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Comparative Study of Hexavalent Chromium Induced Biochemical Changes With and Without EDTA in *Sesbania grandiflora* L. Pers.

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ABSTRACT

The toxic effects of chromium in plants, animals and human beings in the environment have been widely studied. In the present study, pot experiment was conducted to determine the effects of chromium on photosynthetic pigments, Nitrate Reductase (NR) activity and total amino acid, proline, total protein and leghaemoglobin content of *Sesbania grandiflora* (L.) Pers. The seedlings were treated with Chromium Cr (VI), concentrations ranging from 0.38-1.92 mM Kg^{-1} of soil with 0.35 mM Ethylene Diamine Tetra Acetic acid (EDTA) and without EDTA. The efficacy of EDTA in its presence and absence was compared for periods of 30, 60 and 90 days. Our results in comparison with our control indicate the inhibitory effect of chromium to *S.grandiflora*. From the results it has been observed that, increasing concentrations of chromium in the presence of EDTA showed a significant increase in proline and total amino acid contents, while the total chlorophyll, leghaemoglobin content and total protein content decreased and the NR activity of the plant was also affected greatly.

Keywords: Chromium, EDTA, Nitrate reductase, Photosynthetic pigment, *Sesbania.*

INTRODUCTION

Soil contamination with heavy metal is a leading problem worldwide largely affecting agriculture (Azmat and Khanum, 2005). Heavy metals like Cd, Cr, Cu, Hg, Pb and Zn, are common contaminants but the hexavalent Chromium Cr (VI) can be said to be the worst in polluting the environment. The industries discharging chromium are those that manufacture alloys, dyes and pigments, electroplating, metal finishing, petroleum refining, leather tanning and wood preservation factories (Aleves *et al.*, 1993, Mishra and Doble, 2008).

Chromium enters the food chain as well because of the consumption of plants which causes hazardous health problems, since chromium is a non-essential element which is highly toxic for both microorganisms as well as plants (Cervantes *et al*., 2001, Azmat *et al.*, 2007). A high concentration of chromium has been found to be harmful to vegetation, since the metal interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced root growth, biomass, chlorosis, photosynthesis impairing, stunting and finally plant death (Vajpayee *et al*., 2000).

The traditional physio-chemical methods to clean the contaminated soil are often too expensive, very difficult and inefficient. Phytoremediation is a method that uses plants to absorb, transform and detoxify heavy metals and this method is simple, efficient, cost effective and environment friendly (McCutcheon and Schnoor, 2003). Among all the chelates, the use of EDTA has been extensively studied (Cooper *et al.,* 1999, Shen *et al.*, 2002). The presence of EDTA alters the metal

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speciation and metal phytotoxicity (Huang *et al.,* 2008, Wang *et al.,* 2008). *Sesbania grandiflora* is a fast-growing, small soft wooded tree, indigenous from Malaysia to North Australia and cultivated in many parts of India. It is a tropical plant that grows well in a hot humid temperature.

The present work focuses on the toxic effects exerted by Cr (VI) on *Sesbania grandiflora* (L.) Pers. and its effect on photosynthetic pigments, NR activity, total amino acid, proline, leghaemoglobin and total protein content in the plant. The role of EDTA in efficient enhancement of Cr (VI) uptake at elevated conditions was simultaneously analyzed.

MATERIALS AND METHODS

Seed Collection and Pot Experiments

The seeds of *Sesbania grandiflora* used in this study were procured from the Agri-clinic, Coimbatore, India. Two sets of pot experiments were conducted. Different concentrations of chromium with 0.38, 0.76, 1.15, 1.53 and 1.92 mM Kg^{-1} chromium without EDTA (represented as C1-C5) were maintained for the first experimental set. The second set consisted of the presence of 0.35 mM EDTA with increasing concentration of chromium (represented as C1+EDTA-C5+EDTA). A comparative study between both chromium treatments with and without EDTA was carried out with their biochemical activities.

Plant Harvesting and Biochemical Parameters Studied

The plant samples were removed from the treatment pots at various time periods of growth as 30, 60 and 90 days after sowing. The effect of chromium in the presence of EDTA and without EDTA in *Sesbania grandiflora* was analyzed for various biochemical activities.

Estimation of Chlorophyll a, b and Total Chlorophyll

About 0.5 g of fresh leaf material was taken and grinded with 10 mL of pre-chilled 80% acetone using a mortar and pestle. The extract was centrifuged at 5,000×g for 5 minutes. The supernatant was collected and the residue was re-extracted with 10 mL of 80% acetone. The extraction was repeated several times and all the supernatant was pooled together and made into a final volume of 25 mL with 80% acetone. This final solution was utilized for chlorophyll estimation. The absorbance was read at 645, 652 and 663 nm using a UV-VIS spectrophotometer model Elico SL-210. The chlorophyll "a", chlorophyll "b" and total chlorophyll were estimated according to Arnon (1949) and Witham *et al.* (1971).

