiently remove HMs from contaminated sites is the use of plants with potential of heavy metal accumulation and tolerance capacity (Salt et al. 1995). This strategy, called phytoremediation, has several advantages over physical remediation methods in costs, practice and the scale at which the processes operate (Salt et al. 1995; Khan et al. 2009). Suitable plants for phytoremediation purpose are identified on the basis of biochemical mechanisms related to heavy metal tolerance and accumulation. Important biological mechanisms of HM detoxification involves the induction of metal-binding ligand and antioxidative defence mechanisms.

Phytochelatins (PCs) are a family of peptides with the general structure (γ-Glu-Cys)n-Gly (n = 2–11). PCs are synthesized enzymatically from glutathione by the constitutive enzyme PC synthase, which requires posttranslational activation (Grill et al. 1989; Zenk 1996; Cobbett 2000; Schat et al. 2002). Heavy metals do not bind directly to the enzyme to activate PC biosynthesis, but instead act as substrate ligands for a bisubstrate-substituted enzyme transpeptidation reaction in which free glutathione and its corresponding heavy metal thiolate are cosubstrates (Vatamaniuk et al. 2001). PCs are rapidly induced in a wide range of plant species by heavy metal ions, such as As^{5+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Hg^{2+}, and Pb^{2+} (Zenk 1996; Rauser 1999; Cobbett 2000; Schmoger et al. 2000; Hall 2002; Raab et al. 2004). Induction of phytochelatin in response to Cr exposure has not been reported so far in any plant species.

Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species (ROS) (Sanitadi-toppi et al. 2002; Arvind and Prasad 2003). ROS are partially reduced forms of atmospheric oxygen and under normal conditions their production in cells is low and tightly controlled (Dat et al. 2000). Heavy metal toxicity enhances the production of ROS up to 30-fold (Mittler 2002). These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. The deleterious effects resulting from the cellular oxidative state may be alleviated by the enzymatic and non-enzymatic antioxidant machinery of the plant (Halliwell 1987). The antioxidant protection in plant cells is complex and highly compartmentalized. Superoxide dismutases (SODs) are a family of metalloenzymes catalyzing the dismutation of O_2^- to H_2O_2 (Alscher et al. 2003). A fine regulation of the H_2O_2 level is achieved by the enzymes and metabolites of the ascorbate–glutathione cycle (Shigeoka et al. 2002). The balance between the activities of SOD and enzymes of ascorbate–glutathione cycle may be crucial for determining the steady-state level of O_2^- and H_2O_2. This balance, together with the sequestration of metal ions, is considered to be important in preventing the formation of the ROS via the metal-dependent Haber–Weiss or Fenton reactions (Mittler 2002).

Over 200 terrestrial species are endemic to metalliferous soils and can tolerate and accumulate high amounts of heavy metals such as Cd, Zn, Cu, and Ni in their organs (Baker et al. 2000; Kumar et al. 1995; Hajiboland 2005). Species from the genus *Brassica* are good toxic metal accumulators, allocating large amounts of Cd, Pb, and Ni into the shoots (Salt et al. 1995, 1996; Jiang et al. 2000; Panwar et al. 2002; Ghosh and Singh 2005; Diwan et al. 2010). *V. radiata* (Mung bean) is one of the major grain legume crops in the poorer countries of Asia (Lawn and Ahn 1985). For their high nutritive value, they are important agricultural crops as a food source of proteins, calcium, phosphorus, and certain vitamins, and some cultivars possess excellent aroma (Gupta 1983; Simonová et al. 2007). On the other hand, the seedlings of *V. radiata* can possibly be used as accumulators of arsenic (Van den Broeck et al. 1998), lead (Rout et al. 2001; Singh et al. 2003), and chromium (Samantary 2002). Since, plants are known to respond differently in their heavy metal accumulation and tolerance levels, the present study was undertaken to assess the induction of phytochelatin and antioxidant defence system in response to Cr stress in *B. juncea* and *V. radiata.*

Materials and methods

Plant material

Seeds of *Brassica juncea* (L.) Czern. Coss. cv. Pusa Jai Kisan and *Vigna radiata* L. cv. Pusa Ratna were surface sterilized with 0.5% (w/v) mercuric chloride for 10 min. These seeds were sown in soilrite and kept for germination for 4 days. Seedlings were transferred to 2.5-l plastic pots with nutrient solution with the following composition: 2 mmol l^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, 1 mmol l^{-1} $MgSO_4{\cdot}7H_2O$, 0.9 mmol l^{-1} K_2SO_4, 0.2 mmol l^{-1} KH_2PO_4, 10^{-6} mol l^{-1} H_3BO_3, 2×10^{-7} mol l^{-1} $MnSO_4{\cdot}H_2O$, 10^{-6} mol l^{-1} $ZnSO_4{\cdot}7H_2O$, 2×10^{-7} mol l^{-1} $CuSO_4{\cdot}5H_2O$, 2×10^{-8} mol l^{-1} $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ and 10^{-4} mol l^{-1} $C_{10}H_{12}FeN_2NaO_8$ and allowed to grow in growth chambers under controlled environmental conditions (20°C in the light and 18°C in the dark, 16 h light/8 h dark photoperiod with a photon flux density of 380 µmol m^{-2} s^{-1} and 60% humidity). After 7 days of growth, Cr in the form of $K_2Cr_2O_7$ was supplied at concentrations of 50 (T1), 100 (T2) and 200 µM (T3). The concentration of these

treatments was chosen because these are the environmentally relevant concentrations of Cr commonly found in polluted soils (Dheri et al. 2007). A control treatment contained only nutrient medium. The treated and control plants were analysed at 1 Day After Treatment (DAT), 3 DAT, 5 DAT and 7 DAT.

Measurement of growth and chromium accumulation

Fresh weight was recorded on an electronic top pan balance (Sartorius BL-210S, Germany) and expressed in g per plant. For dry weight determination, the plants were dried separately in a hot air oven at 65 ± 2°C for 72 h. Dried samples were weighed and expressed in g per plant. Plants were cut at the root-shoot junction and the root and shoot lengths were recorded. For Cr accumulation, chromium concentration of the dried root and the shoot samples was measured by atomic absorption spectrometer (Model ZEEnit 600/650, Analytik Jena, Germany) after wet digestion of samples with concentrated HNO_3 at 300°C for 1 h.

Estimation of lipid peroxidation

The level of lipid peroxidation in the shoot was determined through estimation of malodealdehyde (MDA), a major 2-thiobarbaturic acid reactive substances (TBARS), by the method of Heath and Packer (1968). The MDA level was expressed in nmol g^{-1} fresh weight.

