IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 12 Ver. I (Dec. 2014), PP 58-63
www.iosrjournals.org

Physiological responses induced by chromium^{+6} toxicity to *Cucumis sativus* L. and *Macrotyloma unifloroum* Lam.

Soumyashree Pati[1], Ayashkant Ghadei[1], Atia Arzoo[1],
Sandeep Kumar Nayak[1], Ashirbad Mohapatra[2], Kunja Bihari Satapathy[1*]
[1]*Post Graduate Department of Botany, Utkal University, India.*
[2]*Sri Jayadev College of Education and Technology, India.*

Abstract: *The aim of the present study was to examine the possible effect of Chromium (Cr^{6+}) stress on the rate of germination, growth, total chlorophyll, protein, and proline content in the seedling of Macrotyloma unifloroum* Lam. *and Cucumis sativus* L. *Upon exposure to graded levels of Cr^{+6} toxicity by using liquid culture medium. Seedling of both plants exhibited a gradual decline in the growth, total chlorophyll content, protein content along with enhanced proline content with increasing levels of Cr^{+6}. Rate of germination was 68% in case of Macrotyloma unifloroum* Lam. *whereas 1% in Cucumis sativus* L. *at 100 ppm of Cr^{+6}. The root and shoot growth showed a decline trend with the rise in concentrations (5 to 20 ppm) in both the plants. It was noticed that Macrotyloma unifloroum was less affected by Cr^{+6}in comparison to Cucumis sativus. The study of biochemical analysis revealed that the total chlorophyll and protein content of Macrotyloma unifloroum and Cucumis sativus were reduced gradually with the rise in concentration (5-20 ppm) and also time duration (7-21days). A decrease in proline content was observed at lower concentrations (5 ppm) of Cr^{+6} whereas at higher concentrations (20 ppm) enhanced proline content was reported in both the species, but comparatively more in Cucumis sativus. The outcome of the present study indicates that the effect of hexavalent chromium (Cr^{+6}) on Cucumis sativus was high, whereas moderate on affecting Macrotyloma unifloroum.*

Keywords: *Hexavalent Chromium, Toxicity, Macrotyloma unifloroum, Cucumis sativus, Seed germination, Growth.*

I. Introduction

Heavy metals are the group of elements having a density greater than 5g/cm^3 (Alloway, 1995) and their contamination in soil and water from anthropogenic sources is a growing problem for mankind (Rouphael *et al.*, 2008). The common anthropogenic sources of heavy metals are waste water irrigation, sludge applications, solid waste disposal, automobile exhaust and industrial activities (Shi *et al*., 2005). Crops grown in or close to the contaminated sites can uptake and accumulate these metals in their organs (Jarup, 2003). The effect of heavy metals on crop plants and human beings caused functional disorders in their body organs due to exposure of low dosage over a long period of time (Jianjie *et al*., 2008).

There are mainly two stable oxidation states of chromium (viz., Cr^{+6} and Cr^{+3}); Cr^{+6} is considered to be more toxic than Cr^{3+} (Panda & Patra, 2000). In addition, Cr^{+6} can be reduced to Cr^{3+} by redox reactions (Buerge & Hug, 1997). Cr is not considered as an essential element for plant nutrition. Both forms, $Cr^{+3}$and Cr^{+6}, may be taken up by plants. Uptake of Cr^{+3} is considered passive, while that of $Cr^{+6}$is considered to be active (Liu et al., 2008). It had been estimated that about 1,12,000 tons of Cr was discharged annually into the world's aquatic ecosystems and worldwide annual mining of the chromate ($FeCr_2O_4$) has exceeded a level of 10 million tons. The leather industry is the major cause for the high influx of Cr to the biosphere, accounting for 40% of the total industrial use (Barnhart, 1997). Many countries including India facing problems of contamination of soil and water with Cr^{+6} from different sources including steel industries, electroplating, tanning industries, oxidative dyeing, cooling water towers, refractory materials production, metallurgy, pigments and mining and its concentration above 0.05 mg/L considered to be toxic.