Estimation of Total Amino Acid, Total Protein and Proline Content

The amino acid content in the chromium treated plants was estimated according to Moore and Stein (1948). A standard curve was prepared from glycine with different concentrations and the amino acid content was expressed in mg g^{-1}.

The total protein content of the shoot and roots was estimated by Lowry *et al.* (1951), with different concentrations of Bovine Serum Albumin (BSA) used as the standard for standard curve preparation. The protein content was expressed in µg g^{-1}.

The proline content in the leaves was estimated by Bates *et al.* (1973) and Chinard (1952). The standard curve was prepared by taking different concentrations of pure proline and used for standard graph calculation. The results were expressed in µg g^{-1}.

Estimation of Legheamoglobin content

The leghaemoglobin content in fresh nodules was recovered from the roots under

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chromium stress without EDTA, chromium in the presence of EDTA and control was quantified at 60 and 90 days after seeding according to Sadasivam and Manickam (1992). The leghaemoglobin was extracted with 0.1M potassium phosphate buffer (pH 7.4). The extract was divided equally into two glass tubes (2 mL $tube^{-1}$) and an equal amount of alkaline pyridine reagent was added to each tube. The haemochrome formed was read at 556 and 539

nm after adding a few crystals of sodium dithionite and potassium hexacyanoferrate, respectively.

Estimation of Nitrate Reductase (NR) Activity

Nitrate reductase activity was assayed according to Sadasivam and Manickam (1992). The enzyme extract was prepared by homogenizing 1 g of the plant material in 6 mL of medium containing 1 mM EDTA, 1-25 mM cysteine and 25 mM potassium phosphate with a final pH 8.8. The homogenate was filtered and centrifuged. 0.5 mL phosphate buffer (Ph 7.5), 0.2 mL potassium nitrate solution, 0.4 mL NADH solution and 0.7 mL water was added to 0.2 mL of the enzyme extract and the test tubes were incubated at 30°C for 15 minutes. The reaction was terminated with the addition of 1 mL sulphanilamide and 1 mL naphthyl ethylenediamine reagent. The absorbance was measured at 540 nm after 30 minutes. A standard graph was prepared using sodium nitrite. NR activity is expressed as micromole nitrite produced per min per mg protein.

Analysis of Chromium Accumulation in Plant Parts

The shoot and root samples were removed from the pots and washed under running water and then with distilled water to remove the adhered soil particles. The shoot and roots of the collected plant samples were dried separately at 80°C for 48 hours. 1gram of the powdered sample was digested in an acidic mixture of HNO_3:$HClO_4$ and the chromium analysis was done using an Atomic Absorption Spectrophotometer, Perkin Elmer 3100 model.

Statistical Analysis

The effect of Cr (VI) and EDTA on *S. grandiflora* was evaluated by a two-way ANOVA using Microsoft Excel 2007 and the Least Significant Difference (LSD) was calculated. The data presented in the table and figures are the mean±SE of at least three independent replicates. Significance between control and treatment plants were compared at 0.05 and 0.01 probability levels.

RESULTS AND DISCUSSION

Effect of Ahromium on Photosynthetic Pigments

The effects of chromium on the photosynthetic pigments, chlorophyll 'a', chlorophyll 'b' and total chlorophyll, are presented in Figures 1-a, -b, and -c respectively. The figures show that the pigment content of the *Sesbania grandiflora* leaves was maximum at lower concentrations of Cr (VI) but declined with the increase in Cr (VI) concentration and in the presence of EDTA. The highest content of Chlorophyll a (2.81 mg g^{-1}) was observed for Control and the lowest was reported for C5+EDTA (0.1 mg g^{-1}) at the end of 90 days. The total chlorophyll content of the control plants at 90 days was found to be 5.21 mg^{-1} g and the treatment C5+EDTA that had the lowest range was 0.59 mg^{-1} g. Statistically there is a significant difference between 30 and 90 days and the significant difference was calculated at both the levels $P \leq 0.01$ and $P \leq 0.05$. Sinam *et al.* (2011) reported a significant decrease in the total chlorophyll content in the leaves of the plant

