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Understanding leaf biochemical traits for Sunflower (*Helianthus annuus* L.) cultivars grown in chromium stressed environment

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Abstract

Environmental pollution is big curse for plants, animals and other living organisms. Being atmospheric pollutant, different levels of $Cr6^{+}$ (50-500mg/kg) were applied to sunflower plants to determine its toxic effects. It was found that there was imbalanced distribution of leaf nutrients. XRF studies for various elements revealed alteration in leaf appearance due to nutritional deficiency. Leaf protein was reduced and proline increased pretentiously ($p \leq 0.05$) along Cr concentration. Viability of Cr enriched plants strongly showed the environmental protective role of proline. Present study mainly expresses chromium metal toxicity for carbon and nitrogen metabolism predicting poor growth, biomass and yield attributes in Cr treated plants.

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Introduction

Plants require essential elements for their growth and development. They acquire these elements from atmosphere, soil water and minerals. The depletion of these essential elements due to environmental stress develops various symptoms in plants (Uchida, 2000). Environmental pollution due to chromium has become a serious issue from last few years as this metal is widely used in metallurgy, electroplating, tanning, chemical processing, paints and dyes, pulp and paper industries (Zayed and Terry, 2003).

Cr metal is assumed to be phytotoxic non-essential element and shows pronounced structural affinity with some essential nutritional elements, interacts these elements and causes their depletion (Gardea-Torresdey *et al.,* 2005). Cr application develops different injurious effects i.e. inhibition of seed germination, leaf chlorosis, necrosis, poor seedling & root growth and affected plant biomass (Sharma *et al.,* 1995; Cervantes *et al.,* 2001; Atta *et al.,* 2013b). Such metal pollutants persist in soil to alter its characteristics and inhibit plant growth & development. Plant performance regarding chlorophyll, photosynthesis, nitrate reductase (NR) activity and protein biosynthesis is highly affected due to Cr toxicity (Rai *et al.,*1992; Adriano, 2001; Atta *et al.,* 2013a). Proline is produced in affected plants being an environmental stress indicator and its accumulation indicates protein degradation (Alia and Sarhadi, 1991; Charest and Phan, 1990).

Pakistan and many other countries in the world are facing crop losses due to Cr pollution; as Cr from many sources is contributing to pollute soil and water (Sulwalsky *et al.,* 2008). In present study, we used varying Cr levels to assess its toxicity on leaf biochemical traits in sunflower plants as plant growth and yield is highly attributed with leaf performance.

Materials and methods

Experimental design and soil preparation

Selected seeds of two sunflower hybrids (Hysun-33-A & SF-5009-B) were imbibed and sown in prepared earthen pots of 16 inch diameter. The pots were filled with a mix of canal sand and garden clay soil (3:1 part). The soil was humified (3:1 soil and humus) and Cr doses (50-500mg/kg of soil) were thoroughly mixed with it. Each treatment was followed by seven replicates and selected plants were used for analysis. Thinning was executed at 20th day with seedling size of six inch and final data was collected at post flowering stage of crop.

Soil analysis

Four averaged Cr free sandy loam soil samples (1 kg each) were collected from experimental site and their analysis was executed for different characteristics; pH 7.21, EC 1.74 ds cm^{-1}, O.M 7.06%, C 5.85%, N 1.1%, silica sand 67.55%, clay 18.74%, Fe 3.95%, Ca 3.01%, Mg 2.13%, K 2.42%, Na 1.95% and S 0.26%. XRF-spectrometer (PW-1660/Phillips-Holland) was used to analyze various soil elements (as oxides) after grinding and pelletization of 8g oven dried soil sample following standard operation procedure (SOP). International soil textural triangle (USDA-USA and UK-ADAS) was used to classify the soil. Soil organic matter and carbon (ash) was determined by loss on ignition (LOI) method using "Veckstar-Germany" electric furnace (Goldin, 1987; Konare *et al.,* 2010). Soil nitrogen was assessed by Kjeldahl method using following formula.

$$N\,(\%) = \frac{\text{Acid used for sample} - \text{acid used for blank} \times \text{acid normality} \times 14.01 \times 10 \times 100}{\text{Volume of sample}}$$

Plant analysis

Leaf protein was assessed by method of Bradford (1976) at 595 nm while proline was estimated by method of Bates *et al.*, (1973) following Zengin *et al.,* (2005) at 520 nm using a spectrophotometer. Leaf phosphorous was determined by method of Ryan *et al.,* (2001) and Cr contents were estimated by method of Panichev *et al.,* (2005) following combustion and digestion of plant material.