Plants have a remarkable ability to absorb, translocate and accumulate heavy metals and organic compounds from the environment. In order to maintain their change balance, roots release protons whenever they take up more cations than anions, and take up protons when the opposite occurs (Hinsinger *et al.*, 2003). pH have a distinct impact on bioavailability of many pH-dependent nutrients and potentially toxic metals including Chromium (Cr), Cadmium (Cd), and mercury (Hg) according to Calba et al. (2004). When Cr entered into plant body, it could disturb many biochemical and physiological process and caused oxidative stress to plants that ultimately reduced the growth and yield (Arun *et al*., 2005).

In case of humans, Cr^{+3} is included in micronutrients; on the other hand, Cr^{+6} has toxic effects on biological systems and has been classified by the International Agency for Research on Cancer (IARC) as a

Group-1 human carcinogen. The toxic and carcinogenic properties of Cr^{+6} compounds arise from the possibility of free diffusion of chromate (CrO_4^{-2}) ions across cell membranes and its action as an oxidizing agent, as well as from the formation of free radicals during the reduction of Cr^{+6} to Cr^{+3} inside the cell (O'Brien et al., 2003). Levels of metal particle sand concentration of metal ions in distant organs were highest in patients with worn and loose prostheses (Gunaratnam & Helen, 2008), and about 11 ppm total Cr in the liver of a patient with a loose corroding cobalt chrome hip prosthesis has been observed (Case *et al*.,1994). Elevated circulating Cr concentrations in patients with metal orthopedic implants have been observed.

Heavy metals taken up by plants from contaminated soil and water are toxic to growth performance of plants and possess a hidden threat to consumers (Stobrawa *et al*., 2008). Vegetable crop (*Cucumis sativus* L). and pulses (*Macrotyloma unifloroum* Lam.) are important around the globe including India due to its consumption as food by humans. This research program was envisaged to assess the effects of Cr^{+6} in different plant parts that may be antagonistic for the plant growth particularly in the contaminated areas.

II. Materials And Methods

Graded dry seeds of *Macrotyloma uniflorum* L. and *Cucumis sativus* L. were obtained from Orissa University of Agriculture and Technology, Bhubaneswar. For germination study uniform sized seeds were selected and surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for about five minutes and then was washed several times with tap water followed by distilled water (Mitra et al., 2004). The surface sterilized seeds were placed in Petri plates containing cottons with different concentration (0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm) of Hexavalent Chromium for germination. Then germination percentage were calculated.

The growth parameters like root length and shoot length of seven days old seedlings were used for study. Different Hexavalent Chromium concentrations (0 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm) were used during growth parameter study. For study of root and shoot length, the root and shoot were first detached from each other. Individual length of root and shoot was measured in centimeters. Similarly shoot length and root length of 14 days and 21 days old plant were measured. Biochemical analysis was done in both control and treated seedlings grown in different concentrations (5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm) of Hexavalent Chromium.

For protein estimation 0.5 g of leaf sample was taken and homogenized in 10% ice cold TCA by a pre-chilled mortar and pestle incubated overnight at 40^0 C. Then centrifuged at 10,000 rpm for 10 minutes, successively washed with 80% ethanol/chloroform, diethyl ether to remove phenolic compounds. Pellet was washed and suspended in a known volume of 0.1N NaOH. Then protein was estimated by standard method. (Lowry *et al*, 1951)

For proline estimation the plant materials (0.5 g) were grinded in 10 ml of 3% sulfo-salicylic acid then the homogenized mixture was centrifuged at 3000 rpm for 10 minutes. Proline was estimated as per the method (Bates *et al*. 1973). Then to the 2 ml of supernatant 2 ml of acid ninhydrin reagent and 2 ml glacial acetic acid were added. This mixture was boiled in water bath at 100^0 C. The reaction was terminated, by placing the tubes in ice bath. 4ml of toluene was added to each of the test tube containing samples of different treatments. It was then followed to separate into phases by mixing vigorously using a cyclo-mixture. The chromophore containing upper toluene layer was collected carefully with the help of micro-pipette and the absorbance was measured at 520 nm. Total chlorophyll was estimated by SPAD-502 as the non-destructive plant type. All the experiments were done in triplicates and the data were analyzed statistically and standard errors of mean (SEM) was calculated.

