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Induced Toxicty and Bioaccumulation of Chromium (VI) in Cluster Bean: Oxidative Stress, Antioxidative Protection Strategy, Accumulation and Translocation of Certain Nutrient

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Abstract

(Language note by the journal's language editor: the whole manuscript should be checked for accurate use of active and passive sentence structures!!!!!)

The present investigation was carried out to examine the noticeable phytotoxicity symptoms, growth behavior, metabolic changes, sulfur, phosphorus, iron, chromium translocation and accumulation in the cluster bean (*Cyamopsis tetragonoloba* L) plants under different concentration of Cr (VI) treatment. Initially plants were grown after sowing of seeds for 90 d with supplying essential nutrients solution. On the day 91 after plant growth, Cr treatment was given as potassium dichromate at 0.05 mM, 0.25 mM and 0.50 mM concentration along with a set of control (0.00 mM Cr). At 0.5 mM of Cr (VI), visual toxicity symptoms were noticed 8-9 days after exposure expressed as loss of turgor, old as well as central leaves became chlorotic, wilted and reductions in leaf dimension. Later phases of toxicity symptoms appear on newly young upper leaves followed by chlorosis and necrosis in patches at subsequent stages. After 15 days of Cr exposure, toxicity symptoms showed at lower levels (0.25 mM) with decreased leaf area, RWC, biomass, yield, concentration of chlorophyll, non-reducing sugar, protein nitrogen, total nitrogen, starch, protein, hill activity, glutathione reductase and non-protein thiol; however, the concentration of reducing sugar, total sugar, non-protein nitrogen, catalase activity, peroxidase, ribonuclease, acid phosphatase, proline and lipid peroxidation increased in the Cr treated plants. Excess levels of Cr concentration resulted in reduction of iron (Fe) accumulation in the leaves from 497to 176 μg/g^{-1}dw along with sulfur (S) and phosphorus (P) translocation significantly at 0.50 mM Cr concentration. Maximum Cr accumulation recorded in the roots (197μg g^{-1} dw) and leaves (142 μg g^{-1} dw) and minimum in the shoots (69 μgg^{-1}dw) at higher concentration of Cr (0.50 mM) and 30 days of treatment duration. The present study concludes that uptake and accumulation of Cr at higher concentration affect the plant growth, metabolic processes, translocation of essential nutrients, crop yield and may lead to health hazards.

Keywords : Chromium; Phytotoxicity; RWC; Glutathione reductase; Non protein thiol

1. Introduction

The chromium (Cr) is a metal known for its toxic effects due to its detrimental effects on living organisms including human beings and persistent nature in the soil and water for a long time once contaminated (Kapoor et al.,2022). *Cr (VI) and Cr (III) are more stable among several chromium oxidation state (Shanker et al., 2005), and hexavalent chromium causes more toxicity in the living being as compared to trivalent chromium due to its more reactivity and mobility (Cervantes et al., 2001;* Von Handorf et al., 2021*). Cr has been listed as one of the 14 most dangerous substances due to its toxic and detrimental effects on plants and other organisms (EPA 2000). Chromium (Cr) in trace amount required in animals including human beings for certain metabolism (Mertz 1969), however, no known biological role reported in the plant metabolism (Reale et al., 2016). Presence of chromium in water bodies is largely due to discharge of industrial effluent particularly from tanneries (Nriagu, 1988) and electroplating industries. On average, 2000–3200 tons of Cr beings discharged in the aquatic environment by tanneries effluent annually in India (Chandra et al., 1997). Discharge of more Cr in the water and soil leads to serious health concern through environmental and food chain contamination (Dube et al., 2003; Ahmed et al., 2016;* Singh et al., 2021*).*

Effects of chromium in relation to phytotoxicity have been investigated by Tiwari et al., (2008; 2009; 2013) on

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** ***Abbreviations :*** *Cr Chromium. Cr (VI) Hexavalent chromium , RWC Relative Water Content, d Days*

important crop plants due to its uptake from soil, water and subsequent accumulation in the plant tissues. Cr has detrimental impacts on the plant's growth due to imbalances in nutrients uptake and metabolic processes due to its competing uptake with other essential elements having similar structure and absorption pathway. Also, Cr uptake in plants leads to formation of more reactive oxygen species (ROS) due to oxidative damage of plant tissues, ultrastructural changes in the chloroplast, cell membrane and subsequent phytotoxicity (Sharma et al., 2020a). Phytotoxicity leads to retarded growth of plants, seed germination inhibition, degradation of photosynthetic pigments, nutrient imbalances, and oxidative injuries (Panda et al., 2003; Tiwari et al., 2009; Tiwari et al., 2013). Trivalent chromium (Cr III) treatment induces stunted growth and phytotoxicity with smaller leaves, wilting and chlorosis (Chatterjee and Chatterjee 2000). Similarly, Cr induced biochemical changes and enzyme activities in the crop plant under different concentrations leads to chlorosis and necrosis of leaves (Dube et al., 2003).

