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Chromium-induced alterations in photosynthesis and associated attributes in Indian mustard

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Abstract

Contamination of soil and water by chromium (Cr) is increasing enormously due to anthropogenic activities. The potential of plants to accumulate or stabilize Cr compounds for the purpose of remediation of Cr contamination has been recognized in recent years. We conducted pot experiments to study photosynthesis and associated attributes in cv Pusa Jai Kisan of Indian mustard under natural as well as Cr-loaded environmental conditions. High doses of Cr caused toxic effects in plants, as evident by a reduction in photosynthetic rate (24.3 to 8.7 μmol CO_2 m^{-2} s^{-1} at 80 DAS), nitrate reductase activity (3.76 to 1.30 μmol nitrite g^{-1} f. wt. h^{-1} at 80 DAS) and the contents of chlorophyll (1.49 to 0.86 mg g^{-1} f. wt. at 80 DAS) and soluble protein (2.96 to 1.93 mg g^{-1} f. wt. at 80 DAS). Since plants lack a specific Cr-transport system, mineral nutrient contents also changed due to Cr toxicity. Cr accumulation in different plant parts was affected by both duration and dose of Cr treatments, with a maximal localization of Cr in roots (up to 0.77 mg g^{-1} d. wt) at initial stages (40 DAS) and in stem (up to 4.19 mg g^{-1} d. wt) at the later stage (80 DAS) of plant growth. Thus, Indian mustard was able to withstand Cr stress and protect itself from Cr toxicity by altering various metabolic processes. Owing to its ability to accumulate large amounts of Cr, it may be useful in the process of land reclamation.

Key words

Chlorophyll, Chromium, Indian mustard, Nitrate reductase, Phytotoxicity, Phytoremediation

Introduction

Soils are the main terrestrial sinks for metal pollutants. Many pollutants, particularly the toxic and persistent heavy metals, affect soil characteristics (Adriano, 2001). The soil contamination ultimately reflects in retarded growth of plants. Thus, there is a risk of transfer of available toxic metals to humans and animals through food chain. These heavy metals are present in the soil as free metal ions, soluble metal complexes (sequestered to ligands), exchangeable metal ions, organically bound metals, precipitated compounds such as oxides, carbonates and hydroxides, or as constituents of silicate materials, and find their way into environment through multiple sources such as metal smelters, industrial effluents, use of fertilizers and a variety of pesticides. Metal effects on plants thriving on these soils become inevitable (Edwards, 2002; Jabeen *et al*., 2009). Of the borderline elements, chromium (Cr) is a highly significant pollutant discharged by the tanning and plating industries. Tannery sludge application to land has become a common practice in the recent years, which alters the physico-chemical characteristics of the soil and deprives it of its fertility. The effluents, inadvertently used by farmers for irrigation, find their way into the food chain (Singh and Sinha, 2005). Chromium (VI), used in electroplating operations, is highly toxic and a proven carcinogen. Breathing high levels of hexavalent chromium can damage and irritate nose, lungs, stomach, and intestine (ATSDR, 1993). In plants, toxic Cr concentrations inhibit growth and alter a number of physiological and biochemical characteristics (Sharma *et al*., 2003; Panda and Choudhury, 2005a). Chromium blocks the photosynthetic electron transport, inhibits photophosphorylation and decreases membrane integrity (Shanker *et al.,* 2005), affects

nitrogen and protein metabolism (Caravaca *et al*., 2003, 2005) and reduces chlorophyll contents (Davies *et al*., 2002; Panda *et al*., 2003; Choudhary and Panda, 2005; Panda and Choudhary, 2005b; Vernay *et al*., 2007). Since Cr enjoys structural similarity with other essential elements like iron (Fe) and sulphur (S), it interferes with the uptake of these elements (Gardea-Torresdey *et al*., 2005), leading to mineral imbalance. It impedes nitrogen metabolism by altering the nitrate levels and nitrate reductase (NR) activity in plants (Caravaca *et al*., 2003, 2005). Studies on toxic effects of Cr on plants have been carried out mostly under controlled conditions and therefore, information is scanty on physiological responses of hyperaccumulator plants to heavy metal stress under realistic field conditions. Since Indian mustard has been identified as a potential plant for phytoremediation of Cr under controlled environmental conditions (Baker and Brooks, 1989; Zhang *et al.,* 2005, 2007; Diwan *et al.,* 2008, 2010a,b), it is germane to work out its behaviour under natural field conditions. This study, investigates the Cr-induced alteration in key metabolic processes, *viz*. photosynthetic rate, stomatal conductance, Cr accumulation and the chlorophyll, nitrate and amino acid contents, which have relevance to plant growth and development.