III. Results

3.1. Germination studies

There was concentration dependent decrease in number of seed germinated for hexavalent chromium treatment both in *Macrotyloma uniflorum* Lam. and *Cucumis sativus* L. The least number of seed germination was noticed in 20 ppm treated plants. The effect of Cr^{+6} on germination is presented in Fig. 1. Decline in germination ranged from 88.33% (with 20 ppm Cr^{+6}) to 68.33% (with 100 ppm Cr^{+6}) in *Macrotyloma uniflorum*. Similarly, in *Cucumis sativus* germination declined from 53.33% (with 20 ppm Cr^{+6}) to 2.22% (with 100 ppm Cr^{+6}).

3.2. Effect of Cr^{+6} on shoot length of *Macrotyloma uniflorum* Lam. and *Cucumis sativus* L.

The effect of Cr^{+6} on shoot length of both the plant is presented in Fig. 2 and 3. Decline in shoot length ranged from 7.13 cm (7 day after treatment), 5.80 cm (14 day after treatment) and 8.17cm (21 day after treatment) with 5 ppm Cr^{+6} to 1.93 cm (7 day after treatment), 1.40 cm (14 day after treatment) and 2.23 (21 day after treatment) with 20 ppm Cr^{+6} in *Macrotyloma uniflorum* at the pre-flowering stage. Similarly, in *Cucumis sativus* shoot length declined from 6.10 cm (7 day after treatment), 7.37 cm (14 day after treatment)

and 9.97 cm (21 day after treatment) with 5 ppm Cr^{+6} to 3.5 cm (7 day after treatment), 0.77 cm (14 day after treatment) and 1.67 (21 day after treatment) with 20 ppm Cr^{+6}respectively.

3.3. Effect of Cr^{+6} on Biochemical parameters of *Macrotyloma uniflorum* Lam. and *Cucumis sativus* L.

The levels of total chlorophyll in control plants were found maximum in both the species of plant at the pre-flowering stage. Cr^{+6} proved to be less toxic in 5 ppm as compared to 20 ppm as decrease in chlorophyll content was from 22.85 to about 17.01 (SPAD unit) in *Macrotyloma uniflorum* (Table 1). Similarly at higher levels of Cr^{+6} treatment (20 ppm) total chlorophyll content in *Cucumis sativus* declined from 39.7 to 26.25 (SPAD unit) at the pre-flowering stage (Table 2). The results related to the effect of Cr^{+6} on protein content is depicted in both the species presented in Table 1 and Table 2. In *Macrotyloma uniflorum* a decline in protein content from 14.05 mg/g fr. wt. to 7.83 mg/g fr. wt. was observed at the pre-flowering stage (Table 1). Similarly, in *Cucumis sativus* a decline in protein content from 21.60 mg/g fr. wt. to 5.98 mg/g fr. wt. was reported at the pre-flowering stage (Table 2).

The results pertaining to the effect of Cr^{+6} on proline content is presented in Table 1 and Table 2 for both the species. The higher concentrations of Cr^{+6} (20 ppm) increased proline content from 7.65 µg/g fr. wt. to 46.10 µg/g fr. wt in *Macrotyloma uniflorum*. Similarly, in *Cucumis sativus* increased proline content was observed from 0.43 µg/g fr. wt to 39.98 µg/g fr. wt. at the pre-flowering stage (Table 2).

IV. Discussion

4.1. Germination study

Hexavalent chromium adversely affected the germination of *Cucumis sativus* L. whereas the *Macrotyloma uniflorum* Lam. was less affected. The reduced germination of seeds under Cr stress would be due to the depressive effect of Cr on the subsequent transport of sugars to the embryo axis (Zeid, 2001). Protease activity increases simultaneously with the chromium treatment which could also contribute to the reduction in germination of chromium treated seeds.