Cr effects nutrients and water uptake in plants leads to reduction in cell division, reduced translocation of essential elements, decrease uptake of selective inorganic nutrients, increase in generation of reactive radicals and oxidative stress, essential nutrients substitution with ligands and other key molecules, damage to plant tissues and organelles including chloroplasts, mitochondria and nucleic acids (Cervantes et al.,2001; Shanker et al., 2005;Tiwari et al.,2013). Although Cr induced stress may be alleviated by activities of antioxidants, it is more susceptible to Cr toxicity in plants at higher concentration (Dong et al., 2007). Toxicity of Cr basically dependents on the oxidation state, mobility in rhizosphere, uptake, translocation, and accumulation in the plant tissues. Cr (VI) follows the same pathway of active transport for plant uptake as other anions like sulphate (Cervantes et al., 2001). Iron (Fe), Sulfur (S) and phosphorus (P) compete with chromium for binding sites of carriers (Wallace et al., 1976). Micronutrient deficiencies in different crops were reported from agricultural fields irrigated with tannery effluent (Sujatha and Gupta 1996; Broadwayet al., 2010). Present study was undertaken to evaluate tolerance level of Cr (VI), visual toxicity symptoms, physiological and metabolic changes, nutrient uptake and translocation in the plant, cluster bean at different concentration of Cr (VI) exposures under sand pot culture growth condition.

2. Materials and Methods

2.1. Sand pot culture experimental setup

The plant Cluster bean (*Cyamopsis tetragonoloba* L) cv. Agaita Guara-112 grown in pre-washed sand as per procedure of Hewitt (1966) with slight modification (Agarwala and Chatterjee 1996) to maintain ambient temperature optimum (25^0-30^0C) under glasshouse-controlled condition for Indian climatic condition. Polyethylene made containers with 10 L size having a hole at center used to grow plants having roofed with a reversed watch glass and glass wool. Nutrient solution was prepared (control) with a composition of 4m $MKNO_3$ (Potassium nitrate), 4 mM Ca $(NO_3)_2$ (Calcium nitrate), 2 mM $MgSO_4$ (Magnesium sulfate), 1.33 mM NaH_2PO_4 (Sodium dihydrogen phosphate), 100 µM Fe EDTA (Ferric Ethylene Diamine Tetra Acetic Acid), 10 µM $MnSO_4$ (Manganese sulfate), 30 µM H_3BO_3 (Boric Acid), 1 µM $CuSO_4$(Copper sulphate), 1µM $ZnSO_4$ (Zinc sulphate), 0.2 MNa_2MoO_4 (Sodium molybdate), 0.1 µM $CaSO_4$ (Calcium sulfate), 0.1µM $NiSO_4$ (Nickel sulphate) and 0.1 Mm NaCl (Sodium chloride). Concentration of iron in the nutrient solution is maintained with Fe EDTA (Ferric ethylene diamine tetra acetic acid) chelate as per the recommendation of Jacobson (1951) and pH maintained to 6.8±0.2 in the prepared nutrient solution during the entire treatment duration.

2.2. Plant growth and chromium treatment

Based on the growth condition, life cycle of test plant and assessed phytotoxic parameters under treatment of Cr (VI), plants were initially grown for 90 d along with complete nutrient solution after sowing of planting seeds in the pots to acclimatize and achieve uniform growth under sand culture experimental condition. Prepared nutrient solution was supplied on regular basis during the experiment duration except in case flushing of pots with deionized water removing the roots exudates and settled salts. After acclimatization under normal growth sand culture conditions, on the day 91, pots were kept in a group of three pots and three lots along with one lot to serve as the control, in which no Cr was added. In the remaining lots, Cr (VI) was supplied at 0.05, 0.25, 0.50 mM concentration superimposed along with base nutrient solutions. Nutrient solution was supplied to plant pots every day on a regular basis except during the removal of salts and other substances from the sand culture medium by flushing with deionized water. Visible phytotoxic symptoms and other phytotoxic parameters of Cr treatment were observed continuously for 30 d after Cr exposures with short treatment duration to complete the study before the achieving maturity stage of the test plant. Plant pots were regularly monitored and maintained under sand culture growth condition for 120 days throughout during the Cr treatment experiment.

2.3. Measurement of growth parameters and biochemical analysis

*Cr induced effects on growth behavior, metabolic changes, imbalances in uptake and translocation of sulphur, phosphorus, iron and chromium were studied in detail in the present investigation. On 106 d (16 d of chromium treatment), the Relative Water Content (RWC) was analyzed in the analogous middle leaves as per procedure of Barrs and Weatherley (1962) during between 9.00 and 11.00 AM under waterlogged sand with nutrient solution in the pots. Temperature (Ambient) was recorded between 25-30^{o}C along with atmospheric humidity at the range of 65-75%. As an index of plant growth, leaf area (cm^2) deliberated by Delta-T leaf area dimension system was measured at d 16 after Cr treatment. At d 107 (17 d after Cr treatment), mature young leaves were harvested from the plants and processed with crude leaf extract for determination of chlorophyll (a, b and total) and Hill activity, sugars and starch content, nitrogen and phenol concentration, and enzyme activities (peroxidase, catalase, ribonuclease, and acid phosphatase), lipid peroxidation, proline, non-protein thiol and soluble protein by following the prescribed standard procedures. C*hlorophyll a, b and