Materials and Methods

Seeds of Indian mustard [*Brassica juncea* (L.) Czern and Coss. cv Pusa Jai Kisan] were collected from Indian Agricultural Research Institute, New Delhi. Sowing and cultivation of plants were carried out as described by Diwan *et al*. (2010a). Forty-day-old plants were treated with five Cr concentrations in line with some earlier works (Shanker *et al*., 2005). Concentrations of 100 (T1), 200 (T2), 300 (T3), 400 (T4) and 800 (T5) mg Cr kg^{-1} soil were prepared by dissolving potassium dichromate (a source of Cr-VI) in double distilled water (DDW). Sampling of plant material was done at pre-flowering (40 DAS), flowering (60 DAS) and post-flowering (80 DAS) stages to study the various physiological and biochemical parameters.

Chromium content: After harvesting, plant material was rinsed thoroughly in Milli Q water, dried at 80°C for 48 hr, and then ground to fine powder. An amount of 0.25 g of dry material of each treatment was added to a 3 ml of concentrated HNO_3 in a 50 ml digestion tube and mixed gently by swirling. The digestion tubes were placed in a heating block set at 150°C for 1 hr, 2 ml of a 30% H_2O_2 was added to each digestion tube after cooling. These were heated further for 3 hrs at 150°C and then cooled to room temperature. Upon complete digestion of the plant tissue, the solution was diluted to 50 ml and the upper clear part was separated from the lower sand-grit portion and used for determination of Cr content in roots, stem and leaves with the help of atomic absorption spectrometer (Model ZEEnit 600/650, Analytik Jena, Germany).

Chlorophyll content: Chlorophyll (Chl) content was estimated in fresh leaf samples by the method of Hiscox and Israelstam (1979), which involves estimation of plant pigments without maceration. Leaves kept in a moist filter paper in an icebox were washed with cold DDW and chopped. The chopped leaf material (0.1 g) was taken in vials, containing a 5 ml of dimethyl sulfoxide (DMSO), to be kept in oven at 65°C for 1 hr, to achieve a complete leaching of pigments. Thereafter, the volume of DMSO was made up to 10 ml and the chlorophyll content was measured immediately. The absorbance of DMSO, containing the pigments, was recorded at 663 and 645 nm, using a UV-Vis spectrophotometer (BIO 20, Perkin Elmer, Germany). Values of optical density were used in computing the chlorophyll contents with the help of formulae given by Arnon (1949).

Net photosynthetic rate: The net photosynthetic rate and stomatal conductance in the leaves was recorded by using a portable infra red gas analyzer (LICOR 6400, LICOR, Lincoln, USA) that works on the basis of the net exchange of CO_2 between leaf and atmosphere through stomata, as measured by enclosing the leaf in the leaf chamber, and monitoring the rate at which the CO_2 concentration changes over a short time interval (10-20 s). Photosynthetic rate was expressed in μmol CO_2 $m^{-2}s^{-1}$, and stomatal conductance in mmol CO_2 m^{-2} s^{-1}.

NR activity and contents of nitrate, free amino acids, and soluble protein: The NR activity was determined by the method of Klepper *et al*. (1971). Concentrations of nitrite were determined against the standard curve prepared by using sodium nitrite solution. The enzyme activity was expressed in μmol nitrite g^{-1} f.wt. hr^{-1}.

Extraction of nitrate from plants was done by the method of Grover *et al.* (1978). Nitrate in the aliquot was reduced to nitrite with the help of hydrazine sulphate, following the method of Fishman *et al*. (1964). Concentrations of nitrate were determined against the standard curve prepared by using potassium nitrate solutions, and expressed in μmol g^{-1} f.wt.