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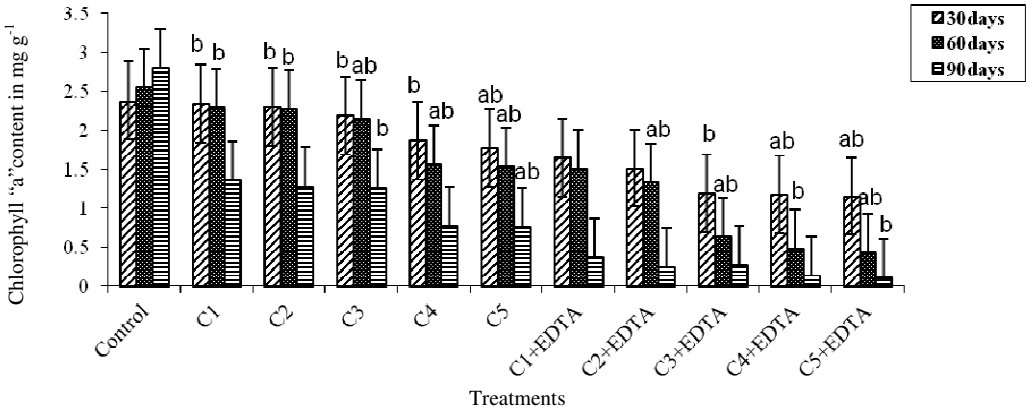
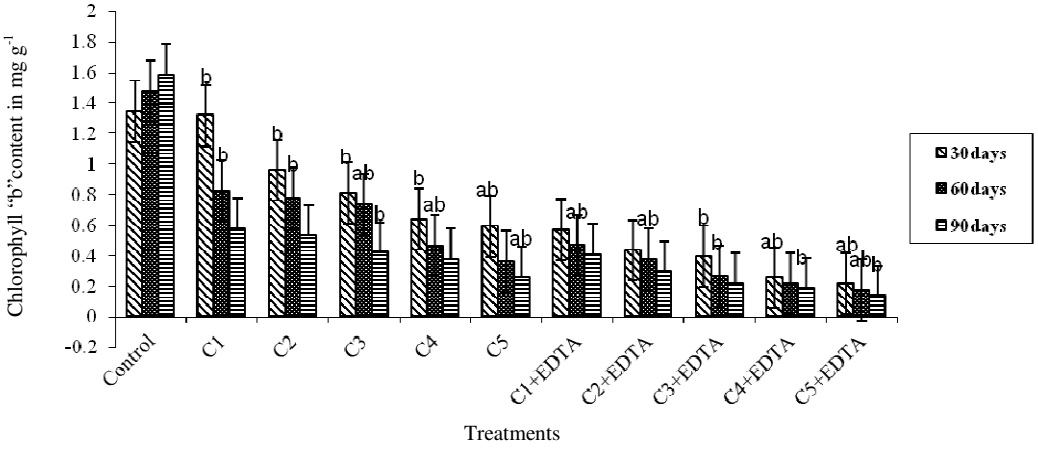


(a)



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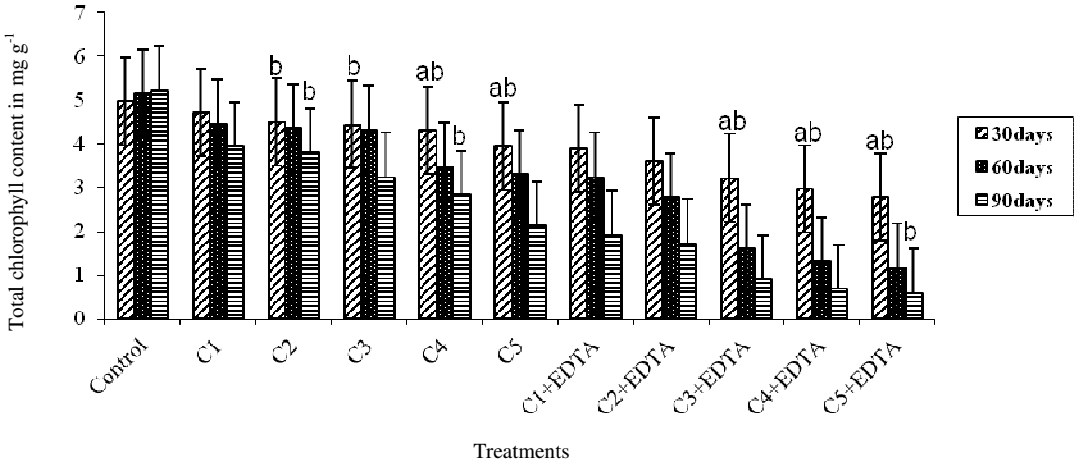

(c)

Figure 1. Effect of different concentration of chromium with and without EDTA on chlorophyll ''a'' (a); chlorophyll ''b'' (b), and total chlorophyll contents (c) (mg g^{-1}) on *Sesbania grandiflora.* Significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

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Cucumis utillissimus grown in garden soil and contaminated soil in the presence of EDTA when compared with substrates without EDTA, but treated with 150 µg g^{-1} chromium. Similar to our results it is clear that the photosynthetic pigments chlorophyll a, b and total chlorophyll content of chromium spiked soil in the presence of EDTA is lower when compared to chromium spiked soil without EDTA, due to diminished chlorophyll biosynthesis and Fe deficiency due to Cr (VI), which caused the inhibition of photosynthesis (Barcelo *et al.,* 1985) in the presence of high metal content. Analogous to our study a decline in both *Chl a* and *Chl b* contents was reported by Vajpayee *et al.* (2000) at enhanced chromium levels and with longer duration of treatment. Similar results were demonstrated by Baszynski *et al.* (1981) in *Lemna minor* L. with the increase of Chromate (CrO_4^{2-}) in nutrient medium.