total content were determined in the leaves extracted from harvested fresh leaves by crushing in 80% acetone by following the procedure of Arnon (1949). Similarly, Hill activity was determined at 620 nm by using calorimeter with reaction of 2,4,6-Dichlorophenol Indo Phenol (DCPIP) and determined changes in optical density (O.D./10 min/100mg fw) by following the method of Brewer and Jogendorf 1965.Reducing,non-reducing and total sugar concentration in tested plants was determined by the method of Nelson (1944).Starch concentration was measured as per the method prescribed by Montgomery (1957).Nitrogen fraction was analyzed as per the method given by Chibnall et al. (1943). Phenols content is analyzed by using the method of Swain and Hillis (1959). *Enzymatic activities of* peroxidase, catalase, ribonuclease, and *acid phosphatase* were determined in the harvested fresh leaves extract after homogenizing leaf sample in the ice-cold glass distilled water (1:10) and grinding with help of pestle and mortar at 4^0C. Peroxidase and catalase activity was assayed by the method of Luck (1963) and Bisht et al.(1989), respectively. Similarly, ribonuclease was assayed by the method of Tuve and Anfinson (1960) and acid phosphatase by method of Schmidt (1955). Lipid peroxidation is determined in terms of malondialdehyde (MDA) content in the leaves by the reaction of Thiobarbituric Acid (TBA) as described by Heath and Packer, 1968. Proline content was determined by following the method of Bates *et al.,*1973. Glutathione reductase was determined in the leaves extract by following the procedure of Smith *et al.,* 1988. Non protein thiol (NP-SH) and soluble protein were determined in leave homogenate precipitated in TCA following the method of Boyer (1954) and Bradford (1976) using bovine serum albumin (BSA) as standard, respectively.

2.4. Estimation of iron and chromium

At 120 d of growth (30 d after Cr treatment), Cluster Bean plants are harvested for analysis of tissue. Harvested treated plant samples, washed with tap water, were wiped with 0.01 N HCl and rinsed with deionized water then roots, shoots and leaves were separated, chopped, and kept in a forced-draught oven at 70^0C for complete dryness. *For analysis of Fe and Cr, harvested dried plant sample was digested in a mixture of* $HClO_4$*:* HNO_3 *(1:4v/v), up to dryness (Piper, 1942) to get c*lear digests samples and then *diluted with distilled water (milli-Q grade)* for estimation of iron and chromium *using ICP-OES (Optima 3300 RL).*

2.5. Quality control and assurance

For quality control and quality assurance, the instrumental techniques and standard calibration reference materials of Cr (EPA, quality control samples from E-Merck, Germany) used, and for Iron (BND, 1101.02) from National Physical Laboratory (NPL), India used. Analytical data quality of the tested element was ensured by multiple analysis (n=5) of standard reference samples and data established about ±2.01% of certified value. Blanks were taken in triplicate for all sets of samples to ensure accuracy of the method with the detection limit of chromium (0.5 ppb) and iron (0.3 ppb). Mean resurgence was observed around 98 and 96 for chromium and iron, respectively.

2.6. Analysis of data

Experiment conducted in a randomized block design and all measurements were conducted in triplicate and twice. A*ll data was analyzed for two-way analysis of variance (ANOVA) to measure significance between treatments and to ensure variability and validity of results.* Standard deviation of the means was calculated and given along with the mean according to the method of Panse and Sukhatme (1954).

3. Results

3.1. Periodic observation of phytotoxic symptoms

To investigate Cr induced phytotoxicity, the plants of cluster bean grown under refined sand pot culture condition with Cr (VI) treatment at different concentration (0.05, 0.25, 0.50 mM) along with a set of control. The sand culture technique was very useful for specific symptoms-based study, plant nutrition, plant-metal interaction, plant physiology and plant biochemistry study of a single or mixed metal treatment under controlled conditions as it provides better growth conditions as soil without any interference of other elements. Phytotoxic effects of chromium treatment in the treated plants were observed in terms of grain yield production up to the maturity stage. After d 8 of Cr treatment, toxicity symptoms were observed as chlorosis on the middle leaves followed by wilting at 0.25 mM and 0.50mM Cr of treatment. On 10^{th} d of Cr treatment, older leaves changed into golden yellow color with reduced number and small size followed by chlorosis intensification and severe necrosis in subsequent days and formed large necrotic areas. Further after a few days, chlorotic leaves observed were permanently dried and wilted leading to leaf fall. Similarly, the next upper young leaf showed the same pattern of toxicity spread over a large area. After 15-16 d of Cr treatment, chlorosis symptoms are relatively delayed in the leaves of plants grown at low concentration of Cr.

3.2. Growth Responses of cluster bean under Cr treatment

Growth responses of cluster bean plant under chromium treatment in terms of biomass production, leaf area index and relative water content (RWC), grain yield are given in the Table 1. In the present study, dry biomass of cluster bean is recorded to decrease with increased chromium in the growth medium (Nutrient solution) from 0.05 mM to 0.50 mM. At 0.5 mM Cr (VI) concentration, and after 120 days of maturity, significant reduction (76.9%) in biomass was observed as comparison to the control plants. In term of grain yield, pods produced only in the pots treated with 0.05mM and 0.25 mM Cr (VI), pods were not produced at higher level (0.50 mM). Similarly, grain weight decreased considerably from 0.05mM and 0.25 mM of Cr treatment which was more pronounced at 0.25 mM Cr (VI) in comparison to control plant. Seed size and shape were found abnormal with more deformed and shriveled seed in plants treated with higher Cr concentration with reduction grain yield (85.94%) at 0.25 mM of Cr (VI) concentration. At d 118 (28 d after metal exposure), leaf area of cluster beans observed decrease with increasing Cr (VI) concentration in the solution in comparison to control plants and recorded a

depression of 55.58% in the plants treated with 0.50 mM of Cr (VI). Similarly, relative water content (RWC) was observed to decrease gradually in leaves with increasing Cr concentration as compared to control plants which showed a decrease of 4.26%, 42.9% and 60.3% at 0.05mM, 0.1 mM and 0.25mM Cr concentration, respectively. Biomass reduction in Cr treated cluster beans may be due to profound loss of moisture content and tissue damage.