The amino acid content was estimated using the method of Lee and Takahashi (1966). Standard curve was prepared from glycine of different concentrations and the amino acid content was expressed in μmol g^{-1} f.wt.

The total soluble protein content of leaves was estimated by the method of Bradford, (1976), using a standard curve prepared from the standard of bovine albumin serum (BSA). The protein content was expressed in mg g^{-1} f.wt.

Nitrogen (N), carbon (C) and sulphur (S) contents: For estimating the nitrogen, carbon and sulphur contents, the plant material was rinsed thoroughly with Milli Q water, oven-dried at 80°C for 48 hr, and then ground to a fine powder. A standard was prepared by using sulfanilic acid over a varied range of concentrations. A 0.25 mg of the finely ground plant material was analyzed by the elemental analyzer (Vario EL, CHNOS Elemental Analyzer, Germany) for estimating the contents of N, C and S.

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

Results and Discussion

Chromium accumulation: In roots, Cr accumulation progressed with increase in plant age and Cr dose. Cr levels rose by 0.29 to 0.89 mg g^{-1} d. wt. with T1 on different sampling days. The maximum (1.62 mg g^{-1} d. wt.) chromium accumulation was observed with T5. The accumulation of Cr in the stem also increased continuously and the increase was both dose and time-dependent. The maximum Cr accumulation (4.19 mg g^{-1} d.wt.) was observed with T5 at 80 DAS. Leaves also exhibited a similar pattern of Cr accumulation. With increase in the treatment dose, Cr accumulation was enhanced, showing the highest increment (0.99 mg g^{-1} d. wt.) with T5 (Table 1). Analysis of Cr uptake and accumulation in different plant parts readily reveals the suitability of Indian mustard for remediation of HM-contaminated soils. The maximum accumulation at early stages of plant growth occurred in roots, followed by the stem and leaves, which could be due to vacuolar sequestration of Cr metal in the roots as they act as a barrier against excessive metal translocation, a potential tolerance mechanism operating in plants under metal stress. With increase in the age of the plant, however, the ability to transfer the metal to aerial parts increased, leading to a higher Cr accumulation in the aboveground plant parts, especially in the stem.

Chlorophyll content: No significant change relative to control was noticed in the Chl *a* and total Chl contents of leaves by Cr treatments up to the 300 mg Cr kg^{-1} (T1, T2 and T3) on any of the sampling days. However, with higher Cr doses (T4 and T5) Chl *a* content was reduced by 25-30, 25-32 and 21-34% at 40, 60 and 80 DAS, respectively, all changes being significant. Chl *b* content was not affected significantly at 40 DAS with any Cr dose. A significant decline of 57-59 and 60-64%, with respect to the control, was observed with T4 and T5, respectively, at 60 and 80 DAS (Table 2). The changes in the chlorophyll content were concomitant with the development of Cr toxicity symptoms. Chromium degrades animo-levulinic acid dehydratase, which reduces the availability of prophobilinogen required for chl biosynthesis, thereby affecting the amino levulinic acid (ALA) utilization. This causes ALA build up and finally reduces the chlorophyll level (Vajpayee *et al.*, 2001). Decline in chlorophyll content could also be linked to inhibition of biosynthesis of lipids and carotenoids (Palle *et al.*, 1992; Marschner, 1995). Similar observations were reported in *Nelumbo nucifera* and *Spirodela polyrhiza* (Vajpayee *et al.*, 1999; Appenroth *et al.*, 2003). The decline in Chl *b* could be due to destabilization and degradation of proteins of the peripheral part. Higher concentration of Cr caused a breakdown of chlorophyll and an increase in membrane permeability and membrane damage (Vajpayee *et al.*, 2000; Bertrand and Poirier, 2005; Shanker *et al.*, 2005).