Effect of Chromium on Total Amino Acid

The total amino acid content in the leaves was observed at two stages, 60 and 90 days of its growth as given in Figure 2. The highest content of (0.26 mg g^{-1}) amino acid was observed after 60 days for C5+EDTA treated plants and the lowest was recorded (0.10 mg g^{-1}) in control plants. At 90 days a similar pattern was observed with, C5+EDTA treated plants which exhibited the highest content of amino acid (0.30 mg g^{-1}) and the lowest amino acid concentration (0.20 mg g^{-1}) in control plants. The results were significantly analyzed by LSD at both levels of $P \leq 0.01$ and $P \leq 0.05$ for which the values showed significant difference at 60 days of treatment. From the results it is observed that the amino acid content increased with the increase in chromium concentration and also with the increase in the time period. Reports by Diwan *et al.* (2012) showed similar results that with increasing concentrations of chromium the free amino acid content increased in Indian mustard, irrespective of the age of the plants, when compared to the control. Authors also report that the increase in free amino acid content in their study was due to decreased protein synthesis or increased proteolysis.

Effect of Chromium on Proline Content

The proline content of *Sesbania grandiflora* was analyzed at two different growth periods 60 and 90 days as shown in Figure 3. From the figure it is clear that when compared to the control, the proline content increased with the corresponding increase in chromium concentration. The highest proline content (84 µg) was recorded

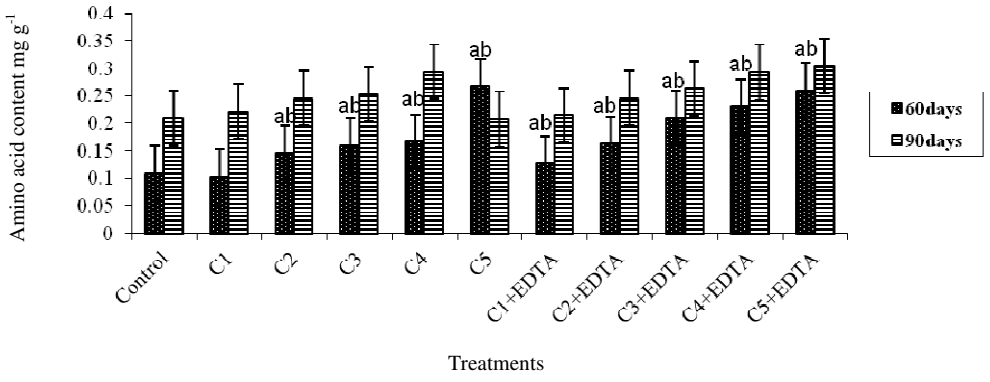

Figure 2. Effect of different concentration of Chromium with and without EDTA on total amino acid content (mg g^{-1}) on *Sesbania grandiflora.* Significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

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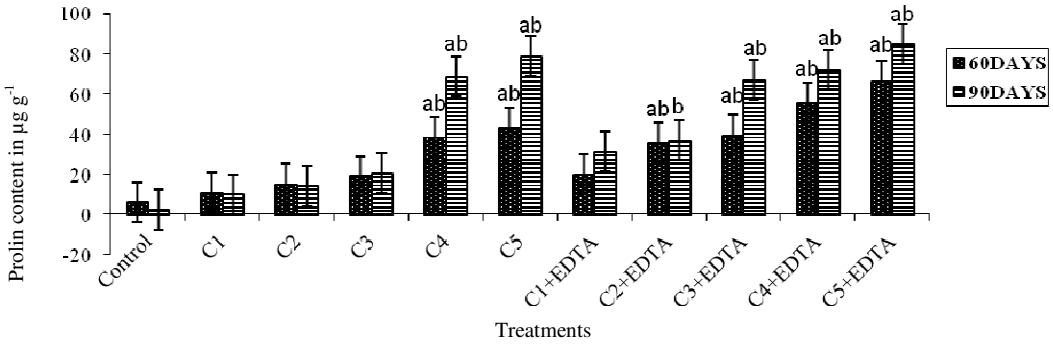