Table 1. Effect of variable chromium exposure on biomass, grain yield, leaf area and relative water content of cluster bean plants.

Days of growth	Days after metal supply		mM chromium treatment				LSD (*P*=0.05)
			Control	0.05	0.25	0.50	
120	30	Biomass: g plant^{-1}	28.52 ±1.80	21.75 ±1.03	10.46 ±0.48	6.58 ±0.21	0.81
120	30	Grains: g plant^{-1}	9.75 ±0.14	5.21 ±0.11	1.37 ±0.01	-	0.27
118	28	Leaf area: cm^2	93.15 ±4.72	77.24 ±3.84	58.12 ±2.93	41.37 ±1.89	2.75
106	16	RWC: %	96.28 ±5.63	92.17 ±4.05	54.96 ±2.42	38.18 ±0.94	3.51

Values are means ± SE (n=5).

3.3. Chlorophyll content and biochemical changes in cluster bean

Effects of Cr treatment on the photosynthetic pigment and biochemical changes in the cluster bean under different concentrations of Cr (VI) are shown in Table 2. In the present study, concentration of chlorophyll total, a and b decreased variably and manifestly with increase of Cr (VI) concentration in the solution supplied to the treated plant. However, decrease in content of photosynthetic pigment in leaves of the cluster bean observed more at 0.25 mM and 0.50 mM of Cr treatment with 53.09 % reduction in total chlorophyll in comparison to control plant. Decrease in chlorophyll content might be due to inhibition of chlorophyll biosynthesis enzymes or lipid peroxidation of chloroplast membrane by ROS exposed with high concentration of Cr (VI) treatment. Carotene content in fresh leaves of cluster bean plant recorded decreased with increasing concentration of Cr (VI) in the solution. Highest decline was observed at 0.50 mM treatment by 82.09%. Similarly, reducing sugars content was recorded appreciably in higher order. Compared to control leaves, Cr treated plants showed higher reducing sugars with increasing Cr (VI) concentration.

Table 2. Variable chromium exposure on concentration of chlorophyll, carbohydrate fraction, starch, nitrogen and phenol in leaves of cluster bean leaves (at d 107; 17 d after metal exposure).

Parameters	mM chromium treatment				LSD (P=0.05)
	Control	0.05	0.25	0.5	
Chlorophyll: mg g^{-1} fresh wt					
A	0.985 ±0.06	0.639 ±0.04	0.418 ±0.42	0.356 ±0.02	0.07
B	0.518 ±0.05	0.347 ±0.03	0.287 ±0.03	0.175 ±0.02	0.03
Total	1.503 ±0.13	0.986 ±0.11	0.705 ±0.09	0.531 ±0.03	0.09
Carotene (mg g-^{1}fw)	4.86±0.23	4.12±0.27	2.27±0.15	0.87±0.08	0.03
Sugars: % fresh weight					
Reducing	0.27 ±0.02	0.34 ±0.02	0.41 ±0.03	0.49 ±0.04	0.04
Non reducing	0.08 ±0.01	0.07 ±0.01	0.05 ±0.01	0.03 ±0.01	0.01
Total sugars	0.31± 0.02	0.45 ±0.03	0.49 ±0.05	0.49 ±0.04	0.03
Nitrogen: % fresh weight					
protein nitrogen	1.131 ±0.13	0.927± 0.12	0.655± 0.10	0.572± 0.06	0.08
non protein nitrogen	0.253± 0.01	0.288 ±0.02	0.396 ±0.04	0.465 ±0.03	0.04
total nitrogen	1.384 ±0.15	1.215 ±0.12	1.051 ±0.11	1.037 ±0.07	0.07
Starch: % fresh weight	1.175 ±0.13	0.962 ±0.11	0.537 ±0.07	0.381 ±0.02	0.06
Phenols: % fresh weight	0.002± 0.001	0.004± 0.001	0.005±0.001	0.007± 0.001	0.001

Values are mean ±SE (n=5).

In the same trend, total sugars content also increased gradually with increasing chromium in the treatment solution. In contrast, Cr treated plants showed a declining trend of non-reducing sugars with increasing concentration of Cr in the medium. Concentration of protein N decreased with an increasing Cr (VI) level in the nutrient medium, however, the value of non- protein N was recorded with increasing order as compared to non- treated plant. In case of total nitrogen content, a decreasing trend is observed with increasing Cr concentration in comparison to control plant. The starch content decreased in the Cr (VI) treated plants and a maximum 67.57% reduction was recorded in the plant grown at 0.50 mM of chromium treatment. In the present study phenols level was found to increase with Cr (VI) concentration in the medium in cluster bean plants in comparison to non-treated plants and observed increase by 47% at higher Cr (VI) concentration (0.50 mM).