Rate of photosynthesis: No significant effect was found in the net photosynthetic rate with low Cr treatments (T1, T2 and T3). With higher doses (T4 and T5), however, the photosynthetic rate declined by 15-36, 43-60 and 40-64%, as recorded at 40, 60 and 80 DAS

Table - 1: Chromium accumulation in the roots, stem and leaves (mg g^{-1} d.wt.) of cv Pusa Jai Kisan of Indian mustard under different treatments and at different stages of plant growth

Treatments (Cr kg^{-1} soil)	Days after sowing (DAS)		
	40	60	80
		Root	
T0	0	0	0
T1	0.29±0.024^{a}	0.66±0.04^{a}	0.89±0.01^{a}
T2	0.38±0.030^{ac}	0.69±0.02^{a}	1.11±0.05^{a}
T3	0.53±0.053^{ab}	0.88±0.06^{a}	1.29±0.12^{a}
T4	0.63±0.10^{bc}	1.03±0.07^{b}	1.13±0.17^{a}
T5	0.77±0.05^{bc}	1.02±0.12^{b}	1.62±0.20^{b}
		Stem	
T0	0	0	0
T1	0.15±0.02^{a}	0.49±0.02^{a}	0.97±0.08^{a}
T2	0.27±0.03^{a}	0.87±0.11^{a}	2.29±0.66^{ab}
T3	0.44±0.03^{a}	1.10±0.12^{ab}	2.86±0.31^{bd}
T4	0.56±0.06^{a}	1.86±0.22^{b}	3.52±0.25^{bc}
T5	0.64±0.03^{a}	2.34±0.56b	4.19±0.14^{c}
		Leaf	
T0	0	0	0
T1	0.05±0.01^{a}	0.21±0.03^{a}	0.37±0.03^{a}
T2	0.10±0.01^{ab}	0.18±0.01^{a}	0.40±0.02^{a}
T3	0.11±0.01^{ab}	0.30±0.09^{a}	0.55±0.33^{a}
T4	0.13±0.01^{bc}	0.33±0.05^{a}	0.60±0.47^{a}
T5	0.20±0.03^{c}	0.62±0.06^{b}	0.99±0.11^{b}

The values are mean of three replicates ± SE. Different letters indicate significantly different values at a particular DAS ($p \leq 0.05$)

Table - 2: Effect of chromium treatments on chlorophyll contents (mg g^{-1} f. wt.) of cv Pusa Jai Kisan of Indian mustard at different stages of plant growth

Treatments (Cr kg^{-1} soil)	Days after sowing (DAS)		
	40	60	80
		Chl *a*	
T0	1.23±0.14^{a}	1.38±0.19^{a}	1.07±0.01^{a}
T1	1.32±0.01^{a}	1.40±0.28^{a}	1.17±0.02^{a}
T2	1.35±0.01^{a}	1.48±0.01^{a}	1.26±0.08^{a}
T3	1.12±0.020^{a}	1.39±0.04^{a}	1.09±0.04^{a}
T4	0.92±0.001^{b}	1.03±0.001^{b}	0.85±0.04^{b}
T5	0.86±0.005^{b}	0.93±0.0002^{b}	0.71±0.05^{b}
		Chl *b*	
T0	0.29±0.003^{a}	0.36±0.026^{a}	0.41±0.005^{a}
T1	0.30±0.012^{a}	0.38±0.015^{a}	0.44±0.021^{a}
T2	0.31±0.005^{a}	0.39±0.008^{a}	0.45±0.017^{a}
T3	0.31±0.032^{a}	0.42±0.013^{a}	0.47±0.018^{a}
T4	0.29±0.049^{a}	0.15±0.010^{b}	0.16±0.013^{b}
T5	0.28±0.003^{a}	0.14±0.002^{b}	0.14±0.005^{b}
		Total Chl	
T0	1.52±0.14^{a}	1.74±0.18^{a}	1.49±0.02^{a}
T1	1.63±0.01^{a}	1.79±0.03^{a}	1.62±0.02^{a}
T2	1.66±0.01^{a}	1.88±0.06^{a}	1.71±0.08^{a}
T3	1.44±0.04^{a}	1.82±0.06^{a}	1.56±0.05^{a}
T4	1.22±0.05^{b}	1.19±0.01^{b}	1.01±0.06^{b}
T5	1.15±0.004^{b}	1.08±0.003^{b}	0.86±0.06^{b}