4.2. Effect on root and shoot length

Presence of Cr^{+6} in water with higher concentrations (up to 20 ppm) reduced the growth of plants. Reduction in root and shoot length in both the plants were clear after seven days of incubation at different concentration in ppm of hexavalent chromium which demonstrated that high concentration of Cr^{+6} inhibit the growth of plants. As noted, plant root growth was more susceptible compared to shoot growth may be due to heavy accumulation of Cr in them. This might interfere with the function of genes that govern the synthesis of enzymes, which in turn controlled the chemistry of cell. Cr^{+6}seems to act principally on plant roots, resulting in intense growth inhibition. Increasing concentration of Cr caused significant reduction in root length and shoot length. The general response of decreased root growth due to Cr toxicity could be as a result of inhibition of root cell division, root elongation or the extension of cell cycle in root.

4.3. Effect on total chlorophyll content

The chlorophyll concentration significantly decreased with the increase in Cr concentration in both the plants i.e. *Macrotyloma uniflorum* and *Cucumis sativus.* The chlorophyll pigments are present in thylakoid within chloroplast, and any damage brought to these structures could lead to denaturation of these pigments. It may be suggested that observed decrease in chlorophyll content at higher concentration of chromium might be due to breakdown of thylakoid and chloroplast envelope as was previously reported (Dodge and Law, 1974).

4.4. Effect on total soluble protein

The protein concentrations were found to decrease in both the plant species with the increase in Cr concentration (Table 1 & 2). Cr decreased protein is dose- and time-dependent manner. It may be the degradation of proteins in plants which could result in inhibition of nitrate reductase activity (Choudhury and Panda, 2004) and it could be correlated with reduced photosynthetic activity, nitrogen metabolism and nucleic acid damage under Cr^{+6} stress.

4.5. Effect on proline content

Proline apparently is the only amino-acid that accumulates to a great extent in the leaves of many plants under stress. Higher proline content was observed with increasing chromium concentrations in both the species. Thus proline accumulation under such condition might also be operative as usual in osmotic adjustment while accumulation of proline in tissues can be taken as a dependent marker for genotypes tolerant to stress.

V. Conclusion

The present study revealed that the application of hexavalent chromium on *Macrotyloma uniflorum* and *Cucumis sativus* seedlings led to reduced growth, metabolism and enhanced proline accumulation. Hexavalent chromium also negatively affected the soluble protein content which may be due to some hindrance in protein synthesis at gene level. *Macrotyloma uniflorum* exhibited better tolerance to Cr^{+6} than *Cucumis sativus*. These findings may lead for further study towards regarding the phytoremediation strategy and efficiency of the two plant species.

Acknowledgement

We are thankful to Head, Post Graduate Department of Botany, Utkal University for providing Laboratory facilities to conduct this experiment.