Leaf elements were assessed as oxides; selected leaf samples were completely converted into ash at high ignition temperature using muffled electric furnace. Similar to soil, leaf ash samples were subjected to

XRF-spectrometer for elemental analysis. Leaf carbon & nitrogen was determined by Konare *et al.,* (2010) and Kjeldahl method, respectively. Alteration in leaf color/appearance was justified by comparing the affected leaves with lush green leaves of control plants (Table 2).

Statistical analysis

Using SPSS-20 statistical software, the data was subjected to one-way ANOVA and the difference of treatment means was determined by DMRT (α-0.05). The graphs were prepared by MS-Excel, 2003.

Results and discussion

Effect on leaf protein and proline

In both test hybrids, leaf soluble protein was more in control plants (T0) than in Cr treated plants. Reduction line for protein was pretentiously ($p \leq 0.05$) elevated at higher Cr doses than lower. At 100-150mg/kg Cr, both varieties revealed a less protein decline i.e. 6-11.7%. At 250-500 mg/kg Cr application, protein reduced by 22.6-41.1% in variety-A, and 20.5-38.2% in variety-B (Fig. 1). In contrast to protein; leaf proline quantification found less in control plants than in Cr affected plants. Proline level increased along increasing Cr level. At 250-500mg/kg of Cr; a rapid increase in proline level was noted for variety A and B i.e. 31.2-46.3% and 26-45.2%, respectively. There was a less increase in proline level at 100-150 mg/kg i.e. 8.3-18.5% (Fig. 1).

Effect on leaf carbon & NPK

Figs. 1 and 2 showed a significant effect ($p \leq 0.05$) of increasing Cr levels on leaf carbon, N, P, and K. In variety-A, at 100-150 mg/kg carbon declined by 4.8-9.7% while decline in NPK was 3.25-8.8%, 1.41-7% and 0.5-1.43%, respectively. At 250-500mg/kg the reduction line was 17-43.9%, 12.9-35%, 11.2-28.1% and 3.4-5.28%, respectively. For variety-B, reduction in these nutrients at 100-150mg/kg was 2.4-9.2%, 3.1-8.7%, 4.1-4.2%, 0.5- 1.43% and at 250-500mg/kg, reduction increased upto 41.5%, 34.3%, 27.7% and 4.8%, respectively.

Table 1. One-way ANOVA for various leaf bio-chemical traits under Cr-stress.

SOV	df	MS		F-ratio	
		Var-A	Var-B	Var-A	Var-B
Protein	23	1.00	0.80	151.6***	134.7***
Proline	23	1.59	1.61	336.8***	431.8***
C	23	1.29	1.38	367.9***	389.3***
N	23	0.52	0.53	130.7***	133.9***
P	23	0.02	0.02	8.850***	15.99***
K	23	0.56	0.58	125.9***	145.0***
Na	23	0.53	0.53	36.50***	19.83***
Mg	23	0.60	0.62	43.21***	42.50***

***highly significant results at α-0.05.

Effect on leaf sodium & magnesium

Our study data shows a remarkable effect of Cr metal both on leaf sodium and magnesium contents of test varieties (Fig. 2). Along Cr application, sodium increased upto 23% and magnesium decreased upto 26.3%. The rate of increase & decrease found less at 100-150mg/kg (4.3-8.3% and 3.03-5%) than at 250-500mg/kg (15.3-23% and 10-26.3%). Sodium increase was slightly more in variety-B than variety-A.