The values are mean of three replicates ± SE. Different letters indicate significantly different values at a particular DAS ($p \leq 0.05$)

Table - 3: Effect of chromium treatments on photosynthetic rate (µmol CO_2 m^{-2} s^{-1}) and stomatal conductance (mmol CO_2 m^{-2} s^{-1}) in the leaves of cv Pusa Jai Kisan of Indian mustard at different stages of plant growth

Treatments (Cr kg^{-1} soil)	Days after sowing (DAS) 40	60	80
	Net photosynthetic rate		
T0	28.0±0.08^a	31.6±1.54^a	24.3±0.95^a
T1	28.5±0.29^a	32.2±1.71^a	24.6±2.13^a
T2	28.4±1.13^a	30.7±1.35^a	23.3±0.72^a
T3	26.9±1.41^a	27.9±2.07^a	20.7±1.48^a
T4	23.8±1.00^b	17.9±1.12^b	14.5±1.32^b
T5	17.8±1.23^c	12.5±0.78^c	8.7±0.47^b
	Stomatal conductance		
T0	0.031±0.001^a	0.046±0.001^a	0.019±0.0003^a
T1	0.032±0.009^a	0.048±0.001^a	0.018±0.0004^a
T2	0.035±0.005^a	0.041±0.006^a	0.017±0.0002^a
T3	0.030±0.005^a	0.040±0.002^a	0.015±0.0003^a
T4	0.016±0.002^b	0.028±0.004^b	0.012±0.0007^b
T5	0.011±0.002^b	0.018±0.002^b	0.011±0.0006^b

The values are mean of three replicates ± SE. Different letters indicate significantly different values at a particular DAS ($p \leq 0.05$)

Table - 4: Effect of chromium treatments on nitrate content (µmol g^{-1} f.wt.) and nitrate reductase activity (µmol nitrite g^{-1} f.wt. h^{-1}) of the leaves of cv Pusa Jai Kisan of Indian mustard at different stages of plant growth

Treatments (Cr kg^{-1} soil)	Days after sowing (DAS) 40	60	80
	Nitrate content		
T0	1.90±0.28^a	1.99±0.12^a	0.14±0.01^a
T1	2.23±0.29^a	2.11±0.05^a	0.16±0.005^a
T2	2.35±0.16^b	2.24±0.12^a	0.18±0.004^b
T3	2.33±0.14^b	1.95±0.33^a	0.22±0.014^b
T4	2.09±0.11^a	1.91±0.20^a	0.19±0.006^b
T5	1.23±0.23^c	1.86±0.04^a	0.18±0.005^b
	NR activity		
T0	5.70±0.52^a	6.69±0.47^a	3.76±0.49^a
T1	4.70±0.20^a	6.12±0.33^a	3.29±0.20^a
T2	4.36±0.11^b	6.07±0.12^a	2.28±0.18^b
T3	4.25±0.06^b	5.92±0.12^a	2.26±0.12^b
T4	2.27±0.17^c	2.13±0.057^b	2.14±0.06^b
T5	2.83±0.05^c	3.45±0.019^b	1.30±0.17^c

The values are mean of three replicates ± SE. Different letters indicate significantly different values at a particular DAS ($p \leq 0.05$)

respectively, differing significantly from the control. A similar trend of variation was observed for stomatal conductance (Table 3). Chromium toxicity affects plant metabolism, including synthesis of photosynthetic pigments due to metal binding to protein sulphydryl groups (Van Assche and Clijsters, 1990), or by direct destruction of photosynthetic pigments through generation of highly active oxygen radicals (Pinto *et al*., 2000) Progressive decline in the photosynthetic rate was observed with application of Cr concentrations. Cr is a strong oxidant with a high redox potential of 1.38 eV, and may cause serious oxidative damage to the photosynthetic apparatus, as reflected

Table - 5: Effect of chromium treatments on the free amino acid (µmol g^{-1} f.wt.) and soluble-protein contents (mg g^{-1} d.wt.) of the leaves of cv Pusa Jai Kisan of Indian mustard at different stages of plant growth