Figure 3. Effect of different concentration of Chromium with and without EDTA on proline content (µg g^{-1}) on *Sesbania grandiflora.* Significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

for C5+EDTA having 1.92 mM of Cr (VI)+0.35 mM EDTA and the lowest value was noted for the control (0.283 µg) at 90th day of treatment. Increase of proline in plant is a defensive mechanism. The mechanisms, by which the plant tolerance is increased by proline, are by the elimination of hydroxyl radicals, osmosis adjustment, inhibition of enzyme lysis and maintaining protein synthesis (Kuzentsov and Shevyakova, 1997). Similar results were reported by Mohanty and Patra (2013) in Mung bean seedlings, where the proline content of plants with Cr:EDTA showed a greater accumulation of chromium. Ganesh *et al.* (2009) reported that higher proline content was observed in tolerant genotypes and a gradual increase in proline in the soybean variety JS 355 could be noted followed by P1, CO1 and CO2 varieties with increasing chromium concentrations. Proline is the only amino acid that accumulates to a greater extent in leaves of many plants under stress. In the present investigation, statistical observations at 60 days shows that a significant difference is observed for the treatments C4 and C5 in the absence of EDTA, but in the presence of EDTA the treatments having 0.76-1.92 mM Cr^{6+} Kg^{-1} concentrations showed significant difference at both significant levels ($P \leq 0.01$ and $P \leq 0.05$). Similarly at 90 days the values differed significantly at both the levels as noticed for 60 days, except for C2+EDTA which showed a significant difference only at one level. The results obtained in the present study are similar to the study conducted by Najafian *et al.* (2012) in which the proline content in the root and the aerial part of *Brassica napus* L increased in the presence of increasing concentrations of chromium, but the increase in the root was more than in the aerial part.

Effect of Chromium on Total Protein

Proteins play an essential role in the growth and development of a plant (Unnikannan *et al.,* 2013). The protein content of *Sesbania grandiflora* shoot at 30, 60 and 90 days presented in Table 1, was found to decrease with the increase in the chromium concentration and it decreased further in the presence of chromium in the presence of EDTA. The protein content in the roots at 30, 60 and 90 days are presented in Table 2. The highest protein content in shoot (311.25 µg) was observed in control at 90 days. The lowest content of protein (146.87 µg) was observed in 1.92 mM chromium with EDTA at 90 days. Similarly the roots of the control plants had the highest total protein content (175.05 µg), but for treatments with higher concentrations of chromium in the presence of EDTA, the

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Table 1. Effect of different concentration of Chromium with and without EDTA on total protein content (µg g^{-1}) of *Sesbania grandiflora* in shoot.[a]

Concentration of Chromium (Cr, mg L^{-1})	30 days	60 days	90 days
	Cr	Cr	Cr
Control	257.91±(0.003) [ab]	264.3±(0.001) [ab]	311.25±(0.005) [ab]
20	247.15±(0.002) [ab]	239.78±(0.001) [b]	172.6±(0.001) [ab]
40	244.95±(0.003) [ab]	231.43±(0.001) [b]	170.21±(0.005) [ab]
60	231.25±(0.001) [ab]	211.9±(0.04)	169.35±(0.0001) [ab]
80	235.1±(0.003) [ab]	191.9±(0.0009)	164.91±(0.001) [ab]
100	212.6±(0.003) [ab]	162.25±(0.04) [ab]	159.15±(0.001) [ab]
Concentration of Cr (mg L^{-1})+EDTA (mM)	Cr+EDTA	Cr+EDTA	Cr+EDTA
20 +0.35	237.85±(0.001) [ab]	240.08±(0.0002) [b]	160.96±(0.003) [ab]
40 +0.35	235.8±(0.005) [ab]	212.45±(0.04)	151.98±(0.001) [ab]
60 +0.35	235.65±(0.001) [ab]	197.05±(0.008)	148.55±(0.001) [ab]
80 +0.35	198.15±(0.001) [ab]	189.8±(0.08)	147.2±(0.003) [ab]
100 +0.35	172.35±(0.0003) [ab]	165.76±(0.001)	146.87±(0.0003) [ab]

[a] Figures in parenthesis are (Mean±SE), significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

Table 2. Effect of different concentration of Chromium with and without EDTA on total protein content (µg g^{-1}) of *Sesbania grandiflora* in roots.[a]