3.4. Protein content and antioxidant enzyme activities in cluster bean

In the present study, data of protein contents and antioxidant enzyme activities in the plant cluster bean under Cr (VI) treatment in the sand culture condition were quite evident (Fig.1). Results indicate that Cr (VI) treatment affects protein content which was observed to decrease gradually with increasing Cr (VI) concentration in the growth medium and maximum 32.2% and 47.9% reduction observed in the plants grown at 0.25mM and 0.50 mM of Cr (VI) treatment. At d 107 (17 d of treatment), catalase activity was found to decrease with differential Cr (VI) concentration in treated plants. In contrast, peroxidase activity at the same treatment duration and concentration of Cr (VI) is recorded to increase in treated plants as compared to control plants. Excess treatment of Cr at 0.25 mM resulted in slight decrease of ribonuclease activity; however, the activity further increased gradually up to 0.50 mM. The present study showed an increasing trend of activity of acid phosphatase at 0.05mM to 0.50 mM of Cr treatment. Hill activity in fresh leaves of cluster beans was observed to decrease with increased Cr (VI) concentrations.

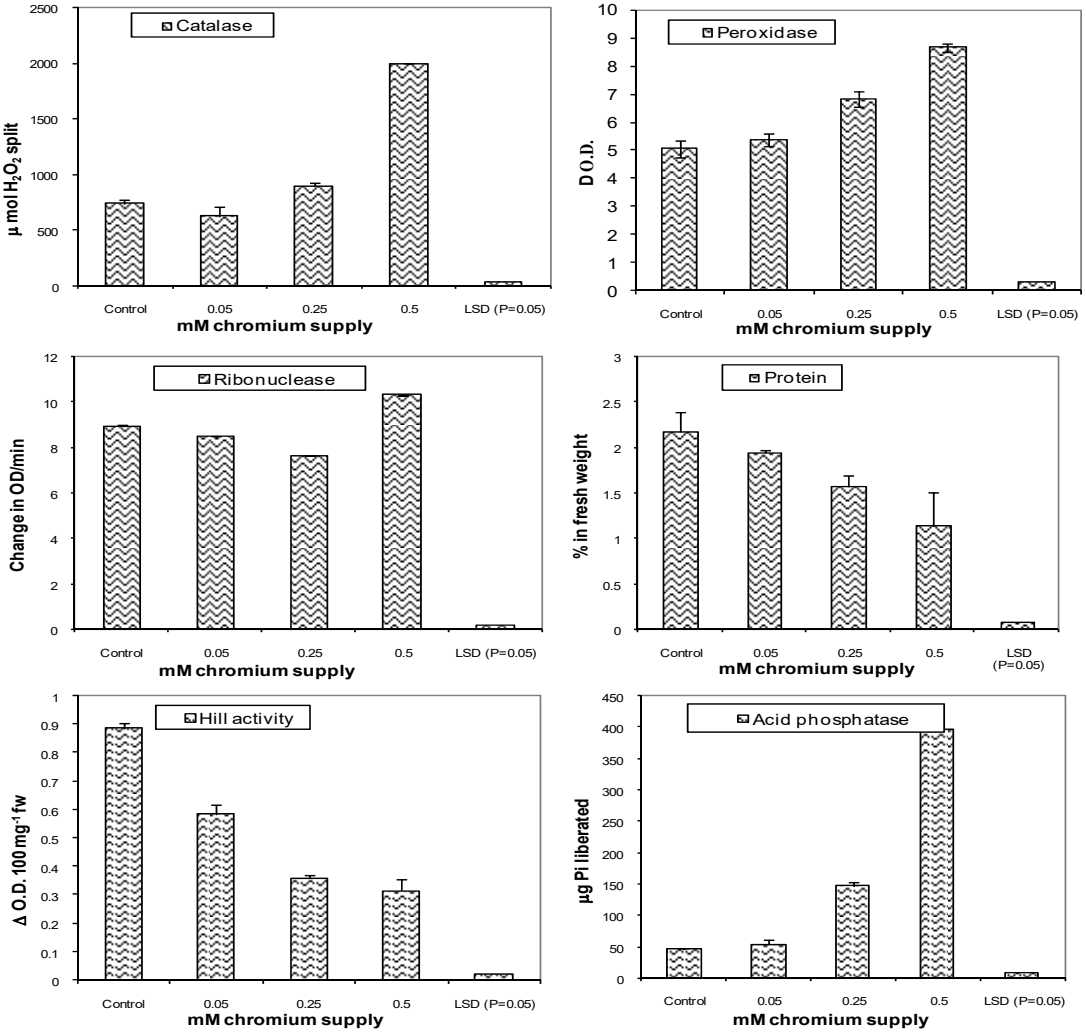

Figure 1. Variable chromium treatment and activities of enzymes, catalase, peroxidase, ribonuclease; hill reaction, acid phosphatase and protein concentration in leaves of cluster beans at 107 d (after 17 days of metal treatment). Values are means ± SE (n=5)

3.5. Proline, glutathione reductase, non-protein thioland lipid peroxidation

Proline content, activities of glutathione reductase, lipid peroxidation and non-protein thiol (NP-SH) in the leaves of cluster beans under different chromium treatment are depicted in Table 3. The present investigation data showed changes in proline content at higher concentration of chromium and increasing trend observed with Cr (VI) concentration in the nutrient solution. However, activity of glutathione reductase in the Cr treated cluster bean is observed to decrease with increasing Cr concentration. At d 107 (17 days of metal supply), the activity of lipid peroxidation increased with increasing level of Cr (VI) from 0.05mM to 0.50 mM, as Cr generates induced phytotoxic results in the plant of cluster beans. The activity of non-protein thiol (NPSH) was found to decrease with excess of Cr (VI) supply in the growth medium.

Table. 3. Variable chromium exposure on proline, glutathione reductase and lipid peroxidation and Non protein thiol in the Cluster Bean plant leaves (at d 107; 17 d after metal exposure).