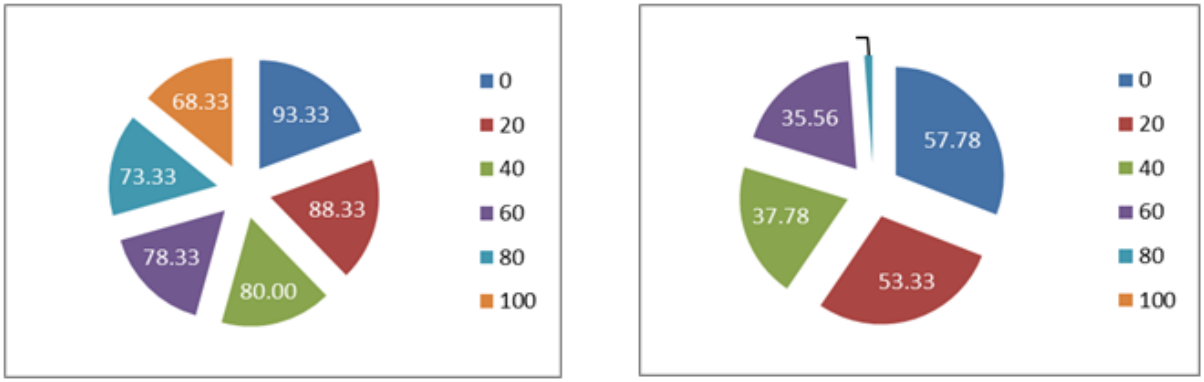
Reference

[1] B.J. Alloway, *Heavy metals in soils*. (2nd edition. USA: Blackie Academic & Professional, 1995)
[2] Y. Rouphael, M. Cardarelli, E. Reab, and G. Colla. Grafting of cucumber as a means to minimize copper toxicity, *Environment And Experimental Botany, 63,* 2008, 49-58.
[3] Z. Shi, S. Tao, B. Pan, W. Fan, X.C. He, Q. Zuo, S.P. Wu, B.G. Li, J. Cao, W.X. Liu, F.L. Xu, X.J. Wang, W.R. Shen, and P.K. Wong, Contamination of rivers in Tianjin, China by polycyclic aromatic hydrocarbons, *Environmental Pollution, 134,* 2005, 97-111.
[4] L. Jarup, Hazards of heavy metal contamination, *Brazilian Medical Bulletin, 68,* 2003, 425–462.
[5] Fu. Jianjie, Z. Qunfang, L. Jiemin, L. Wei, W. Thanh, Z. Qinghua and J. Guibin, High levels of heavy metals in rice from a typical E-waste recycling area in southeast China and its potential risk to human health, *Chemosphere, 71,* 2008, 1269-1275.
[6] S.K. Panda, and H.K. Patra, Nitrate and ammonium ions effect on the chromium toxicity in developing wheat seedlings, *Plant National Academy of Science of India, B70,* 2000, 75-80.
[7] I. J. Buerge and S.J. Hug, Kinetics and pH dependence of chromium (VI) reduction by iron (II). *Environmental Science & Technology, 31,* 1997, 1426–1432.
[8] Y. Liu, J. R. Key, and X. Wang, The influence of changes in cloud cover on recent surface temperature trends in the Arctic, *Journal of Climate, 21,* 2008. 705–715.
[9] J. Barnhart, Occurrences, uses, and properties of chromium, *Regulatory Toxicology and Pharmacology, 26*, 1997, 53-57.
[10] P. Hinsinger, C. Plassard, C. Tang, and B. Jaillard, Origins of root-induced pH changes in the rhizosphere and their responses to environmental constraints, a review. *Plant Soil 248,* 2003, 43–59.
[11] H. Calba, P. Cazevieille, R. Poss, and B. Jaillard, The dynamics of protons, aluminium and calcium in the rhizosphere of maize cultivated in tropical acid soils, experimental study and modeling, *Plant Soil, 260*, 2004, 33-46.
[12] K. Arun, T. Shanker, C. Cervantes, H. Loza-Tavera, and S. Avudainayagam, Chromium toxicity in plants, *Environment International, 31,* 2005, 739-753.
[13] T.J. O'Brien, S. Ceryak and S.R. Patierno, Review, Complexities of Chromium Carcinogenesis, Role of Cellular Response, Repair and Recovery Mechanisms, *Mutation Research. pp. 3-36*, 2003.
[14] M. Gunaratnam, and G.M. Helen. Cr (VI) inhibits DNA, RNA, and protein synthesis in hepatocytes, involvement of glutathione reductase, reduced glutathione and DTdiaphorase, *Toxicology In Vitro, 22,* 2008, 879-886.
[15] C.P. Case, V.G. Langkamer, C. James, Widespread dissemination of metal debris from implants. *J Bone Joint Surg [Br], 76-B,* 1994, 701-12.
[16] K. Stobrawa and G. Lorenc-Plucińska, Thresholds of heavy-metal toxicity in cuttings of European black poplar (*Populus nigra* L.) determined according to antioxidant status of fine roots and morphometrical disorders. *Science of the Total Environment 390(1),* 2008, 86-96.
[17] B. Mitra, P. Ghosh, S.L. Henry, J. Mishra, T.K. Das, S. Ghosh, C.R. Babu, and P. Mohanty, Novel mode of resistance to Fusarium infection by mild dose pre-exposure of cadmium to wheat, *Plant Physiol. Biochem, 42,* 2004, 781-787.
[18] O.H. Lowry, N. J. Rosegrough, A.L. Farr, and R.J. Randall, Protein measurement with Folin phenol reagent, *J. biol. Chem, 193,* 1951, 265-275.
[19] L.S. Bates, R.P. Waldren, and I.D. Teare, Rapid determination of free proline for water-stress studies, *Plant and Soil, 39,* 1973, 205-207.
[20] I.M. Zied, Response of *Phaseolus vulgaris* to chromium and cobalt treatment, *Biol. Plant, 44*, 2001, 111-115.
[21] J.D. Dodge and G.B. Lawes, Plastic ultra-structure in some parasitic and semi-parasitic plants, *Cytobiologie, 9, 1974,* 1-9.
[22] S. Choudhury and S.K. Panda, Role of salicylic acid in regulating cadmium induced oxidative stress in *Oryza Sativa* L. roots, *Bulg. J. Plant Physiol, 30(3-4),* 2004, 95-110.