Assay of antioxidative enzymes

The shoot was ground with a mortar and pestle under chilled conditions in homogenization buffer (2 ml) containing phosphate buffer (0.1 M, pH 7.5), and ethylene diamine tetraacetic acid (EDTA, 0.5 mM). The homogenate, filtered through four layers of muslin cloth, was centrifuged at 12,000*g* for 10 min at 4°C. The resulting supernatant was used for the assay of different enzymes. Soluble protein estimation was carried out by the method of Bradford (1976) using bovine serum albumin as the standard.

For estimation of SOD (EC 1.15.1.1) activity, method of Beyer and Fridovich (1987) was followed. SOD activity was assayed by measuring the inhibition of photo-reduction of nitroblue tetrazolium (NBT) at 560 nm using UV–Vis spectrophotometer (Model λ-Bio-20, Perkin-Elmer, Germany). One unit of SOD is defined as that being present in the volume of extract that caused inhibition of the photo-reduction of NBT by 50%, and was expressed in enzyme units (mg^{-1} protein h^{-1}).

Activity of APX (EC 1.11.1.11) was measured by following the rate of hydrogen peroxide-dependent oxidation of ascorbate in a reaction mixture that contained 0.5 M phosphate buffer (pH 7.0), 0.5 mM ascorbic acid and enzyme extract (Nakano and Asada 1981). The reaction was initiated by addition of 10 µl of 10% (v/v) H_2O_2 and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm for 3 min by using UV–vis spectrophotometer (Model λ-Bio-20, Perkin-Elmer, Germany). APX activity was calculated by using extinction coefficient 2.8 mM^{-1} cm^{-1} and expressed as enzyme units $(mg\ protein)^{-1}$. One unit of enzyme (EU) is the amount necessary to decompose 1 µmol H_2O_2 per min at 25°C.

Catalase (CAT) activity was determined by monitoring the disappearance of H_2O_2, measuring a decrease in the absorbance at 240 nm (Aebi 1984). The reaction was carried in a reaction mixture containing 1.0 ml of the 0.5 M (pH 7.2) phosphate buffer, 3 mM EDTA, 0.1 ml of the enzyme extract and 0.3% H_2O_2, and allowed to run for 3 min. The enzyme activity was calculated using the extinction coefficient 0.036 mM^{-1} cm^{-1}. One enzyme unit (EU) determines the amount of enzyme necessary to decompose 1 µmol of H_2O_2 per mg protein per min at 25°C and expressed as EU mg^{-1} protein.

Activity of GR (EC 1.6.4.2) was determined by the method of Foyer and Halliwell (1976) and modified by Rao (1992). The supernatant was immediately used to assay GR activity through glutathione-dependent oxidation of NADPH at 340 nm. About 1 ml reaction mixture, containing 0.2 mM NADPH, 0.5 mM GSSG and 50 µl of enzyme extract, was run for 5 min at 25°C by using UV–vis spectrophotometer (Model λ-Bio-20, Perkin-Elmer, Germany). The activity was calculated by using extinction coefficient 6.2 mM^{-1} cm^{-1} and expressed in enzyme unit $(mg\ protein)^{-1}$. One unit of enzyme is the amount necessary to decompose 1 µmol of NADPH per min at 25°C.

Estimation of GSH content

Glutathione (GSH) content was determined by the recycling method of Anderson (1985). Fresh plant material (0.5 g) was homogenized in 3.0 ml of 5% (w/v) sulfosalicylic acid under cold conditions and was centrifuged at 10,000 rpm for 10 min. Half ml aliquot was taken in a microfuge tube, to which 0.5 ml reaction buffer [0.1 M phosphate buffer (pH 7.0), 3 mM ethylenediaminetetraacetic acid (Na_2EDTA)] and 50 µl of 5′ dithio-bis-(2-nitrobenzoic acid) (0.15% DTNB) were added. After 5 min, absorbance for determination of GSH was read at 412 nm using UV–Vis spectrophotometer (Model λ Bio-20, Perkin-Elmer, Germany). The level of GSH were expressed in nmol g^{-1} fresh weight.

Estimation of phytochelatins

Phytochelatins were analyzed by pre-column derivatization using monobromobimane (mBBr). About 500 mg of tissue

(each of roots and shoot) was frozen in liquid nitrogen, pulverized and transferred to a microfuge tube. 1 N NaOH containing 1 mg of sodium borohydride ($NaBH_4$) per ml was added. After thorough mixing the solution was centrifuged at 11,000*g* for 5 min at 4°C. Supernatant was collected and acidified with 3.6 N HCl (ratio 5:1). The tubes were incubated in ice bath for 15 min followed by centrifugation at 11,000*g* for 5 min at 4°C. The 200 µl of this supernatant was diluted with 400 µl of 200 mM HEPES buffer (pH 8.2), then derivatized by adding 10 µl of 25 mM mBBr and incubated for 30 min in dark. The reaction was stopped by adding 60 µl of 10 mM acetic acid. The final mixture was filtered through 0.45 µm filter and used for analysis of PCs. Separation and analysis of PCs was carried on reverse phase HPLC (Waters, model Water Corp, Milford, USA) with purospher RP-18e column (Merck) using a gradient of solution A and B (A containing 0.05% trifluoroacetic acid and B containing 26% acetonitrile in solution A) at a flow rate of 1.5 ml min^{-1}. Fluorescence intensity with an excitation wavelength of 380 nm and an emission wavelength of 470 nm was recorded using a fluorescence detector. Concentration of PCs was expressed as nanomoles of GSH equivalent g^{-1} fw.

Statistical analysis

Each treatment was analyzed using three replicates of each treatment ($n = 3$). To confirm the variability of data and validity of results, analysis of variance (ANOVA) was conducted. To determine whether differences between treatments were significant as compared to control, least significant difference (LSD) was determined (Cochram and Cox 1957).

Results

Fresh weight (FW) and dry weight (DW) of the *B. juncea* was not significantly affected by any level and duration of the Cr treatments. There was significant reduction in the FW and DW of *V. radiata* at all levels of Cr with maximum decline with T3 (200 µM). There was 5–16% reduction in the root length of *B. juncea* by Cr treatments. However, the significant reduction was reported with T3 only. The per cent reduction in root length of *V. radiata* was 5–26% over various days of Cr treatments with significant difference observed with T2 (100 µM) and T3 (200 µM)Cr treatments on 5 and 7 DAT. Similarly, shoot length of *B. juncea* declined slightly (3–13%) but non-significantly at all levels and duration of Cr treatments. In case of *V. radiata*, all the Cr treatments significantly decreased the shoot length with increasing levels and durations of Cr. Maximum decline (12.5-25%) occurred with T3 treatment (Fig. 1).