Parameters	mM chromium treatment				LSD (P=0.05)
	Control	0.05	0.25	0.5	
Proline (µg g-^{1}fw)	0.79±0.06	1.32±0.09	2.05±0.11	3.14±0.15	0.05
Glutathione reductase (GR) (unit/ min/ mg/ protein)	1.29±0.08	1.17±0.06	1.12±0.07	0.92±0.04	0.03
Lipid Peroxidation (µg g^{-1}fw)	26.74±2.75	33.62±3.18	39.54±2.54	51.21±4.71	0.09
Non protein thiol (NPSH) (µg g^{-1} fw)	98.61±4.82	91.53±3.86	72.19±4.19	57.42±3.07	0.07

Values are mean ±SE (n=5).

3.6. Phosphorus, sulfur, iron and chromium concentration in cluster bean

Translocation of phosphorus (P) and sulfur(S) from roots to other parts of the cluster beans is affected by higher concentration of Cr (VI) treatment. At higher concentration of Cr, the plant cluster bean showed higher concentration of P and S in different plant parts as compared to the control plant; however, analytical results showed more accumulation of P and S considerably in the roots (Fig 2).

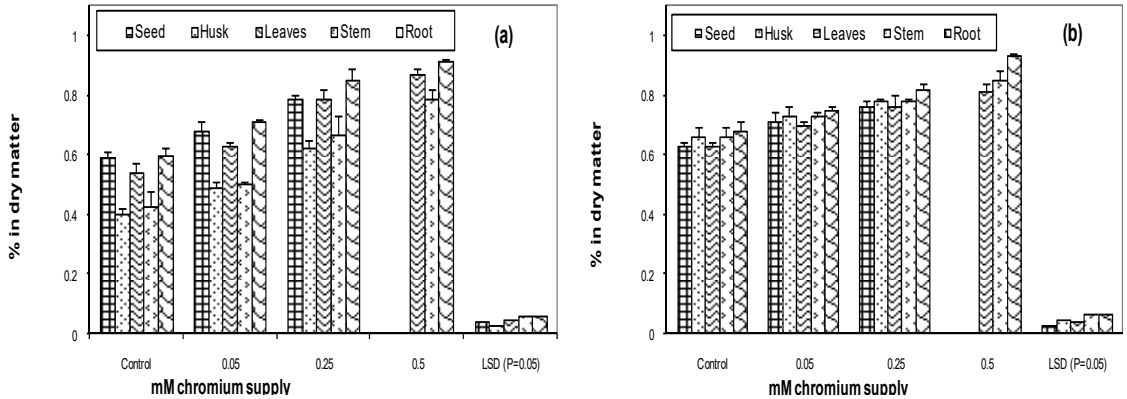

Figure 2. Variable chromium treatment and S (a) and P (b) uptake and accumulation in different parts of the plant of cluster beans at 120 d growth (after 30 days of chromium exposer). Values are means ± SE (n=5)

Higher concentration of Cr (VI) treatment resulted in less accumulation of iron in the upper portion of the plant including stem, leaves, husk and seed and more accumulation in the roots (Fig 3). Chromium accumulation in different parts of cluster bean plants varied with response to Cr (VI) concentration in the medium (Fig 3) and roots, shoots, leaves and other parts of the tested plants. Maximum Cr accumulation observed in the roots, followed by leaves at higher concentration of Cr treatment (0.50 mM). Cr induced toxicity as resulted in reduction of economic yields in terms of seed quality and quantity observed in the present study. High concentration of Cr ranged from 44 to 197 µg/g dw recorded in the roots, however, least accumulation of Cr in the shoots observed with a range from 49 to 69 µg/g dw at different concentration of Cr(VI) treatment.

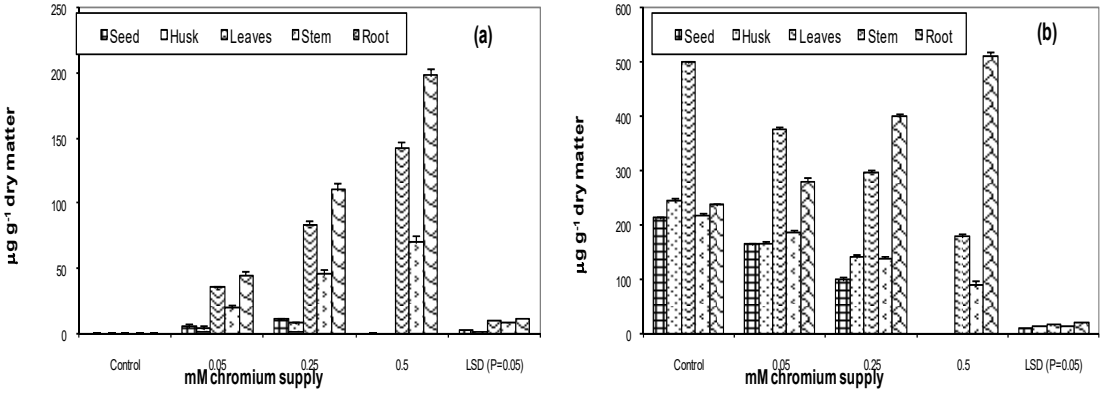

Figure 3: Variable chromium supply and accumulation of chromium (a) and iron (b) in different parts of the plant of cluster beans at 120 d growth (after 30 days of chromium exposer). Values are means ± SE (n=5)