Fig. 1: 21 days old *Macrotyloma uniflorum* Lam. seedlings treated with different concentration of Hexavalent Chromium

Fig. 2: 21 days old *Cucumis sativus* L. seedlings treated with different concentration of Hexavalent Chromium

.



Macrotyloma uniflorum *Cucumis sativus*

Fig. 3: Effect of Cr^{+6} on germination of *Macrotyloma uniflorum* Lam. and *Cucumis sativus* L.

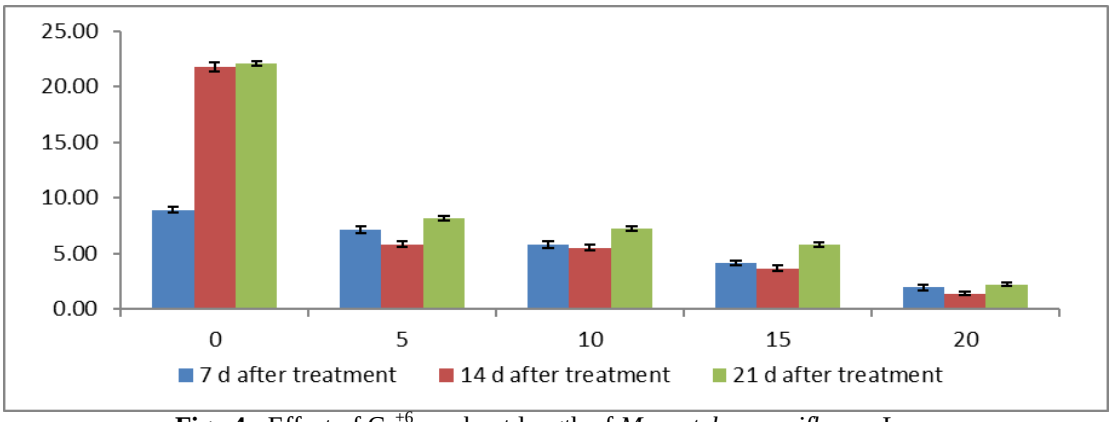


Fig. 4: Effect of Cr^{+6} on shoot length of *Macrotyloma uniflorum* Lam.