Treatments (Cr kg^{-1} soil)	Days after sowing (DAS) 40	60	80
	Free amino acid		
T0	2.42±0.01^a	2.27±0.12^a	1.98±0.17^a
T1	2.49±0.01^a	2.34±0.05^a	2.38±0.083^b
T2	2.50±0.04^a	2.51±0.08^a	2.57±0.123^b
T3	2.57±0.01^a	2.63±0.02^a	2.61±0.056^b
T4	3.61±0.02^b	3.42±0.15^c	3.61±0.025^c
T5	3.97±0.15^c	4.24±0.05^d	3.74±0.050^c
	Soluble protein		
T0	1.64±0.12^a	2.14±0.06^a	2.96±0.01^a
T1	1.68±0.001^a	2.23±0.13^a	2.85±0.01^a
T2	1.91±0.001^a	2.26±0.01^a	2.67±0.02^a
T3	1.31±0.004^b	1.99±0.01^a	2.37±0.01^b
T4	1.02±0.004^c	1.80±0.29^a	2.16±0.12^c
T5	0.98±0.001^d	1.75±0.35^a	1.93±0.02^d

The values are mean of three replicates ± SE. Different letters indicate significantly different values at a particular DAS ($p \leq 0.05$)

Table - 6: Effect of chromium treatments on the nitrogen, carbon and sulphur contents (%) of the leaves of cv Pusa Jai Kisan of Indian mustard at different stages of plant growth

Treatments (Cr kg^{-1} soil)	Days after sowing (DAS) 40	60	80
	N content		
T0	0.30±0.004^a	0.38±0.036^a	0.49±0.011^a
T1	0.28±0.026^a	0.36±0.013^a	0.45±0.031^a
T2	0.30±0.004^a	0.38±0.010^a	0.49±0.023^a
T3	0.32±0.002^a	0.34±0.006^a	0.45±0.032^a
T4	0.29±0.038^a	0.28±0.005^b	0.39±0.010^a
T5	0.27±0.031^a	0.26±0.01^b	0.27±0.012^b
	C content		
T0	3.95±0.18^a	5.43±0.24^a	4.86±0.04^a
T1	3.68±0.15^a	5.02±0.13^a	4.44±0.19^a
T2	3.48±0.23^a	5.26±0.10^a	4.33±0.27^a
T3	3.57±0.01^a	5.09±0.08^a	3.57±0.02^b
T4	3.57±0.26^a	4.18±0.03^b	3.15±0.02^b
T5	2.99±0.21^b	3.77±0.14^b	2.49±0.19^b
	S content		
T0	0.148±0.017^a	0.21±0.003^a	0.209±0.005^a
T1	0.142±0.010^a	0.23±0.014^a	0.207±0.001^a
T2	0.17±0.002^a	0.27±0.014^b	0.17±0.002^a
T3	0.16±0.002^a	0.26±0.012^b	0.14±0.001^b
T4	0.13±0.006^a	0.18±0.005^a	0.13±0.001^c
T5	0.11±0.004^b	0.14±0.005^c	0.058±0.001^d

The values are mean of three replicates ± SE. Different letters indicate significantly different values at a particular DAS ($p \leq 0.05$)

by the results of this study and of Vernay *et al*. (2007). The reduced stomatal conductance in our experiment could be the cause of reduction in photosynthetic rate under Cr treatments, (Liu *et al.,*

2008). The treated plants resisted Cr toxicity in pre-flowering and flowering stages; the decline in the above parameters appeared during late flowering probably because the defence mechanism of plants might have grown weak with time.

NR activity and contents of nitrate, free amino acids and soluble protein: Depletion in NR activity was positively correlated to Cr concentrations. The maximum inhibition of NR activity was recorded at 60 DAS with T4 treatment. In Cr-treated plants, nitrate content increased (10-23%) with T1, T2, T3 and T4 as observed at 40 DAS. However, it was 35% less with T5 in comparison with the control. At 60 DAS, Cr treatment had no significant impact on nitrate content. At 80 DAS, nitrate content of treated plants increased by 13-53%, showing statistically significant differences from the control except at T1. Chromium toxicity significantly impaired NR activity at all treatments except T1 (Table 4). The activity of NR, reducing nitrate to nitrite, depends upon efficiency of photosynthesis or production of photosynthetate and requires photosynthetically generated reductase (NADPH) and energy (Vijayaraghavan *et al*., 1982; Raghuram and Sopory, 1995), thus any alteration in photosynthesis would reflect in the activity of NR enzyme (Rai *et al.,* 2004). The decline in NR activity in Cr-treated Indian mustard could be due to inhibition of chlorophyll biosynthesis, leading to lower photosynthetic rates. Our results are in agreement with earlier ones on Cr toxicity to chlorophyll, NR activity and protein content in eel grass (Vajpayee *et al*., 2001). The reduced NR activity due to Cr treatments allows for nitrate accumulation in the Indian mustard plants, thus resulting in high nitrate levels.