Effect on leaf coloration/ appearance

Observations/symptoms for treated plants regarding change in leaf coloration was compared with lush green leaves of control plants (Plate 1). Variety-A showed light green coloration at 100-350mg/kg, yellow green/chlorosis and yellow brown/burning was prominent at 250-500mg/kg and 350-500mg/kg, respectively. For variety-B, light green coloration was noted at 100-500mg/kg. Yellow green leaves were observed at 250-500mg/kg while yellow brown leaves were prominent at Cr dose 400-500mg/kg. In both varieties, yellowing/burning was noted on leaf tips, margins and along midrib (Table 2 and Plate 1).

Table 2. Alteration in leaf coloration under Cr stress.

Cr^{6+} (mg kg^{-1})	Leaf Cr (ppm)		T. Chlorophyll (Atta *et al.,* 2013a)		leaves treatment^{-1} with particular colour appearance							
					Gr		L.Gr		Y.Gr		Y. Br	
	A	B	A	B	A	B	A	B	A	B	A	B
To	0.00	0.00	47.1	47.6	+	+	-	-	-	-	-	-
50	1.51	1.46	47.3	47.7	+	+	-	-	-	-	-	-
100	2.23	2.10	43.0	44.3	+	+	+	+	-	-	-	-
150	3.31	3.00	41.7	42.3	+	+	+	+	-	-	-	-
250	5.54	4.91	37.6	38.7	+	+	+	+	+	+	-	-
350	6.02	5.47	36.5	37.1	+	+	+	+	+	+	+	-
400	6.21	6.00	34.2	36.5	+	+	-	+	+	+	+	+
500	6.97	6.23	32.5	33.2	+	+	-	+	+	+	+	+

Gr = lush green, L.Gr = light green, Y.Gr = yellow green, Y.Br = yellow brown,+/- = observed/ not observed.

At Cr 50mg/kg of soil, no alteration in leaf appearance was noted. Similarly, other parameters (leaf protein, proline, carbon, N, P, K, Na and Mg) well respond towards this dose like control (Figs. 1and 2). We also calculated mean reduction/increase values for leaf parameters to assess Cr metal interaction in sunflower test varieties (Fig. 3).

At Cr dose 1.0 & 2.0 mM, leaf protein increased in paddy plants (*Oryza sativa* L.) and its decline was observed with increasing Cr application i.e. 4.0 mM (Singh *et al.,* 2006). Najafian *et al.,*(2012) reported increasing proline in *Brassica* was due to increasing Cr doses (0, 25, 50, 75 and 100 µm). Plant tissues having more proline predicted its biosynthesis following reduced protein with more hydrolysis (Charest *et al.,*1990). In present study, our results also agreed these findings. Protein depletion was recorded upto 41.1% which increased along increasing Cr doses. On the other hand, elevated proline was upto 46%. Alteration in protein and proline was less at lower Cr doses than higher. Leaf protein and proline remained unaltered at 50 mg/kg Cr (Fig. 1).