Cr accumulation in the roots of *B. juncea* was in the range of 100–300, 224–450, 398–754 and 550–972 µg g^{-1} DW, respectively at 1, 3, 5 and 7 day of Cr treatments (T1-T3). The corresponding figures in shoot were 80–120, 152–289, 274–419 and 414–504 µg g^{-1} DW. In *V. radiata*, the roots accumulated Cr in the range of 23–296, 31–301, 51–189 and 144–224 µg g^{-1} DW at 1, 3, 5 and 7 days of Cr treatments (T1–T3). The corresponding figures in shoot were 113–241, 120–238, 106–241 and 141–238 µg g^{-1} DW (Fig. 2).

Malondialdehyde (MDA) level in the shoot of *B. juncea* was enhanced by 8–34, 9–85, 26–70 and 21–58% at 1, 3, 5, and 7 days of Cr treatments (T1–T3). However, the enhancement in MDA was non-significant with T1. In *V. radiata*, there was drastic increase in the level of MDA following exposure to Cr. The percent increase in MDA level was 50–80, 243–275, 125–149 and 116–164% respectively at 1, 3, 5 and 7 days of Cr treatments (T1–T3) (Table 1).

Activity of SOD in both the plants increased by Cr treatments, when compared with control. There were fourfolds to sevenfolds increase in SOD activity by Cr treatments, when compared with the control. In *V. radiata*, the range of significant enhancement in SOD activity was 57–127, 47–120 and 42–101% at 1, 3 and 5 days of Cr treatments, respectively. At 7 DAT, no significant increase in SOD activity was observed in *V. radiata* (Fig. 3).

Catalase activity was significantly enhanced (up to six-fold over the control) in *B. juncea* by Cr treatments. In *V. radiata*, the CAT activity increased in the range of 13–80% in a dose dependent manner. At 7 DAT, however, increase in CAT was non-significant (Fig. 3).

There was a induction of APX activity (up to eightfold) in the *B. juncea* by Cr treatments. Maximum induction, however, was reported at T2. In *V. radiata*, significant increase (8–30%) in the APX activity was observed at 1 DAT only (Fig. 3).

Glutathione reductase activity in the *B. juncea* was increased in the range of 111–178% with all the Cr treatments, when compared with control. Maximum increase, however, was observed with T2. In *V. radiata*, the GR activity was increased significantly at 1 DAT only, which was 56–77% over control (Fig. 3).

The content of GSH in *V. radiata* was higher than *B. juncea*. Chromium treatments increased the GSH content in the *B. juncea*, compared to control. In *V. radiata*, on the contrary, GSH content decreased with increase in the Cr treatments (Fig. 4).

Analysis of PCs was performed after 7 days of Cr treatments. PC_2 and PC_3 in shoot and roots of treated as well as control plants were identified and there were also some unidentified thiol peaks. Induction of PCs was higher in roots than shoot in both the plants at all the Cr

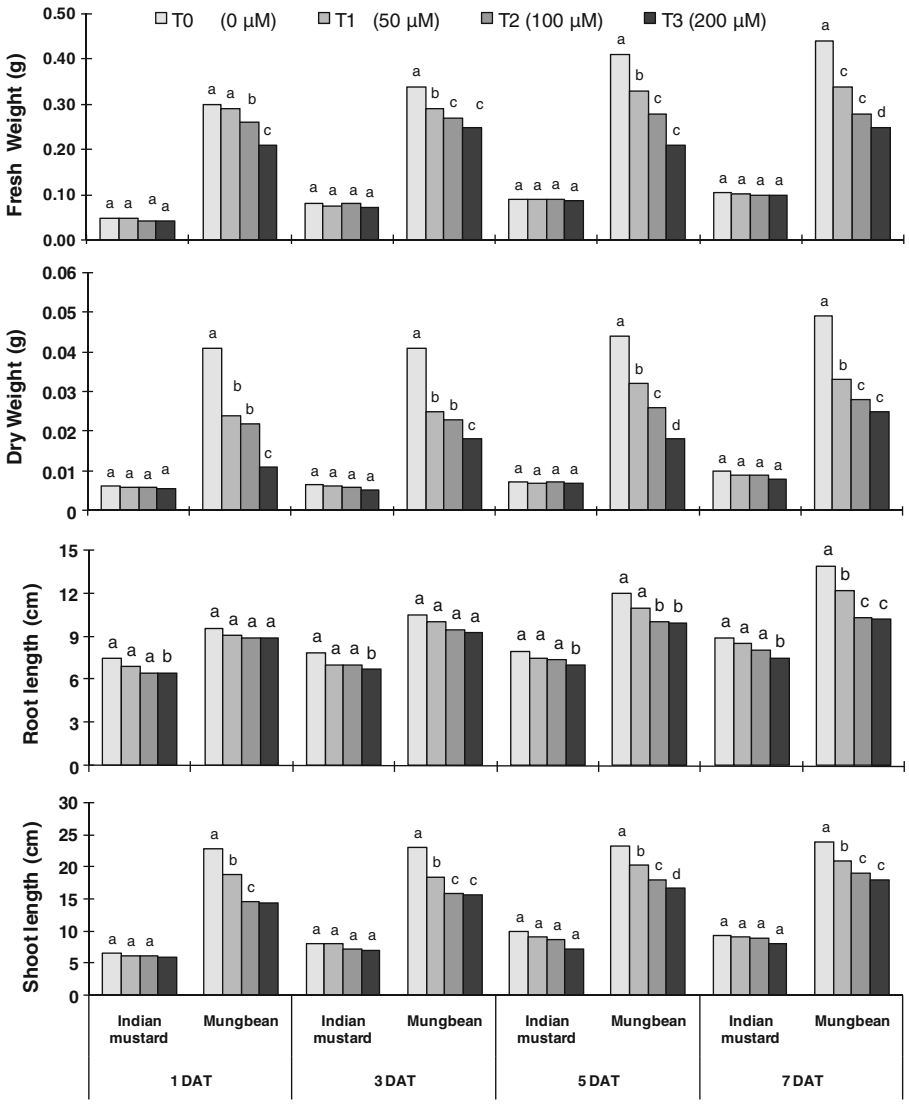

Fig. 1 Effect of chromium treatments on the fresh weight (g), dry weight (g), root length (cm) and shoot length (cm) of the *Brassica juncea* and *Vigna radiata* at various days after treatment (DAT). All the values are mean of triplicates. Different letters indicate significantly different values at a particular DAT ($P \leq 0.05$)

treatments, and in all observations PC_2 was more dominant Cr-binding ligand. With the increase in the level of Cr treatments, there was increase in the induction of PCs in *B. juncea*. Contrary to this, the induction of PCs in *V. radiata* increased with 50 and 100 μM Cr treatments. However, the induction of PCs was lesser with 200 μM Cr treatment than that observed with 50 and 100 μM Cr treatments in both shoot and root (Tables 2, 3).