Free amino acid content in the treated plants increased with increasing Cr doses irrespective of the age of plants, when compared with the control. The enhancement continued till 60 DAS, beyond which either it stayed almost constant or declined. When observed at 40 DAS, the increase (3-64%) was statistically non-significant except with T4 and T5. At 60 and 80 DAS, the increase was 3-87 and 20-89% respectively, being statistically significant except with T1, T2 and T3 at 60 DAS (Table 5). The soluble protein content of leaves was not significantly altered at T1 and T2 doses, as recorded at 40 and 80 DAS. Nonetheless, treatments T3, T4 and T5 caused a significant decline of 20-40% relative to the control at 40 DAS and 20-35% at 80 DAS. Similarly, a decline of 7-18% was apparent at 60 DAS, which, however, was non-significant in comparison with the control (Table 5). The increase in free amino acid content in our study might be because of a decreased protein synthesis or increased proteolysis. Palma *et al*. (2002) elucidated the importance of proteolysis and protein oxidation in situations of HM-induced oxidative stress. Depleted protein content in the leaves of Indian mustard at high Cr doses may be attributed to a variety of factors, including the lowered NR activity, which influences nitrogen assimilation. The increased lipoxygenase may lead to the production of free radicals by dioxygenation of membrane lipids and unsaturated fatty acids, which in turn might damage the chloroplast membrane and cellular constituents such as proteins. Another reason of protein decline could be the Cr-induced and H_2O_2-mediated oxidization of proteins or an increased proteolytic activity.

Nitrogen, carbon and sulphur contents: The N content of leaves increased with plant age. It was always lower in the treated plants than in control. The decline was non-significant except with T4 and T5 at 60 DAS and with T5 at 80 DAS. The C content of leaves increased till 60 DAS followed by a decline at 80 DAS irrespective of treatments. However, a non-significant reduction was observed at 40 DAS except with T5. S content also increased till 60 DAS and declined thereafter under Cr treatment. At 40 DAS, there was no significant effect of Cr treatments except with T5. An increase of 11-29% with T1, T2 and T3, and a decline of 14 and 34% with T4 and T5 respectively, were observed at 60 DAS. At 80 DAS, there occurred a statistically significant decline of 0.77-72%, relative to the control, except with T1 and T2 (Table 6). The declining N content with increase in Cr levels could be due to a low uptake efficiency of roots for nitrate in response to Cr (Arduini *et al*., 2006). As a result of reduced N uptake and reduced plant growth, the amount of N accumulated by Indian mustard decreased with increase in Cr supply. Similar results were obtained with *Miscanthus sinensis* on application of Cr (Arduini *et al*., 2006). Due to its structural similarities with some essential elements, Cr can affect the mineral nutrition of plants in a complex way. It can interfere with the uptake of other ionically similar elements, like Fe and S (Skeffington *et al*., 1976; Scoccianti *et al*., 2006). Decreased uptake of S in Cr-stressed plants could be due to inhibition of activity of the plasma membrane H^+ ATPase (Fernandes *et al*., 2002, Moreira *et al*., 2005). The decrease in the ATPase activity can cause a decrease in proton extrusion, which in turn may decrease the transport activities of the root plasma membrane and the uptake of nutrient elements (Moreira *et al*., 2005; Vernay *et al*., 2007). The present study elucidates a high Cr-uptake potential of Indian mustard, its capability to resist Cr stress till late flowering stage, and its suitability for purpose of land remediation.

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