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# Effect of Chromium ions on the biochemical parameters of cotton plant, *Gossypium hirsutum* (L.)

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**ABSTRACT**

The Cotton plant, *Gossypium hirsutum* (L.) treated different concentrations of chromium [Cr] ions (10, 25, 50, 75, 100 and 250 mgkg$^{-1}$) through soil for 60 days. At the end of the experiment, the biochemical parameters of the control and treated Cotton plant *G. hirsutum* (L.) such as chlorophyll a & b, total chlorophyll, total sugars, starch, aminoacids, protein and proline were analyzed by standard procedures. Results were statistically analysed and the mean values between the groups were evaluated by Duncan post hoc with homogenous subset. In all the treated groups, the biochemical parameters were significantly reduced than control except 10mgKg$^{-1}$ which is representing the below critical concentration of the micronutrients, i.e., heavy metals. Our study evidenced that Cr levels in soil less than 10 mg Kg$^{-1}$ showed no impact on the chlorophyll, total sugars and amino acids in the cotton plants *Gossypium hirsutum* (L.) whereas higher concentrations of the Cr levels showed severe impact on the photosynthetic pigments (chief and total pigments) which resulted in the reduced food production (total sugars and starch) and sugar interconversion, finally the growth and metabolism of the cotton plant were severely altered.

***Key words :*** *Chromium ions, Gossypium hirsutum, Chlorophyll pigments, Total protein, Proline*

## Introduction

Emergence of pollutants into the environment leads to the damage and discomfort for the survivality of the flora and fauna species. Different forms of energies such as noise, heat and light became a source for the various pollutions. Natural and man-made pollutants are always present in the ecosystem whenever the levels are exceeding the permissible limit which is considered as a pollution (Lavanya *et al.*, 2021; Ehi-Eromosele and Okiei, 2012). Environmental pollutants are classified into two major types: biodegradable and non-biodegradable. Living organisms (soil bacterium and saprophytes) utilize organic and inorganic materials present in the biodegradable pollutants by catabolic metabolism. The fragmented and simple acids were eventually taken up by the plants for essential process such as photosynthesis, respiration, transpiration, cell differentiation and PGR synthesis. Non-biodegradable pollutants (metals, radioactive isotopes, glass, pesticides, and plastics) are not utilized or lysed by the biological and microbial process, instead few metals were bioaccumulated in the local plants and microbes (Chen *et al.*, 2020; Barakat, 2011).

Panda *et al.* (2010) indicated the common ways of heavy metal pollutants emergence into the aquatic ecosystem. Both natural and anthropogenic activities such as weathering and bleaching of rocks and sewage from rural, urban, industries respectively. Increased industrialization resulted increased concentrations of heavy metals in aquatic ecosystem

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(Seema *et al.,* 2011). Nature has given the tremendous variety of restorative plants and strong bioactive constituents for mankind as long numerous years (Veerakumar and Muthulingam, 2021). Ram *et al.* (2011) reported the magnification properties of the heavy metals into the flora and fauna. Chromium (Cr) is one of the trace and essential element present in the eukaryotic organisms. Usually present as stable trivalent [Cr(III)] form in foods, but hexavalent [Cr(VI)] forms are considered as a carcinogen, membrane disruptor and toxic to the animals and plants. Chlorophyll a and b are the major photosynthetic pigments responsible for the food production in the autotrophs. Total chlorophyll comprised of chief and accessory pigments present in the leaf regions. Total sugars and starch are the chief assimilatory and respiratory substrates for the plants. Total proteins and amino acid are essential sources for enzyme synthesis used for the various catabolic and anabolic processes. The aminoacid, proline played a major role in the plant metabolism especially against the external stress agents. Proline is essential for the saturation of the stressors in plant cell. The main objective of this study is to treat different concentrations (5, 10, 15) of Chromium to the *Gossypium hirsutum* (L.) cotton plants for 60 days and their biochemical parameters such as Chlorophyll a, b, total chlorophyll content, total sugars, starch, total proteins, amino acids and proline levels in the mature leaves.

## Materials and Methods

### Experimental design

From Cotton Research station (Tamilnadu Agricultural University, Coimbatore), the *Gossypium hirsutum* (L.) belongs to the family: Malvaceae, order: Malvales, which are cultivated widely throughout the world. It is commonly known as ganka kavery cotton whereas certified seeds are procured. The plant *G. hirsutum* (L.) are cultivated under aseptic conditions (Ezhilvannan and Sharavanan, 2020) in Botanical Garden, Department of botany Government Arts College (Dharmapuri, Tamilnadu). Potassium dichromate are purchased from Sigma Aldrich for the preparation of different concentrations (10, 25, 50, 75, 100 and 250 $mgkg^{-1}$) of Chromium are mixed with the potted soil (3 kg).