Discussion

Plant biomass, root length and shoot length are used as indices of growth performance. In our study *B. juncea* and *V. radiata* highlighted significant differences in their responses to the exposure of various levels and durations of Cr treatments. In *V. radiata*, there was a significant reduction in the fresh weight (FW) and dry weight (DW) in response to levels and durations of Cr treatments. On the other hand, *B. juncea* highlighted complete tolerance to Cr regimes. Decrease in the FW may be the outcome of a decreased water uptake or enhanced water loss, both of which may occur following membrane damage since plant cell membranes are generally considered as the primary sites of metal injury (Barcelo and Poschenrieder 1990). The decline in the biomass production in *V. radiata* might be due to reasons like increased tissue permeability, inhibition of cell division resulting in the decline in growth or accumulation of Cr in different plant parts leading to retarded growth (Dube et al. 2003). Root growth has been traditionally considered as a powerful trait for scoring HM tolerance in plants. Results of our study indicated higher degree of Cr tolerance in *B. juncea*, compared to *V. radiata*. A non-significant effect was observed on the root length of *B. juncea* at lower (T1–T2) Cr doses, showing the ability of *B. juncea* to resist any damage by Cr

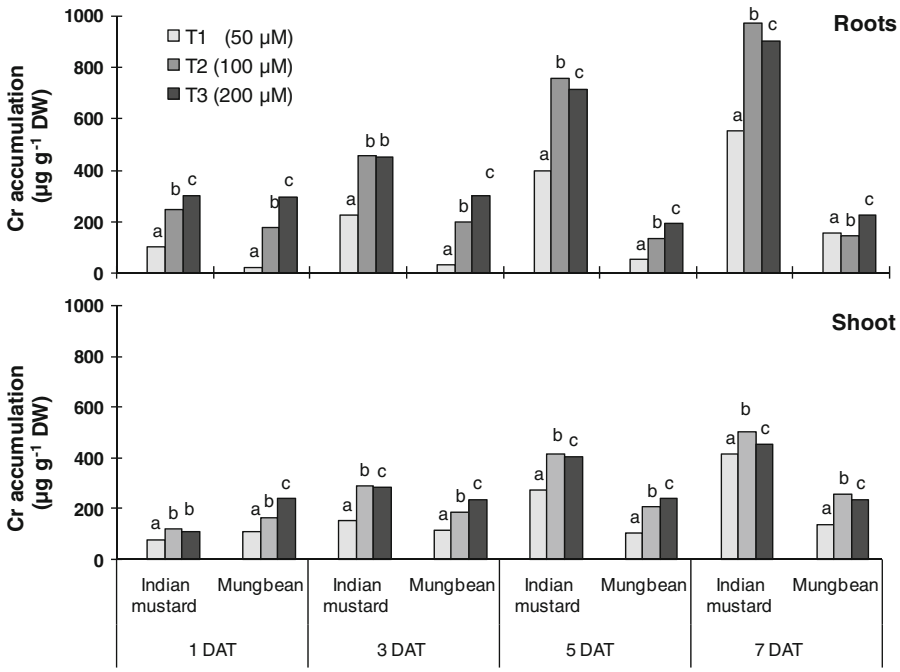

Fig. 2 Chromium accumulation (μg g^{-1} DW) in the root and shoot of the *Brassica juncea* and *Vigna radiata* at various days after treatment (DAT). All the values are mean of triplicates. Different letters indicate significantly different values at a particular DAT ($P \leq 0.05$)

Table 1 Effect of chromium treatments on the MDA content (nmol g^{-1} FW) of the Indian mustard and mungbean shoot at various days after treatment (DAT)

Treatments	1 DAT		3 DAT		5 DAT		7 DAT	
	Indian mustard	Mungbean	Indian mustard	Mungbean	Indian mustard	Mungbean	Indian mustard	Mungbean
T0 (0 μM)	0.87a	31.4a	0.96a	26.6a	1.11a	40.6a	1.21a	26.9a
T1 (50 μM)	0.94a	47.2b	1.04a	91.6b	1.39b	94.9b	1.46a	57.9b
T2 (100 μM)	1.07b	56.3c	1.41b	99.8c	1.69c	101.0c	1.87b	70.9c
T3 (200 μM)	1.17c	51.3d	1.78c	91.3b	1.88c	91.1d	1.90b	63.7d

All the values are mean of triplicates. Different letters indicate significantly different values at a particular DAT ($P \leq 0.05$)

DAT days after treatment

accumulation. However, the root development was affected at the highest level of Cr treatment (200 μM). Root growth arrest caused by Cr at this treatment can be considered as a toxicity symptom. Significant decline in the root length in *V. radiata* was observed at 100 and 200 μM of Cr as the exposure time was increased. This reduction could be due to the accumulation of high HM concentrations in roots, and/or a non-existence of any defined HM-translocation mechanism, thereby enhancing the HM sequestration in the tissue and, thus, inhibiting the root development (Lu et al. 2004). The direct contact of roots with metals also results in a collapse of roots and their inability to absorb water from the media (Barcelo et al. 1986). Shoot development of *B. juncea* and *V. radiata* was affected differentially by Cr treatments. *B. juncea* was able to tolerate the effect of 50 and 100 μM of Cr. In *V. radiata*, a more pronounced effect was observed, probably reflecting its higher degree of sensitivity towards Cr. A reduced shoot length has also been reported in pea, tomato, cauliflower, maize and green gram under Cr stress (Sanita-di-Toppi et al. 2002; Shanker et al. 2004a).