Concentration of Chromium (Cr, mg L^{-1})	30 days	60 days	90 days
	Cr	Cr	Cr
Control	157.8±(0.007) [ab]	169.87±(0.0006) [b]	175.05±(0.04) [ab]
20	148.0±(0.002) [ab]	141.76±(0.01)	115.53±(0.001) [ab]
40	142.8±(0.0007 [ab])	138.16±(0.01)	115.55±(0.0003) [ab]
60	139.8±(0.0004) [ab]	124.8±(0.0004)	111.12±(0.0001) [ab]
80	110.35±(0.001) [ab]	107.11±(0.005)	105.56±(0.0007) [ab]
100	94.6±(0.005) [ab]	89.72±(0.008)	66.33±(0.0004) [ab]
Concentration of Cr (mg L^{-1})+ EDTA(mM)	Cr+EDTA	Cr+EDTA	Cr+EDTA
20 +0.35	121.15±(0.0003) [ab]	119.85±(0.03) [ab]	109.68±(0.0001) [ab]
40 +0.35	116.45±(0.001) [ab]	115.16±(0.05) [ab]	106.93±(0.0003) [ab]
60 +0.35	107.2±(0.0007) [ab]	102.07±(0.001)	101.46±(0.001) [ab]
80 +0.35	95.16±(0.002) [ab]	79.9±(0.006)	77.28±(0.03) [ab]
100 +0.35	90.31±(0.005) [ab]	54.16±(0.001)	60.05±(0.005) [ab]

[a] Figures in parenthesis are (Mean±SE), significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

total protein content decreased further to 60.05 µg. Tables 1 and 2, clearly indicate that the total protein content of the roots is lower than that of the shoots. Reactive oxygen species induced by chromium are known to damage protein (Sinha *et al.,* 2005) and this may be attributed to the decrease in the protein level in the roots of *Sesbania grandiflora*. After 30 and 90 days there was a notable difference at both significant levels $P \leq 0.05$ and $P \leq 0.01$ in the protein content of both the shoots and roots in the presence and absence of EDTA when compared with the control. The significant difference seen in the present study correlates as reported by Vajpayee *et al.* (2001) in which the decrease in protein content might be due to increased protease activity or other catabolic enzymes

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transported during heavy metals stress in plants, or sulphydryl group of protein may be reduced causing deleterious effects in the normal protein form, Rai *et al.* (1992). In the present study the presence of EDTA had a positive effect on the total protein content of the plant, which is indicated by the decreased protein values with increasing chromium concentration.

Effect of Chromium on Leghaemoglobin Content

The changes in leghaemoglobin content are presented in Figure 4. The highest level of leghaemoglobin content (0.07 mM) was determined for control plants at 60 days of growth and the lowest (0.0005 mM) was recorded for 1.53 mM chromium with 0.35 mM EDTA (C4+EDTA) at 90 days of growth. At 60 days of growth the values were significantly different for all the treatments except for C1+EDTA and C4+EDTA at both the levels $P \leq 0.05$ and $P \leq 0.01$, but for 90 days of growth the values significantly differed for all the treatments C1+EDTA, C2+EDTA, C3+EDTA and C4+EDTA at both the levels $P \leq 0.05$ and $P \leq 0.01$. The important role of the leghaemoglobin in the nodule was suggested by Wani *et al.* (2007). According to the author, changes in nodule concentration, affect the entire system of nitrogen fixation. From our previous study on the nodule number, in the presence of increasing concentration of chromium with and without EDTA, it was observed that, when the concentration of chromium increased there was a decrease in the nodule number. When concentrations were above 1.53 mM Cr (VI) Kg^{-1}, no single nodule was formed. Due to the decrease in the nodule number with higher concentrations of chromium, the leghaemoglobin content also decreased with increasing chromium concentrations. From the statistical analysis it is concluded that, the Leghaemoglobin (Lb) content decreased with the increase in the concentration of chromium and with the increase in the age of the plant. From the review, this is the first report given on the effects of chromium in the presence of EDTA on the leghaemoglobin content of the plant.

Effect of Chromium on Nitrate Reductase (NR) Activity

The results of NR activity with increasing concentrations of chromium in the presence and absence of EDTA are presented in Table 3. From the results it is observed that with the increasing chromium concentrations, in

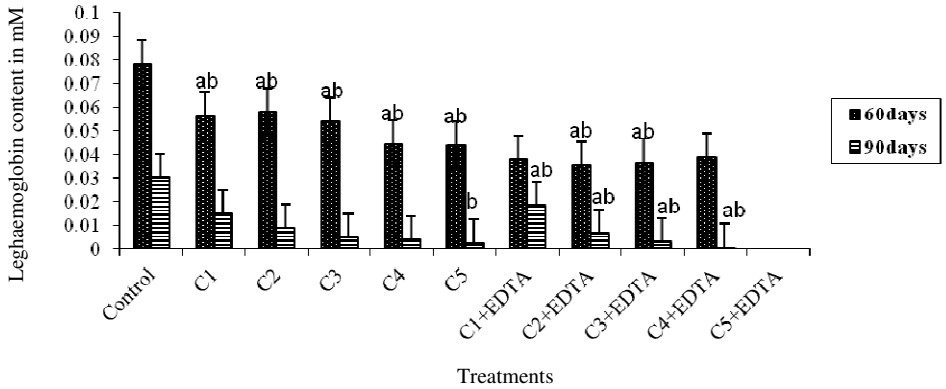

Figure 4. Effect of different concentration of Chromium with and without EDTA on leghaemoglobin content (mM) on *Sesbania grandiflora.* Significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