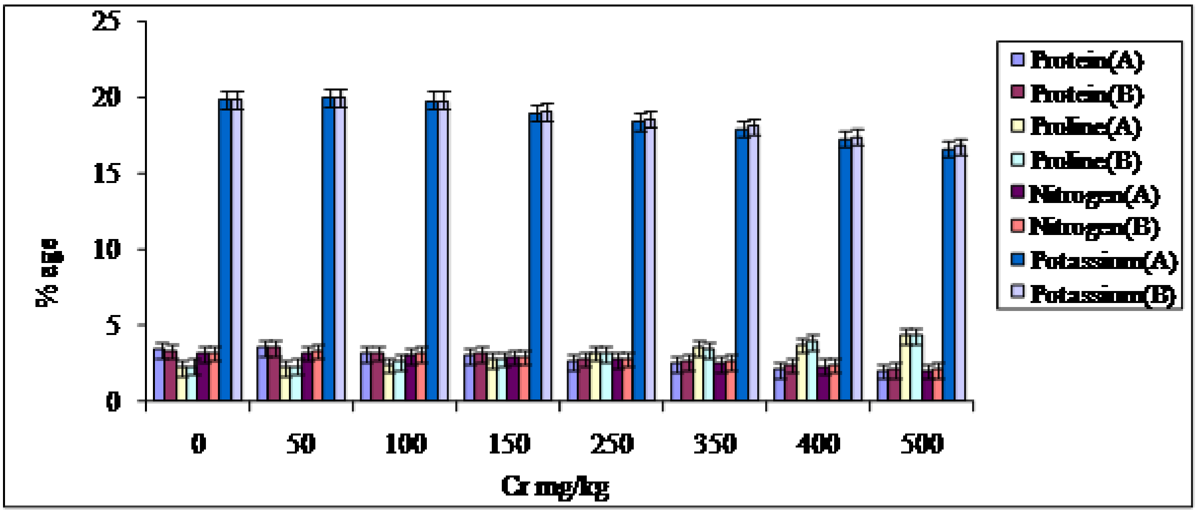

Fig. 1. Effect of Cr metal on some leaf biochemical and elemental characteristics.

Proline production predicts environmental stress and plays a protective role on heavy metals accumulation in plants (Alia and Sarhadi, 1991). Sunflower plants grown in Cr enriched environment remained viable till maturity supported the protective role of proline. Less protein and more proline implements stressed nitrogen metabolism and biosynthesis of free amino acids i.e. proline.

Leaf carbon contents were decreased due to Cr stress in mustard plants remarkably at later growth stages than earlier (Diwan *et al.,* 2012). In rice plants, Cr inhibited level of leaf NPK that was upto 82%, 37% and 42%, respectively (Ahmad *et al.,* 2011). Andaleeb *et al.,* (2008) reported reduction line for Na, K, P and N contents in sunflower roots and shoots at 60mg/kg of Cr. In soya-bean plants, Cr declined Ca, Na, K, Mg, P, N and Cu and left no effects on Fe, Mn and Zn (Turner and Rust, 1971). Our findings agreed these documentations except that Na was increased due to Cr application. For both test varieties, decline in leaf C, N, P, K was recorded upto 43.9%, 35%, 28.1% and 5.28%, respectively. Magnesium reduced upto 26.3% while Na increased upto 23%. We find out a slow reduction/increase in leaf nutrients at Cr dose 100-150mg/kg than 250-500 mg/kg. Results obtained at 50mg Cr/kg soil reveal to be unaffected (Figs. 1and 2).

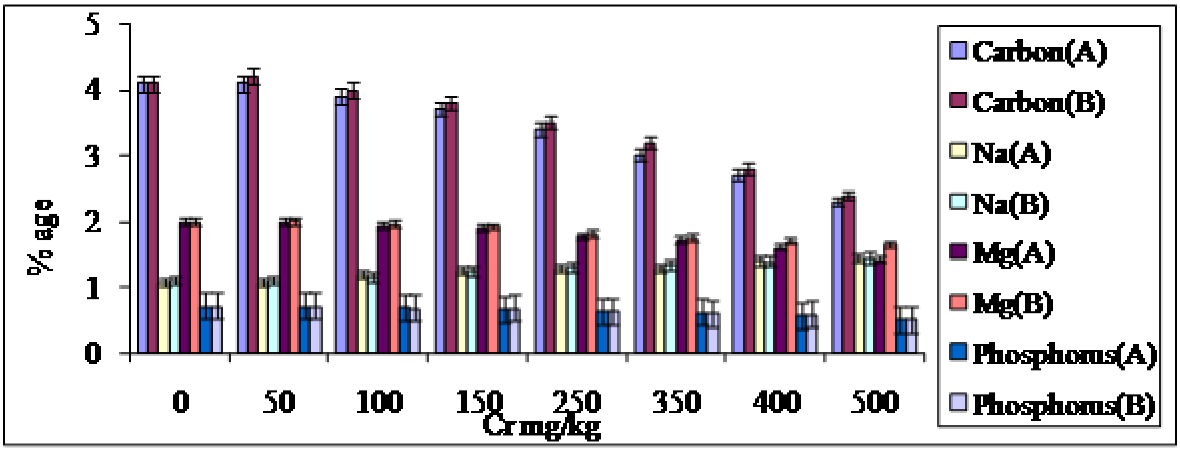

Fig. 2. Effect of Cr metal on leaf elemental composition.