The removal of HMs by plants is based on their ability to take up these potentially harmful heavy metals into their tissues (roots/aerial parts) and modulate their defence system so as to develop optimum tolerance for the accumulated heavy metal as well. In our studies, *B. juncea* accumulated 972 μg Cr g^{-1} DW in roots and 504 μg Cr g^{-1} DW in shoot, whereas *V. radiata* accumulated in the range of 301 and 259 μg Cr g^{-1} DW in the root and the shoot, respectively. Roots accumulated higher amount of Cr in both the plants studied. It could be because the roots serve as an interface between the soil, rhizosphere and the plant and being in direct contact with the metal they quench most of it, thus restricting its mobility to higher plant parts (Barcelo et al. 1986). Poor translocation to the shoot could also be due to the vacuolar sequestration of Cr

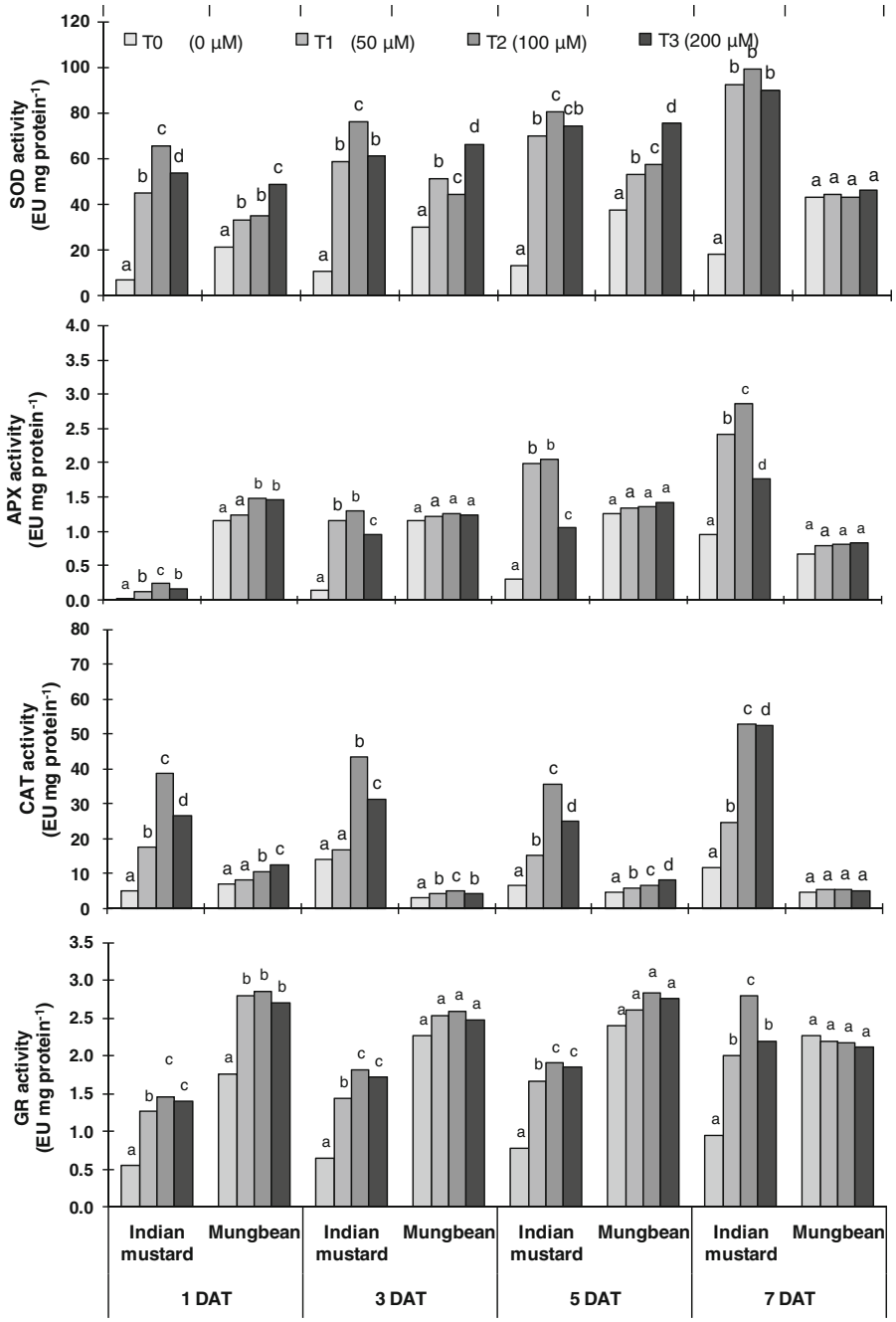

Fig. 3 Effect of chromium treatments on the activities SOD, CAT, APX and GR (EU mg protein^{-1}) in the shoot of the *Brassica juncea* and *Vigna radiata* at various days after treatment (DAT). All the values are mean of triplicates. *Different letters* indicate significantly different values at a particular DAT ($P \leq 0.05$)

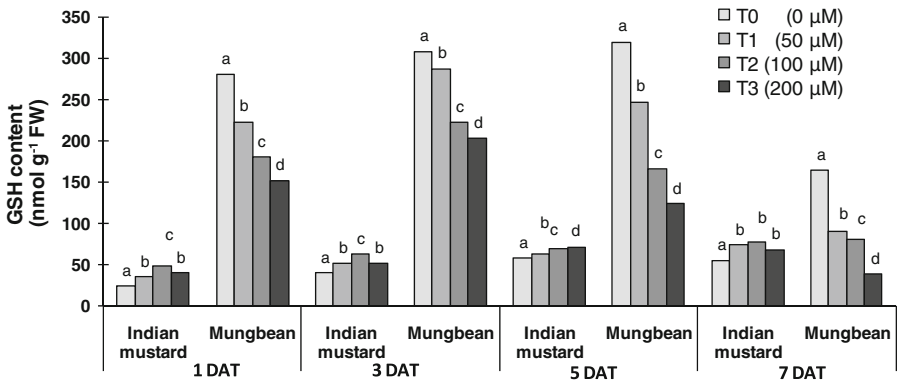

Fig. 4 Effect of chromium treatments on the GSH content (nmol g^{-1} FW) in the shoot of the *Brassica juncea* and *Vigna radiata* at various days after treatment (DAT). All the values are mean of triplicates. *Different letters* indicate significantly different values at a particular DAT ($P \leq 0.05$)

Table 2 Phytochelatin (PC_2 and PC_3) content in root of Indian mustard and mungbean exposed to various levels of Cr for 7 days

Treatments	PC_2		PC_3	
	Indian mustard	Mungbean	Indian mustard	Mungbean
T0 (0 μM)	21.5a	38.7a	10. 9a	27.7a
T1 (50 μM)	95.8b	201.4b	62.8b	87.5b
T2 (100 μM)	164.2c	480.1c	83.2c	122.3c
T3 (200 μM)	247.2d	193.5d	92.3c	103.6d

All the values are mean of triplicates. ANOVA significant at $P \leq 0.01$. Different letters indicate significantly different values

Table 3 Phytochelatin (PC_2 and PC_3) content in shoot of Indian mustard and mungbean exposed to various levels of Cr for 7 days

Treatments	PC_2		PC_3	
	Indian mustard	Mungbean	Indian mustard	Mungbean
T0 (0 μM)	8.1a	19.6a	6.7a	13.5a
T1 (50 μM)	64.8b	141.4b	41.1b	57.7b
T2 (100 μM)	97.3c	276.3c	66.5c	101.6c
T3 (200 μM)	177.5d	103.8d	87.6d	91.0d

All the values are mean of triplicates. ANOVA significant at $P \leq 0.01$. Different letters indicate significantly different values

in the root cells in order to render it non-toxic, which may be a natural anti-toxicity response of this plant against Cr stress.