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Table 3. Effect of different concentration of Chromium with and without EDTA on Nitrate reductase activity (Units mg^{-1} protein) of *Sesbania grandiflora*.[a]

Concentration of Chromium (Cr, mg L^{-1})	30 days	60 days	90 days
	Cr	Cr	Cr
Control	678.65±(0.005)	369.42±(0.006)	424.32±(0.0002)
20	425.93±(0.0007)	318.17±(0.004)[b]	268.17±(0.004)b
40	374.17±(0.004)	309.91±(0.0002)[b]	265.22±(0.0005)b
60	360.50±(0.005)	294.85±(0.01)[b]	253.77±(0.0003)b
80	340.70±(0.006)[b]	279.14±(0.001)[ab]	244.77±(0.001)b
100	337.72±(0.002)	266.41±(0.01)[ab]	228.25±(0.004)b
Concentration of Cr (mg L^{-1})+ EDTA(mM)	Cr+EDTA	Cr+EDTA	Cr+EDTA
20 +0.35	317.55±(0.001)[ab]	281.33±(0.003)[ab]	191.27±(0.008)[ab]
40 +0.35	219.25±(0.01)[ab]	198.49±(0.006)[ab]	161.11±(0.001)[ab]
60 +0.35	210.76±(0.001)[ab]	149.64±(0.008)[ab]	97.16±(0.002)[ab]
80 +0.35	198.33±(0.001)[ab]	83.24±(0.0004)[ab]	82.99±(0.001)[ab]
100 +0.35	181.61±(0.0001)[ab]	82.75±(0.0006)[ab]	56.96±(0.001)[ab]

[a] Figures in parenthesis are (Mean±SE), significant difference at $P \leq 0.01$ (a); $P \leq 0.05$ (b), significant difference at $P \leq 0.01$, and $P \leq 0.05$ (ab).

the absence of EDTA, there is a decrease in the NR activity and similarly the chromium treatment in the presence of EDTA (0.35 mM EDTA) still suffered more in terms of NR activity, and the decrease was also more prominent with the increase in the growth period of the plant.

At 30 days the C4 treatment showed a significant difference at $P \leq 0.05$ level. But when the concentration of chromium increased in the treatment in the presence of EDTA, the activity of NR in the roots of *Sesbania grandiflora* was significantly different at both significant levels $P \leq 0.05$ and $P \leq 0.01$. *However at 60 days there was a significant difference at both the levels* $P \leq 0.05$ *and* $P \leq 0.01$ for all the treatments with chromium in the presence and absence of EDTA, except for C1, C2 and C3 which showed a significant difference only at one level $P \leq 0.05$. For 90 days significant changes were seen for all the treatments of chromium with increasing concentration without EDTA and chromium with EDTA at both the levels $P \leq 0.05$ and $P \leq 0.01$, except for C1-C5 which suffered significant difference, only at $P \leq 0.05$. Our results are in agreement with Panda and Choudhury (2005) who observed the similar effect of NR activity in moss *Polytrichum commune* with the heavy metals Cr, Cu and Zn. Efficiency of photosynthesis or production of photosynthetate and requirement of photosynthetically generated reductase (NADPH) and energy are the important factors for NR activity (Vijayaraghavan *et al.,* 1982; Raghuram and Sopory, 1995). Accordingly the NR activity depends upon the above factors, thus any alteration in photosynthesis would reflect in the activity of NR enzyme (Rai *et al.,* 2004).

Diwan *et al.* (2012) reported similar significant decline in the NR activity in chromium treated Indian mustard due to the inhibition of chlorophyll biosynthesis, by decreased photosynthetic rates and reported that the reduced NR activity due to chromium treatments increase the nitrate accumulation in the Indian mustard plants, thus resulting in high nitrate levels. According to Farissi *et al.* (2014), the lesser nitrate reductase content or its limited transport to shoots as nitrate, has decreased the NR activity. Similarly in our present study, the decrease in the photosynthetic pigments in *Sesbania grandiflora* leaves have directly reduced the NR activity in the plants with increasing concentration of

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chromium in the presence of EDTA. Similarly chromium toxicity to chlorophyll, NR activity and protein content in eel grass was studied by Vajpayee *et al.* (2001). According to Pantawat and Tippawan (2014) in *Ananas comosus* the presence of EDTA enhances and promotes higher chromium than EDDS. Similarly Ebrahimi and Shahsavand (2014) reported that 5 mmol kg^{-1} EDTA was optimum in phytoextracting chromium in plants.