### Biochemical analysis

For chlorophyll a and b analysis, control and treated groups plant leaves (1g) were collected and ground well with 20 ml acetone (80%). The mixture was centrifuged (15 min, 3000 rpm) and the supernatant was collected. The intensity of the solution were calorimetrically read at 645 and 663 nm for chlorophyll a and b. By using the following formula, the chlorophyll a, b and total content were measured by the constant "a" is the length of the light in cm, V is the total volume of the extract.

$$\text{Chlorophyll 'a' (mg g}^{-1}) = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times \text{sample weight}} \times V$$

$$\text{Chlorophyll 'b' (mg g}^{-1}) = \frac{22.9 \times A645 - 4.68 \times A663}{a \times 1000 \times \text{sample weight}} \times V$$

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times \text{sample weight}} \times V$$

For total sugar analysis, 2 g of fresh leaves were boiled in water bath with 5% anhydrous sodium sulphate, cupric sulphate (15 g) and distilled water (1L). After 20 mins of boiling, arseno-molybdate reagent (1 ml) was added and incubated for 2 days at 37 °C. The optical density of the solution was measured at 495 nm.

For Starch analysis, 200 ml of ethanolic leaf extracts were mixed with 3 ml HCl (6N) and autoclaved for 60 mins at 100 °C, finally neutralized with sodium hydroxide. For aminoacid analysis, 1 ml of ethanolic leaf extracts were mixed with ninhydrin reagent (1 ml), NaOH (0.5 ml) and kept in boiling water bath for 20 mins. Diluent solution (5 ml) was added to the solution and the absorbance measured at 570 nm.

For proline analysis, leaf (5 g) homogenized with 10ml aqueous sulphosalicylic acid (3%). The homogenate filtered and the supernatant was collected. Ninhydrin acid (2 ml), glacial acetic acid (2 ml) were added to the supernatant and heated for 60 min at 100 °C. Toulene (4 ml) added to the mixture and vortexed for 20 sec. The absorbance of the aqueous vortex phase were measured at spectrophotometer at 520 nm. Based on the lowry method, the protein levels of the leaf were estimated.

### Statistical analysis

Data collected from control and chromium treated *Gossypium hirsutum* (L.) cotton plant leaves biochemical parameters were statistically analysed by SPSS (17.0) software. Mean and standard deviation

of the data were processed. Comparison of mean between the control and treated groups were analysed by One-way ANOVA. Duncan post hoc test was performed to analyze the homogenous subset between the tested leaf results.

## Results

### Chlorophyll content

The chief photosynthetic pigment chlorophyll a in control *G. hirsutum* (L.) plant leaf was observed as 0.57±0.03mg $g^{-1}$ fresh weight whereas in all the treated groups showed significantly decreased (F=23.78, P<0.05) levels of chlorophyll as ranged between 0.58 to 0.33. Similar results were observed in chlorophyll b contents ranged between 0.37 to 0.20 in the chromium treated groups whereas decreased pattern of chlorophyll b levels were observed as increased concentration of Cr ions as 10, 25, 50, 75, 100 and 250mg/kg. These results which implied on the total chlorophyll levels in the cotton plants as nearly 40% of the total chlorophyll contents than compared to the control (0.92±0.05 mg $g^{-1}$ fresh weight).

### Total sugars and starch analysis

Total sugar and starch representing the photosynthetic products output and storage levels in the control and Cr treated ions. The total sugar levels were found as 9.89±0.50, 9.38±0.47, 8.59±0.43, 8.35±0.42, 7.24±0.36 and 6.13±0.31 mg $g^{-1}$ fresh weight for 10, 25, 50, 75, 100 and 250mg/k Cr treated cotton plants. The stored form of sugars as polysaccharides, starch. In control group, the total sugar and starch levels were 9.55±0.48 and 5.53±0.28 mg $g^{-1}$ fresh weight respectively whereas their ranges were 9.80 to 6.13 (P<0.05, F=6.11) and 6.12 to 2.37 (P<0.01, F=76.99).