That chromium produces oxidative stress is evident from enhancement in lipid peroxidation. Heavy metals may cause molecular damage to plant cells either directly or indirectly through the formation reactive oxygen species (Qureshi et al. 2005; Diwan et al. 2008), which include free radicals as well as non-radical molecules of high reactivity such as H_2O_2, singlet oxygen ($^1O_2$). Membrane lipids are especially prone to attack by free radicals. Protonation of superoxide radical can produce hydroperoxyl radical (•OH, H_2O_2), which can convert fatty acids to toxic lipid peroxides, destroying the biological membranes (Foyer et al. 1994a). Since lipid peroxidation is ascribed to oxidative damage (Zenk 1996), measurement of MDA levels, a common product of lipid peroxidation, is routinely used as sensitive index of oxidative stress (Smirnoff 1993; Metwally et al. 2005; Choudhary et al. 2007). In this study, MDA level rose significantly high when plants were subjected to high level of Cr. This suggests that high level of endogenous Cr induced production of superoxide radicals, leading to increased lipid peroxidation. Increased MDA levels in shoot of *V. radiata* as compared to *B. juncea* indicated an increased lipid peroxidation of cell membrane. The relatively low MDA content in *B. juncea* in contrast to *V. radiata* suggested low oxidative injury and better stress abating tendency of *B. juncea*, which in turn accounted for the limited inhibition of growth. The possible tolerance mechanism operative in both the plants is described below.

ROS production in plants is regulated by the activities of a particular group of enzymes (Van Assche and Clijsters 1990; Gratao et al. 2005). Inadequate regulation of ROS generation potentially leads to oxidative damage. The first line of defence against ROS-mediated toxicity is achieved by SOD that catalyzes the dismutation of superoxide radicals to H_2O_2 and O_2. Enhanced SOD activity in both the plants suggested that Cr caused oxidative stress. The data, however, also revealed that SOD activity was higher in *B. juncea*, indicating that this plant can efficiently detoxify the toxic superoxide radicals produced by the accumulated Cr when compared to *V. radiata*. High SOD activity has been associated with stress tolerance in plants because it neutralizes the reactivity of O_2^-, which is overproduced under oxidative stress. It has been well documented that SOD activity has a protective role in heavy metal plants. In response to higher levels of Cr, however, the increase in SOD activity was not comparable to that evident at lower Cr levels as there might be inactivation of the enzyme by H_2O_2 (Yamaguchi et al. 1995). On the other hand, the up-regulation in the SOD activity in *V. radiata* in response to Cr stress was not strong enough to detoxify the superoxide radicals completely, thus reflecting lesser tolerance towards Cr stress, which also indicated that O_2^- scavenging function of SOD was impaired with duration and levels of Cr treatments. Similar results have emerged from several other studies on plant responses to environmental stress (Labra et al. 2006; Zhang et al. 2005, 2007). H_2O_2, a product of SOD activity, is also toxic to cells and has to be further detoxified by CAT and the ascorbate–glutathione cycle. In the present study, CAT activity was increased in both the plants in response to Cr treatments. However, compared to *B. juncea*, low activity of CAT was observed in *V. radiata*. This may be due to inhibition of enzyme synthesis or change in assembly of enzyme subunits in the later (Ogawa et al. 1997).

Ascorbate–glutathione cycle in chloroplasts is the major defence system for scavenging H_2O_2, which finally converts H_2O_2 to H_2O and O_2. The cycle involves mainly ascorbate peroxidase and glutathione reductase enzymes, ascorbate and glutathione as oxireductants, H_2O_2 as an electron acceptor, and NADPH as an H^+ donor, which are strictly compartmentalized and act in a highly coordinated manner (Asada 1992; Foyer et al. 1994b). In our study, APX activity significantly increased with Cr treatments in both the plants. However, this induction of APX activity was much higher in *B. juncea* (up to eightfolds) than *V. radiata* (8–30%). Increase in APX activity suggested a

role of APX in the detoxification of H_2O_2 and its up-regulation under Cr-induced oxidative stress as established earlier with reference to many other heavy metals (Qureshi et al. 2005; Israr et al. 2006; Diwan et al. 2008; Khan et al. 2009). Importance of APX as a limiting factor of defence against photo-oxidative stress has been confirmed in transgenic tobacco plants (Rizhsky et al. 2002; Yabuta et al. 2002). Both the plants showed an initial increase in GR activity. However, the decrease in GR activity with duration of Cr exposure in *V. radiata* may be the result of a direct reaction of metal with sulfhydryl groups interfering with glutathione cycle since Cr is known to bind to the thiol group and thereby inactivate the thiol-containing enzymes such as GR. Sustained activity in *B. juncea* with duration and levels of Cr treatments proved the ability of hypertolerance in *B. juncea* toward oxidative stress induced by chromium. On exposure to HMs, increased GR activity has been reported in *Phaseolus vulgaris* and *Alyssum* (Srivastava et al. 2004). It was also observed that GR activity in *B. juncea* did not allow any fall in the GSH content as evident from the enhancement of GR enzyme upon exposure to Cr stress. Elevated GSH concentration is correlated with the ability of plants to withstand a metal-induced oxidative stress (Freeman et al. 2004). In case of *V. radiata*, decline in the GSH content was observed which could possibly point towards the formation of PC since GSH serves as the precursor for PC synthesis. We reported the induction of PCs in *B. juncea* and *V. radiata* in response to Cr. Increase in GSH content with a concomitant increase in phytochelatins in *B.juncea* signify that *B. juncea* besides showing an induction in PC generation, simultaneously managed to restore the GSH levels also. This could be explained by an augmented sulphur uptake, which eventually led to GSH biosynthesis, thus maintaining both GSH and PC concentration in this plant (Sharma et al. 2004). Induction of PCs in response to Cr stress has not been reported earlier. However, expression of metallothioneins (MTs)-like proteins has been reported under Cr stress in sorghum (Shanker et al. 2004b). The role of PCs in regulating metal toxicity has been reported earlier for As, Cd, Cu, Ag, Hg and Pb in a wide range of plant species (Cobbett 2000; Cobbett and Goldsbrough 2002; Schmoger et al. 2000; Hall 2002; Raab et al. 2004), and are known to play a major role in the detoxification of metals in plant cells (Shanker et al. 2004b; Srivastava et al. 2007). A sustained increase in PCs level in *B. juncea* with Cr treatments compared to *V. radiata* showed stronger tolerance capacity of the former in detoxification of accumulated Cr than the latter which showed a comparatively higher but inconsistent increase in PCs induction. In both the plants, the induction of PCs in shoot was lesser than those observed in roots. This may be due to binding of metal to other ligands (Salt et al. 1995) or to cell wall (Vecchia et al. 2005) or due to binding with GSH. In *V. radiata*, there was lesser induction of PCs at 200 μM Cr than at 50 and 100 μM Cr treatments. The decrease in the PCs at higher concentration in *V. radiata* may be due to its transport to shoot or due to the fact that PCs might have got degraded due to excessive Cr accumulation (Harmens et al. 1993). The results point towards the putative role of PCs along with antioxidant enzymes in conferring tolerance in plant cells subjected to Cr stress.