Accumulation of Chromium in Plant Parts

The accumulation of chromium in the plant parts like the shoot and the root are represented in Figure 5. From the figure it is clear that the accumulation of chromium increases with the concentration increase of the chromium and it progressed more in the presence of EDTA, with the age of the plant from 30-90 days. In plants cultivated in the presence of chromium or in combination with EDTA, the metal mainly accumulated in the roots. The maximum chromium accumulation in the root was observed for the treatment C5+EDTA (with 0.35 ppm) and the maximum accumulation in the shoots was observed for the treatment C5+EDTA (with 0.22 ppm). The figure also shows that when the concentration of chromium was 1.92 mM chromium Kg^{-1} (C5+EDTA), the addition of EDTA was found to enhance the root chromium accumulation more than that of the shoot. Diwan *et al.* (2012) reported the accumulation of chromium in Indian mustard plant. Similar results by Aydin and Coskun (2013), reported the accumulation of Cr^{+3} in the presence of EDTA in *Nasturtium officinale* plant.

CONCLUSIONS

With increasing concentrations of chromium, the presence of EDTA affected the plant growth in terms of the various biochemical parameters. The parameters like photosynthetic pigment, total protein, leghaemoglobin content and NR activity were found to be decreased in their activity, whereas total amino acid and proline content increased with the increased chromium activity. Thus in the presence of EDTA, increase in metal accumulation was responsible for the changes in the biochemical parameters of the plant. Thus the chelator had a positive effect in the uptake of chromium and the growth of *Sesbania grandiflora* plant.

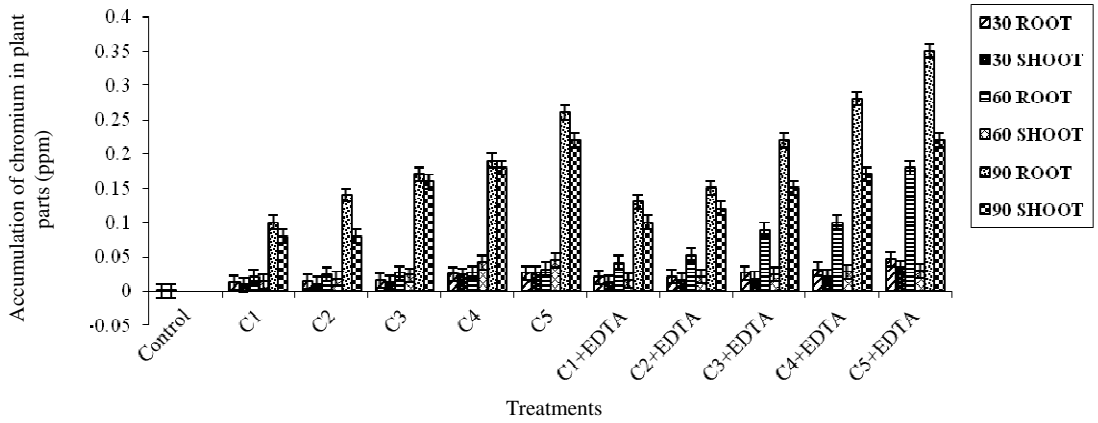

Figure 5. Accumulation of Chromium with and without EDTA in root and shoots of *Sesbania grandiflora* in ppm.

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بررسی مقایسه ای تغییرات وارد شده ی بیوشیمیایی بر کروم شش ظرفیتی با و بدون EDTA در *Sesbania grandiflora* (L.) Pers

س. رواتی، و و. سوبهاشر

چکیده

تأثیرات سمی کروم در گیاهان، حیوانات و انسانها در محیط زیست به نحو گسترده ای مورد مطالعه قرار گرفته است. در این مطالعه ازمایش "pot" برای تشخیص تأثیرات کروم بر رنگدانه های فتوسنتزی، فعالیت های نیترات ردوکتاز، آمینو اسید کلی، پرولین، پروتئین کلی و محتوای لگاموگلوبین (leghaemoglobin) *Sesbania grandiflora* (L.) Pers صورت گرفت. نهال ها با کروم کلر با غلظت های $0.38\text{-}1.92\text{mM Kg}^{-1}$ از خاک و با 0.35mM اسید Ethylene diamine tetra acetic و بدون EDTA مورد درمان قرار گرفتند. تأثیر بودن یا نبود EDTA برای مدت زمان ۳۰، ۶۰ و ۹۰ روز به قیاس گذاشته شد. نتایج در مقایسه با گروه کنترل نشان از تأثیر بازدارنده ی کروم بر *S.grandiflora* دارد. از نتایج چنین برداشت می شود که افزایش غلظت کروم در حضور EDTA، باعث افزایش قابل توجهی در پرولین و محتوای کلی آمینو اسید شد، در حالیکه کلوروفیل کلی، محتوای لگاموگلوبین و محتوای پروتئین کلی کاهش یافته و فعالیت NR گیاه به شدت مورد تأثیر قرار گرفت.

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