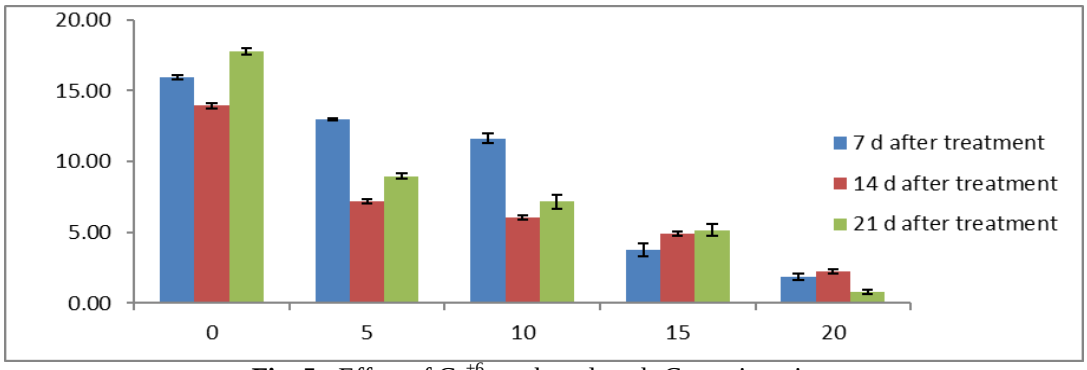


Fig. 5: Effect of Cr^{+6} on shoot length *Cucumis sativus*

Table 1: Biochemical composition of *Macrotyloma uniflorum* under Cr^{+6} stresses

Concentration (ppm)	Protein (mg/g fr. wt)			Proline (µg/g fr. wt)			Total Chlorophyll		
	7 d after treatment	14 d after treatment	21 d after treatment	7 d after treatment	14 d after treatment	21 d after treatment	7 d after treatment	14 d after treatment	21 d after treatment
0	19.36±0.32	26.81±0.48	31.09±1.76	0.08±0.04	0.22±0.06	0.54±0.10	25.03±0.68	22.88±1.36	20.93±0.95
5	14.05±0.33	19.34±0.35	20.11±0.72	7.65±0.45	8.30±0.40	9.99±0.66	22.85±0.96	21.68±1.29	20.02±0.91
10	9.31±0.45	13.55±0.29	11.11±0.64	13.97±0.51	16.92±1.73	22.69±1.16	22±0.97	20.52±1.18	18.54±0.86
15	7.14±0.45	13.47±0.50	12.23±0.22	19.85±1.01	21.22±0.73	34.19±1.24	20.57±0.84	19.41±1.40	17.61±1.09
20	6.50±0.31	7.76±0.89	7.83±0.22	23.88±1.34	27.69±0.57	46.10±2.70	19.2±0.84	18.12±1.26	17.01±1.30

*values in the table are mean ± SE of 3 replicates

Table 2: Biochemical composition of *Cucumis sativus* L. under Cr^{+6} stresses

Concentration (ppm)	Protein (mg/g fr. wt)			Proline (µg/g fr. wt)			Total Chlorophyll		
	7 d after treatment	14 d after treatment	21 d after treatment	7 d after treatment	14 d after treatment	21 d after treatment	7 d after treatment	14 d after treatment	21 d after treatment
0	29.30±1.18	21.24±0.65	21.24±1.15	0.43±0.08	0.92±0.16	1.02±0.23	44.96±0.94	40.98±1.74	31.63±1.09
5	21.60±1.27	17.81±0.19	19.11±0.73	9.46±0.66	8.98±0.92	13.36±0.54	43.34±0.88	39.7±1.64	30.44±1.02
10	12.92±0.84	10.20±0.49	8.15±1.34	14.39±0.51	14.89±1.28	14.81±1.37	41.7±0.91	38.36±1.89	29.4±1.23
15	12.52±0.40	9.15±0.47	8.64±1.95	17.35±2.30	18.74±2.62	28.42±1.38	40.31±0.99	36.6±1.84	28.4±1.29
20	10.02±0.27	8.38±0.42	5.98±1.45	29.73±0.55	26.75±1.28	39.98±1.69	39.34±0.87	34.75±1.91	26.25±1.18

*values in the table are mean ± SE of 3 replicates