Thus, it can be inferred that cumulative effect of both antioxidant enzymes and PCs probably accounted for enhanced tolerance in *B. juncea* over *V. radiata* in combating Cr toxicity. It can be concluded that *B. juncea*, as a hyperaccumulator of Cr, could be potentially used for remediation of Cr contaminated soils and *V. radiata* as a fodder and food under our environmental and climatic conditions.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Alscher RG, Erturk N, Heath LS (2003) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1131–1341

Anderson ME (1985) Determination of glutathione and glutathione disulfides in biological samples. Methods Enzymol 113:548–554

Arvind P, Prasad MNV (2003) Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L., a free floating freshwater macrophyte. Plant Physiol Biochem 41:391–397

Asada K (1992) Ascorbate peroxidase: a hydrogen peroxide-scavenging enzyme in plants. Physiol Plant 85:235–241

Baker AJM, Mc Grath SP, Reeves RD, Smith JAC (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Terry N, Banuelos G (eds) Phytoremediation of contaminated soil and water. Lewis Publishers, Boca Raton, pp 85–107

Barcelo J, Poschenrieder C (1990) Plant-water relations as affected by heavy metal stress: a review. J Plant Nutr 13:1–37

Barcelo J, Poschenrieder C, Gunse B (1986) Water relations of chromium (VI) treated Bush bean plants (*Phaseolus vulgaris* L. cv Contender) under both normal and water stress conditions. J Exp Bot 37:178–187

Bartlett RJ (1991) Chromium cycling in soils and water: links, gaps, and methods. Environ Health Perspect 92:17–24

Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161:559–566

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 2:248–254

Choudhary M, Jetley UK, Khan MA, Zutshi S, Fatma T (2007) Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicol Environ Saf 66:204–209

Cobbett CS (2000) Phytochelatin biosynthesis and function in heavy-metal detoxification. Curr Opin Plant Biol 3:211–216

Cobbett CS, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Cochram WG, Cox GM (1957) Experimental designs. Wiley, New York

Dat J, Vandanabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci 57:779–795

Dheri GS, Brar MS, Malhi SS (2007) Comparative phytoremediation of chromium contaminated soil by fenugreek, spinach and raya. Commun Soil Sci Pl Anal 38:1655–1672

Diwan H, Ahmad A, Iqbal M (2008) Genotypic variation in the phytoremediation potential of Indian mustard for chromium. Environ Manag 41:734–741

Diwan H, Ahmad A, Iqbal M (2010) Chromium-induced modulation in the antioxidant defense system during phenological growth stages of Indian mustard. Int J Phytol 12:142–158

Dube BK, Tewari K, Chatterjee J, Chatterjee C (2003) Excess chromium alters uptake and translocation of certain nutrients in citrullus. Chemosphere 53:1147–1153

EPA US (1984) Health assessment document for chromium. Environmental Criteria and Assessment Office, EPA 600/8-83-014F. NTIS PB 85-115905. US Environmental Protection Agency, Research Triangle Park

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21–25

Foyer CH, Deascouveries P, Kunert KJ (1994a) Protection against oxygen radicals: important defense mechanisms studied in transgenic plants. Plant Cell Environ 17:507–523

Foyer CH, Lelandais M, Kunert KJ (1994b) Photooxidative stress in plants. Physiol Plant 92:696–717

Freeman JI, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in *Thalpsi* nickel hyperaccumulator. Plant Cell 16:2176–2191

Ghosh M, Singh SP (2005) A comparative study of cadmium phytoextraction by accumulator and weed species. Environ Pollut 133:365–371

Gratao PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494

Grill E, Thumann J, Winnacker EL, Zenk MH (1989) Phytochelatins, the heavy metal binding peptides of plants are synthesised from glutathione by a specific gamma glutamylcysteine dipeptide transpeptidase (phytochelatin synthase). Proc Natl Acad Sci USA 86:6838–6842

Gupta YP (1983) Nutritive value of food legumes. In: Arora SK (ed) Chemistry and biochemistry of legumes. Edward Arnold, London, pp 287–328

Hajiboland R (2005) An evaluation of the efficiency of cultural plants to remove heavy metals from growing medium. Plant Soil Environ 51:156–164

Hall JL (2002) Cellular mechanisms for heavy metal detoxification and tolerance. J Exp Bot 53:1–11

Halliwell B (1987) Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. Chem Phys Lipids 44:327–340

Harmens H, DenHartog PR, ten Bookum WM, Verkleij JAC (1993) Increased zinc tolerance in *Silene vulgaris* (Moench) Garcke is not due to increased production of phytochelatins. Plant Physiol 103:1305–1309

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts In: Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Israr M, Sahi SV, Jain J (2006) Cadmium accumulation and antioxidative responses in the *Sesbania drumondii* callus. Arch Environ Contam Toxicol 50:121–127

Jiang W, Liu D, Hou W (2000) Hyperaccumulation of lead by roots, hypocotyls and shoots of *Brassica juncea*. Biol Plant 43:603–606

Khan I, Ahmad A, Iqbal M (2009) Modulation of antioxidant defense system for arsenic detoxification in Indian mustard. Ecotoxicol Environ Saf 72:626–634

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction—the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238

Labra M, Gianazza E, Waitt R, Eberini I, Sozzi A, Regondi S, Grassi F, Agradi E (2006) *Zea mays* L. protein changes in response to potassium dichromate treatments. Chemosphere 62:1234–1244

Lawn RJ, Ahn CS (1985) Mung bean (*Vigna radiata* (L.) Wilczek/*Vigna mungo* (L.) Hepper. In: Summerfield RJ, Roberts EH (eds) Grain legume crops. Collins, London, pp 584–623