4. Discussion

Various symptoms of phytotoxicity were observed in the plant cluster beans grown under different concentrations of Cr (VI) initially as chlorosis on middle leaves and subsequently, wilting of affected leaves appeared and finally leaf fall at higher concentration (0.25mM and 0.50 mM). The color of middle leaves of tested plants started to change from green to golden yellow in the Cr treated plant. Smaller size and lower number of leaves were observed in the Cr treated plants with severe chlorosis and necrosis in successive days of the treatment. Later, necrotic patches were spread with large areas over the entire leaves followed by wilted dry permanently and premature leaf fall. However, chlorosis observed delayed in the plants grown comparatively at low concentration of Cr treatment even after 15 d. The symptoms of high concentration Cr in cluster bean plants are new insight in ecotoxicological studies and more resemble as reported by Dube et al. (2003). Tiwari et al. (2013) reported on radish plants and recommend that the Cr may generate induced phytotoxic symptoms, reduction in leaf development, growth depression and changes in several biochemical activities as common characteristics under Cr stress. Cr treated plants showed reduced growth with gradual decrease of biomass of cluster bean at 0.05 mM to 0.50 mM concentration. Similar observation reported by many investigators with conclusion that Cr (VI) induces toxicity in the plants by effecting physiological and biochemical processes leading to reduced crop yield (Tiwari et al., 2009; Tiwari et al., 2013; Eleftheriou et al., 2015). In our finding, the crop yields produced only at 0.05 mM and 0.25 mM Cr (VI) treatment level; however, abnormal and deformed size and shape of seed observed with a noticeable reduction in grain yield in cluster beans under Cr treatment. Plant yield is reliant on leaf growth and leaf area; in our present observation the depression in leaf area is observed in treated plants at 0.50 mM as compared to control plants. Many studies examined the Cr and its interaction with plant growth and reported that Cr impedes plant growth, leaf development and yield production while going beyond the threshold levels (Arun et al., 2005; Tiwari et al., 2009). Reduced RWC observed at higher concentration of Cr (VI) treatment with respect to control plants. Reduction in the fresh biomass of cluster bean recorded due to loss of water as apparent from the low RWC of the leaves of plants resulted in wilting. Higher concentration of Cr (VI) exhibited lower water potential and relative water content which in agreement with the observation of Rauser and Dumbroff (1981). The extensively high levels of Cr (VI) exposures cause moribund the stomatal conductance might be owing to the higher oxidative ability of Cr (VI) in the nutrient medium, which in turn might be active in hurtful of the cells and membrane of stomatal protector cells.

Decreasing trends of concentration of chlorophyll content a, b and total in the leaves of cluster bean under Cr (VI) treatment observed, which indicates reduction in the photosynthetic synthesis under Cr treatment as compared to non-treated control plants. Reduction of chlorophyll content may be due to interference of chlorophyll biosynthesis (Lushchak, 2011; Sharma et al., 2020b; *Guanet al.,2021*), changes in the chloroplastic structure or due to Cr (VI) competes with Mg at functional site of the porphyrin ring (Mengel and Kirkby, 2001). Similarly, carotene content was also found to decrease as compared with control plants which support comparable observations reported previously (Mondal et al., 2013; Mondal et al., 2015). Concentration of reducing sugars and total sugars in cluster bean plant leaves was appreciably recorded in higher order with increasing Cr (VI) supply; however, non-reducing sugars concentration recorded declining. Similarly, no significant effects of Cr on non-reducing sugars were reported (Agarwala et al., 1977); however, reducing sugars content was found to increase with Cr(VI) concentration in the growth solution. Increase in reducing sugars due to reduction in the vein and inhibition of photoassimilate export with more sugar accumulation (Rauser and Samarkoon, 1980). Reduction in protein nitrogen, total N and increase in non-protein N concentration in cluster bean leaves recorded in the Cr treated plant in comparison to the control plant. Sharma et al. (1995) reported that Cr affects nitrogen accumulation and absorption which is apparent in decline in protein N content in leaves of wheat plant as N is constituent element of protein as well as core element in the different biomolecules. In cluster bean leaves starch content is also found to decrease under different concentrations of Cr (VI) treatment. Similar observation of reduction in biosynthesis of starch was reported in citrullus plant (Tiwari et al., 2008). The concentration of phenols increased in cluster bean plant at 0.50 mM of Cr treatment as compared to control plants which related to previous study reported by Tewari et al. (2001) and reported same trend of increasing phenols level could be due to fast diffusion of H_2O_2 in the cytosol or due to accretion of high phenols and lower protein formation under Cr stress. The content of protein gradually decreased with increasing Cr (VI) concentration in the growth medium. Chatterjee and Chatterjee (2000) reported the reduced plant biomass in cauliflower might be due to low protein synthesis under stress of Cr, Co and Cu. Degradation of protein can also result in the inhibition of activity of nitrate reductase (Solomonson and Barber, 1990; *Nazet al., 2021*). In cluster bean leaves, the catalase activity was found to significantly decrease in Cr (VI) treated plants, and the observation agreed with the conclusion of Adrees et al. (2015). Catalase might be more susceptible to excess levels of Cr (VI) since it binds willingly to thiol groups and thereby inactivates the thiol-containing enzyme. The efficiency of catalase as an H_2O_2 scavenger is lowered; therefore, it would not play a significant role as an antioxidant (Luna et al., 1994). In cluster bean plant leaves, the activity of peroxidase is observed to increase with an increase in concentration of Cr (VI) in the growth medium. Peroxidase is an enzyme which catalyzes reduction of H_2O_2 into H_2O; however, ascorbate restricts the reduction of H_2O_2 (Luna et al., 1994; Tiwari et al., 2008; Shahid et al., 2016). In cluster bean plant leaves, treatment of Cr up to 0.25 mM reduced ribonuclease activity; however, further increased activity expressed at 0.50 mM of Cr treatment. Enhancement of ribonuclease activity contradicts with the earlier finding (Strakhov and Chazova, 1981) in the plant grapevines. However, some other findings by Tiwari et al. (2013) reported the enhanced ribonuclease activity and acid phosphatase activity seems similar as per our observation. This may be owing to reduced protein synthesis or uptake