### Protein, aminoacid and Proline analysis

Proteins and amino acids are the essential molecules for the various activities of the plant cell as catalyst (enzyme), substrate production and metabolic intermediate products in amphibolic pathway. Control plant protein and amino acids were 47.64±2.38 and 5.67±0.30 mg $g^{-1}$ fresh weight respectively. Similar to the other biochemical parameters, the total protein and aminoacids levels were reduced significantly except 10 mg $kg^{-1}$ concentration of Cr ions. The total protein in the Cr treated plants were ranged between 51.40 to 30.31 (P<0.01, F=124.97) whereas the

**Table 1.** Mean ± SD levels of Biochemical analysis (mg $g^{-1}$ fresh weight) of *Gossypium hirsutum* (L.) treated by different concentrations of chromium (60 days)

| Concentration of Cr in soil | Chlorophyll-a | Chlorophyll-b | Total chlorophyll | Total sugar | Starch | Amino acid | Protein | Proline |
|---|---|---|---|---|---|---|---|---|
| Control | 0.57±0.03$^{f}$ | 0.36±0.02$^{d,e}$ | 0.92±0.05$^{f}$ | 9.55±0.48$^{e,f}$ | 5.53±0.28$^{e}$ | 5.67±0.30$^{d,e}$ | 47.64±2.38$^{e,f}$ | 0.56±0.03$^{a,b}$ |
| 10 mg $kg^{-1}$ | 0.58±0.03$^{f}$ | 0.37±0.02$^{d,e}$ | 0.96±0.05$^{f}$ | 9.89±0.50$^{e,f}$ | 6.12±0.31$^{f}$ | 6.12±0.31$^{e,f}$ | 51.40±2.57$^{f}$ | 0.54±0.03$^{a}$ |
| 25 mg $kg^{-1}$ | 0.53±0.03$^{e}$ | 0.34±0.02$^{d}$ | 0.77±0.01$^{e}$ | 9.38±0.47$^{d,e}$ | 5.25±0.26$^{d,e}$ | 5.54±0.28$^{c,d}$ | 44.40±2.22$^{e}$ | 0.59±0.03$^{a,b}$ |
| 50 mg $kg^{-1}$ | 0.46±0.02$^{d}$ | 0.28±0.01$^{b,c}$ | 0.70±0.04$^{d}$ | 8.59±0.43$^{d}$ | 5.11±0.26$^{d}$ | 5.26±0.26$^{c,d}$ | 39.11±2.00$^{c,d}$ | 0.66±0.03$^{c}$ |
| 75 mg $kg^{-1}$ | 0.41±0.02$^{c}$ | 0.26±0.01$^{a,b}$ | 0.68±0.04$^{c}$ | 8.35±0.42$^{c}$ | 4.43±0.22$^{c}$ | 4.87±0.24$^{b,c}$ | 36.86±1.84$^{b,c}$ | 0.80±0.04$^{d}$ |
| 100 mg $kg^{-1}$ | 0.36±0.02$^{a,b}$ | 0.24±0.01$^{a}$ | 0.56±0.03$^{a,b}$ | 7.24±0.36$^{b}$ | 3.57±0.18$^{b}$ | 4.54±0.23$^{a,b}$ | 33.92±1.67$^{b}$ | 0.84±0.42$^{d}$ |
| 250 mg $kg^{-1}$ | 0.33±0.01$^{a}$ | 0.20±0.01$^{a}$ | 0.52±0.03$^{a}$ | 6.13±0.31$^{a}$ | 2.87±0.14$^{a}$ | 4.30±0.22$^{a}$ | 30.31±1.52$^{a}$ | 0.90±0.05$^{e}$ |
| F value (Sig) | 23.78(P<0.05) | 44.12(P<0.05) | 52.81(P<0.05) | 6.11(P<0.05) | 76.99(P<0.01) | 65.11(P<0.05) | 124.97(P<0.01) | 32.88(P<0.05) |

Control – without chromium in soil; Superscripts (a-f) – Duncan post hoc homogenous subset

aminoacids levels were ranged between 6.12 to 2.87 ($P<0.05$, $F= 65.11$). Proline levels in control group were observed as 0.56±0.03 mg $g^{-1}$ fresh weight and the ranges were observed between 0.54 to 0.90 in the Cr ion treated cotton plants. These results evidenced the development of stress in the cotton plant cells due to the external agent i.e, chromium heavy metal exposure for 60 days.

## Discussion

Phytoaccumulation of heavy metal, in order to eliminate heavy metal ions toxicity in the soil regions are considered as a remediation process were gained a lot attention among the researchers. While the toxicity of natural and anthropogenic heavy metal impact on vegetation were reported by various researchers. Heavy metals treatment causes serious effect to the plants due to their respirational toxicity.