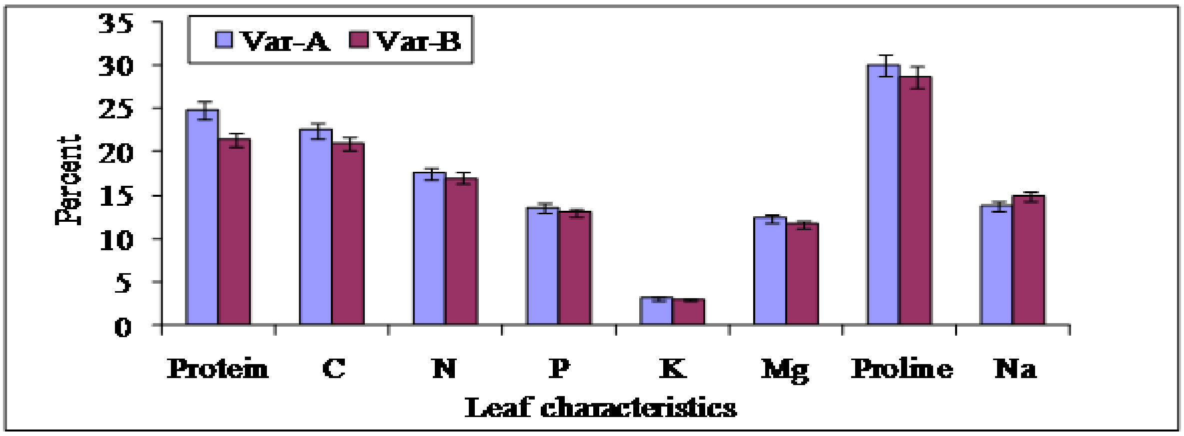

Fig. 3. Mean decrease/increase for various leaf characteristics in Cr affected hybrids.

In our earlier studies, Atta *et al.,* (2013a) reported increased Cr contents in sunflower leaves. The increased Cr and Na contents strongly predict the inhibited transport of minerals from roots to leaves with more osmotic pressure in affected leaves (Table 2). Reduced nitrogen and magnesium level indicates affected photosynthesis as both elements are the essential structural part of chlorophyll pigment. Nitrogen reduction also correlates with affected protein biosynthesis. Less carbon contents reveal Cr affected unstable carbon metabolism mainly carbohydrate accumulation and biosynthesis due to inefficiency of C-fixing enzymes. Carbon and nitrogen decline predicts inhibited metabolism of these in affected leaves. Reduced potassium correlates with stomatal abnormalities for CO_2 intake and transpiration rate. Phosphorous (as energy currency of cell) decline also justify inefficient cell metabolic activities under Cr application upto 500mg/kg. Our earlier findings for decline in sunflower growth and tolerance against Cr metal use (Atta *et al.,* 2013b) also interpret abnormal nutrient accumulation due to Cr stress.

Plate 1. Alteration in leaf coloration due to Cr stress (50-500mg/kg).

Heavy metals change essential mineral composition and induce chlorosis at higher concentration (Vazquez *et al.,* 1987). Burning of leaf tips and margins and reduced leaf size at 60 mg/kg Cr was also reported by Singh (2001). Deficiency regarding N, K and Mg alters leaf coloration (yellow to brownish) either marginal or interveinal (Uchida, 2000). Table 2 and Plate 1 show variations in leaf coloration due to nutrient imbalance under Cr toxicity. Cr outcomes up to100mg/kg reveal no change in sunflower leaf coloration. Burning of leaves was initiated at 350-500mg/kg in variety-A, and at 400-500mg/kg in variety-B. Earlier studies (Table 2) also provided an evidence for sunflower leaf chlorophyll reduction upto 6.99-30.18% along Cr concentration. These findings correlate with nitrogen and magnesium deficiency in affected plants as reported in present study.

Conclusion

Potential outcomes of present study have pointed out Cr as phytotoxic metal. Leaf nutrients are affected by Cr more at higher doses than lower. Leaf physical appearance correlates with its nutritional composition. Reduced C and N contents indicate inhibited metabolism of both with the consequence in abnormal plant biomass and yield attributes. Sunflower variety SF-5009 was less affected by Cr than Hysun-33.

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