of inorganic phosphorus (Marschner, 1995). In our present observation, the hill activity in cluster bean plant was found to decrease with increasing Cr concentration in the medium. Reduced chlorophyll content owing to chromium stress has an impact on the Hill activity in the tested plant. Krupa and Baszynski (1995) investigated that the Cr can also inhibit the Hill reaction disturbing together light and dark reaction. Proline content showed an increasing trend with increase of Cr (VI) concentration in the nutrient solution. Similarly, high levels of amino acids such as cysteine and enhanced synthesis of proline have been observed at high concentration of chromium (Vajpayee et al., 2001) and support other studies suggesting only proline accumulates in the plants grown under metal stress among other amino acids (*Zdunek-Zastockaet al., 2021*). In comparison to the non-treated plant, glutathione activity of reductase is recorded to decrease in the cluster bean due under higher concentration of Cr (VI). Cr (VI) treatment also affected antioxidant enzymes and inhibited activities of glutathione reductase (GR), catalase and peroxidase (Adrees et al., 2015; Ali et al., 2015). In cluster bean leaves, the activity of lipid peroxidation increased from 0.05 mM to 0.50 mM of Cr (VI). Various studies reported increased ROS generation in plants under Cr stress resulted in damage of DNA, pigments, proteins, lipids and initiate the process peroxidation of lipid leads to oxidative burst (Choudhury et al., 2005; Ullah et al., 2009). Similarly, lipid peroxidation and enhanced proline were reported in radish plants under Cr (VI) treatment (Azmat and Akhter 2010). Non-protein thiol (NPSH) activity decreased due to excess amount of Cr in nutrient solution. Cysteine, proline, nonprotein thiol acts as non-enzymaticantioxidants and assist in modulating tolerance under Cr toxicity to protect biomolecules from the free radicals generated during the oxidative stress (Hayatet al., 2012).

In plants, Cr (VI) treatment affects nutrient uptake by interfering uptake of essential nutrients. In excess Cr treatment resulted in increased phosphorus (P) and Sulphur (S) content in various plant parts of cluster bean. However, nutrients element accumulated more significantly in the roots and translocation of P and S from roots to shoot and leaves of plants affected by chromium treatment. Findings of Dube et al. (2003) indicated that high concentration of Cr affects translocation of P, S and other essential nutrients in the Citrullus plants. It is observed that high concentration of Cr (VI) treatment resulted in a decrease accumulation of iron in shoots and increase in the roots. Chlorosis due to Cr treatment has been normally associated with lower plant Fe mobilization and uptake from root to leaves via stems. In iron deficient conditions, leguminous plants enhance root Fe (III) reductase activity to increase the efficiency to reduce Fe (III) to Fe (II), which is more bioavailable and accumulates in the roots (Alcantara et al., 1994). It is also suggested that the excess levels Cr disturbs the nutrient uptake stability from the nutrient sources (Tiwari et al., 2009). This might specify poor utilization of vital nutrients due to Cr toxicity. Translocation and accumulation of Cr in different parts of cluster bean plant from the nutrient solution indicate tolerance responses, which somewhat coincides with an earlier study (Dube et al., 2003; *Aoet al., 2022*) and showed variable uptake and accumulation of Cr roots, shoot and leaves of plant. Our findings are in consonance with the other studied conducted by Huffman and Allaway (1973) who recommended that the mobilization of chromium from roots to shoot and leaves is poor as Cr remains restricted in root.

5. Conclusion

In the present investigation chromium (VI) induced phytotoxicity emphasized in cluster beans with visible symptoms, inhibition of plant growth, reduction of biomass and yield quality. Cr (VI) stress involves particularly with plant metabolism through uptake, translocation, interference in enzymatic activities, and competing essential nutrients on binding sites. It is also concluded that the tolerance limit of Cr (VI) for cluster bean plant was monitored by 0.25 mM level of treatment along with essential nutrient solutions, above which the behind limit plant does not sustain the induced toxicity generated by Cr. Consequently, it is vital to understand the potential strategies to restrict Cr uptake and accumulation in edible parts of crop plants from contaminated sites and to minimize related detrimental effects in the other living beings due to Cr contamination. Further, physiological and biochemical aspects of Cr (VI) toxicity have not been as much considered for study on intact plants in detail. This might lead us to conclude that uptake of Cr from the contaminated agriculture field-grown crop plants may lead to serious health hazards and risk to environmental safety. Importance of present studies could be a new insight in ecotoxicological studies in assessing possible environmental concerns of chromium contamination and ensuring safe and sustainable agriculture.

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Conflicts of interest

The authors declares no conflict of interests in this manuscript.

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