Kumar *et al.* (2022) reported the mechanism of chromium stress in the plants as low concentrations (25µM) showed enhanced growth rate, photosynthetic pigments and enzyme activities while increased concentrations showed remarkable stunned growth in *Ipomoea batatas* (L.) plants. Accumulation of Cr ions developed a stress in plant cell which resulted in the increased proline levels in the cytoplasm.

Wakeel *et al.* (2020) reported Cr induced stress mechanism and their photosynthetic mechanism alterations in the plants. Anjum *et al.* (2017) reported the phytotoxicity of chromium ions in the maize plant by reduced photosynthetic pigment levels with reduced protein levels. Kundu *et al.* (2018) evidenced the stress developed in *Plantago ovato* Forst by *in vitro* studies due to the exposure of chromium hexavalent ions. Ahmad *et al.* (2019) also evidenced the chromium stress in Cauliflower plant with severely altered protein and enzyme levels.

## Conclusion

Our study was focussed about the evaluation of various concentrations chromium metal impact on the biochemical parameters of the cotton plant *Gossypium hirsutum* (L.) treated with 60 days of Chromium ions. After treatments, Cr treated *G. hirsutum* plants chief (Chl. a), accessory (Chl. b) and total photosynthetic pigments were significantly reduced due to the impaired metabolism by Cr toxicity. Carbohydrates (total sugars, starch) and protein (total protein, amino acids) levels were also significantly reduced as increased concentration of Cr ions in treated group leaves than control *G. hirsutum* (L.) plants.

## References


Ahmad, R., Ali, S., Rizwan, M., Dawood, M., Farid, M., Hussain, A., Wijaya, L., Alyemeni, M.N. and Ahmad, P. 2019. Hydrogen sulfide alleviates chromium stress on cauliflower by restricting its uptake and enhancing antioxidative system. *Physiol. Plant.* 168:13001.

Anjum, S.A., Ashraf, U., Khan, I., Tanveer, M., Shahid, M., Shakoor, A. and Wang, L. 2017. Phyto-toxicity of chromium in maize: Oxidative damage, osmolyte accumulation, anti-oxidative defense and chromium uptake. *Pedosphere.* 27: 262–273.

Barakat, M. 2011. New trends in removing heavy metals from industrial waste water. *Arabian Journal of Chemistry*. 4(4) : 361-377.

Chen, J. Ye, Y., Ran, M, Li, Q. Ruan, Z. and Jin, N. 2020. Inhibition of tyrosinase by mercury chloride: spectroscopic and docking studies. *Frontiers in Pharmacology.* 11(81).

Ehi-Eromosele, C.O. and Okiei, W.O. 2012. Heavy Metal Assessment of Ground, Surface and Tap Water Samples in Lagos Metropolis Using Anodic Stripping Voltammetry. *Resources and Environment*. 2(3): 82-86.

Kumar, S., Wang, M., Fahad, S., Qayyum, A., Chen, Y. and Zhu, G. 2022. Chromium Induces Toxicity at Different Phenotypic, Physiological, Biochemical, and Ultrastructural Levels in Sweet Potato (*Ipomoea batatas* L.) Plants. *Int. J. Mol. Sci.* 23 : 13496. https://doi.org/10.3390/ ijms232113496

Kundu, D., Dey, S. and Raychaudhuri, S. Sen 2018. Chromium (VI)-Induced stress response in the plant Plantago ovata Forsk *in vitro*. *Genes Environ*. 40: 21.

Lavanya, K., Gokulprasath, M., Ragul, G., Ranjithkumar, S. and Radha, P. 2021. Analysis of Heavy Metals Contamination in Water: A Review. 8(4) : 201-213.

Ram, S.L., Pravin, U.S. and Deepali, S.P. 2011. Resources and Environment 2011. *International Journal of Ecosystem*. 1(1) : 13-19.

Wakeel, A., Xu, M. and Gan, Y. 2020. Chromium-induced reactive oxygen species accumulation by altering the enzymatic antioxidant system and associated cytotoxic, genotoxic, ultrastructural, and photosynthetic changes in plants. *Int. J. Mol. Sci.* 21 : 728.

Veerakumar, D. and Muthulingam, M. 2021. Preliminary Phytochemical Analysis and Spectroscopic Analysis of Methanolic Extract of *Asteracantha Longifolia* (Nees.). *Journal of Pharmaceutical Research International.* 33(19B): 80-87.