Lu X, Kruatrachue M, Pokethitiyook P, Homyok K (2004) Removal of cadmium and zinc by water hyacinth, *Eichhornia crassipes*. Sci Asia 30:93–103

Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot 56:167–178

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Ogawa K, Kanematsu S, Asada K (1997) Generation of superoxide anion and localization of Cu-Zn superoxide dismutase in the vascular tissue of spinach hypocotyls and their association with lignification. Plant Cell Physiol 38:1118–1126

Panwar BS, Ahmed KS, Mittal SB (2002) Phytoremediation of nickel contaminated soils by *Brassica species*. Environ Dev Sustainability 4:1–6

Pawlisz AV, Kent RA, Schneider UA, Jefferson C (1997) Canadian water quality guidelines for chromium. Environ Toxicol Water Qual 12:123–183

Qureshi MI, Israr M, Abdin MZ, Iqbal M (2005) Responses of *Artemisia annua* L. to lead and salt induced oxidative stress. Environ Exp Bot 53:185–193

Raab A, Feldmann J, Meharg AA (2004) The nature of arsenic-phytochelatin complexes in *Holcus lanatus* and *Pteris cretica*. Plant Physiol 134:1113–1122

Rao MV (1992) Cellular detoxification mechanisms to determine age dependent injury in tropical plant exposed to SO_2. J Plant Physiol 140:733–740

Rauser WE (1999) Structure and function of metal chelators produced by plants. Cell Biochem Biophys 31:19–48

Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermel S, Inze D, Mittler R (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J 32:329–342

Rout GR, Samantaray S, Das P (2001) Differential lead tolerance of rice and black gram genotypes in hydroponic culture. Rost Výroba (Praha) 47:541–548

Salt DE, Prince RC, Pickering IJ, Raskin I (1995) Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol 109:1427–1433

Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, Raskin I (1996) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

Samantary S (2002) Biochemical responses of Cr-tolerant and Cr-sensitive mung bean cultivars grown on varying levels of chromium. Chemosphere 47:1065–1072

Sanita-di-toppi L, Fossati F, Musetti R, Mikerezi I, Favali MA (2002) Effect of hexavalent chromium on maize, tomato and cauliflower plants. J Plant Nutr 25:701–717

Schat H, Llugany M, Vooijs R, Hartley-Whitaker J, Bleeker PM (2002) The role of phytochelatins in constitutive and adaptive heavy metal tolerances in hyperaccumulator and non-hyperaccumulator metallophytes. J Exp Bot 53:2381–2392

Schmoger ME, Oven M, Grill E (2000) Detoxification of arsenic by phytochelatins in plants. Plant Physiol 122:793–801

Shanker AK, Djanaguiraman M, Sudhagar R, Chandreshekhar CN, Pathmanabhan G (2004a) Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram [*Vigna radiata* (L.). Wilczek, cv CO4] roots. Plant Sci 166:1035–1043

Shanker AK, Djanaguiraman M, Sudhagar R, Jayaram R, Pathmanabhan G (2004b) Expression of metallothionein 3 (MT3) like protein mRNA in Sorghum cultivars under chromium (VI) stress. Curr Sci 86:901–902

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–753

Shanker AK, Djanaguiraman M, Venkateswarlu B (2009) Chromium interactions in plants: current status and future strategies. Metallomics 1:375–383

Sharma SS, Kaul S, Metwally A, Goyal KC, Finkemeier I, Dietz KJ (2004) Cadmium toxicity to barley (*Hordeum vulgare*) as affected by varying Fe nutritional status. Plant Sci 166:1287–1295

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot 53:1305–1319

Shukla OP, Rai UN, Singh NK, Dubey S, Baghel VS (2007) Isolation and characterization of chromate resistant bacteria from tannery effluent. J Environ Biol 28:399–403

Simonová E, Henselová M, Masarovičová E, Kohanová J (2007) Comparison of tolerance of *Brassica juncea* and *Vigna radiata* to cadmium. Biol Plant 51:488–492

Singh RP, Tripathi RD, Dabas S, Rizvi SMH, Ali MB, Sinha SK, Gupta DK, Mishra S, Rai UN (2003) Effect of lead on growth and nitrate assimilation of *Vigna radiate* (L.) Wilczek seedlings in a salt affected environment. Chemosphere 52:1245–1250

Sklar FH (1980) A preliminary comparison of the uptake of chromium-51 and zinc-65 by three species of aquatic plants from Louisiana: *Spirodela punctata*, *Eacopa caroliniana*, *Elodea Canadensis*. Proc La Acad Sci 43:46–51

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27–58

Srivastava S, Tripathi RD, Dwivedi UN (2004) Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in *Cuscuta reflexa*-an angiospermic parasite. J Plant Physiol 161:665–674

Srivastava S, Mishra S, Tripathi RD, Dwivedi S, Trivedi PK, Tandon PK (2007) Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. Environ Sci Technol 41:2930–2936

Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13:195–206

Van den Broeck K, Vandecasteele C, Geuns JMC (1998) Speciation by liquid chromatography-inductively coupled plasma-mass spectrometry of arsenic in mung bean seedlings used as a bio-indicator for the arsenic contamination. Anal Chim Acta 361:101–111

Vatamaniuk OK, Bucher EA, Ward JT, Rea PA (2001) A new pathway for heavy metal detoxification in animals—phytochelatin synthase is required for cadmium tolerance in *Caenorhabditis elegans*. J Biol Chem 276:20817–20820

Vecchia FD, Rocca NL, Moro I, De Faveri S, Andreoli C, Rascio N (2005) Morphogenetic, ultrastructural and physiological damages suffered by submerged leaves of *Elodea canadensis* exposed to cadmium. Plant Sci 168:329–338

WHO (1988) Chromium. Environmental health criteria. World Health Organization, Geneva

Witmer CM, Harris R, Shupack SI (1991) Oral bioavailability of chromium from a specific site. Environ Health Perspect 92:105–110

Yabuta Y, Motoki T, Yoshimura K, Takeda T, Ishikawa T, Shigeoka S (2002) Thylakoid membrane-bound ascorbate peroxidise is a limiting factor of antioxidative systems under photo-oxidative stress. Plant J 32:915–925

Yamaguchi K, Mori H, Nishimura M (1995) A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. Plant Cell Physiol 36:1157–1162

Zenk MH (1996) Heavy metal detoxification in higher plants—a review. Gene 179:21–30

Zhang H, Jiang Y, He Z, Ma M (2005) Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). J Plant Physiol 162:977–984

Zhang FQ, Wang YS, Lou ZP, Dong JD (2007) Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). Chemosphere 67:44–50