

Physiological and biochemical changes induced by hexavalent chromium toxicity to *Sesbania cannabina* (Retz.) Pers., *Sesamum orientale* L. and *Ricinus communis* L.

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Abstract

Chromium (Cr) is considered to be a non-essential heavy metal which has a revealing impact on plant. The present study assessed the possible impacts of hexavalent chromium (Cr⁺⁶) toxicity on rate of germination, photosynthetic activity, protein, proline, starch and total sugar content of the test plants. The study has been carried out using three different plant species including a nitrogen fixer plant [*Sesbania cannabina* (Retz.) Pers.], two oil yielding plants [*Sesamum orientale* L.; *Ricinus communis* L.] in the liquid culture medium containing different levels of hexavalent chromium. The experiment revealed that at an interval of 21 days with increased Cr (VI) concentrations in the medium showed decreased germination rate, total chlorophyll, protein, starch and total sugar content but there was increase in proline content of the three test plants. It has been concluded that the species *Sesbania cannabina* (Retz.) Pers. as compared to the other two species i.e. *Sesamum orientale* L. and *Ricinus communis* L. observed to exhibit higher resistance to hexavalent chromium toxicity.

Keywords: hexavalent chromium, toxicity, photosynthetic activity, total chlorophyll, proline, nitrogen fixer

Introduction

Now-a-days heavy metal stress is contemplated as one of the appealing abiotic environmental stress. Besides the different types of natural activities, industrial and anthropogenic activities also contribute for increase in the concentration of heavy metals in the environment. Heavy metals discharged from various activities results in soil pollution, water pollution and also possess greater threat to human beings [1]. In case of plant species the heavy metals compete with other essential minerals and thus disturb plants nutritional value [2]. Plants are inhibited by heavy metal toxicity in several metabolic activities which reduces its growth and development [3, 4]. The occurrence of chromium (Cr) in the irrigated soil and water beyond the permissible limits causes various adverse effects on the growth of crop and its yield [5]. The maximum concentration of chromium in higher plants is 100 µM/kg dry weights, which indicates the toxicity of chromium [6]. The accumulation of Cr in the plant leads to changes in the physiological and biochemical process of the plant which also leads to oxidative stress [7]. There were some phytotoxic Effects observed due to the accumulation of Cr (VI) which results in delayed seed germination, reduction in seedling growth, less pigment, less nutrient content and enzyme activity [8]. At present time, phytoremediation technique has become most effective, environment friendly, potentially cost-effective and reasonable technical solution for the remediation of pollutants from the contaminated soil and water [9-11]. Essentially, phytoremediation technology is the use of plants to clean up contaminants from soil, sediments and water [12]. Plants with exceptional metal-accumulating capacity are known as hyper-accumulator plants. Phytoremediation takes the advantages of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation and contaminant

degradation abilities of the entire plant body. But there are some limitations based on the selection of hyper-accumulative plant species such as plants with low growth rate and having low efficacy in the removal of heavy metal from polluted soil and water on time [13]. For this reason it is very important to select a plant which follows a lesser number of the above mention limitation categories. In the present study three different hyper-accumulative plant species were selected namely *Sesbania cannabina* (Retz.)Pers., *Sesamum orientale* L., and *Ricinus communis* L. *Sesbania cannabina* (common name: Dhandicha) is a multipurpose leguminous crop plant having high growth rate with high biomass production. It is also used as a green manure crop for rice and wheat cultivation. Due to presence of root nodules, harbouring nitrogen fixing bacterium-*Rhizobium*, it can tolerate heavy metal toxicity, control soil erosion and helps in enhanced soil fertility. The second one is *Sesamum orientale* L. (common name is: 'Sesame or Til') is an economically important oil seed crop [14]. From the seeds of this plant, methyl ester is extracted which can be used as petro-diesel [15]. The last one is *Ricinus communis* L. (common name: 'Castor') which is considered as oil yielding plant. It can be utilized for insecticidal and acaricidal purpose [16]. The primary objective of the present study was to investigate and emphasize the toxic symptoms induced by Cr⁺⁶ on various physiological as well as biochemical processes such as germination percentage, photosynthetic activity, protein, proline, total sugar and starch content of *Sesbania cannabina*, *Sesamum orientale* and *Ricinus communis*.

Materials and Methods

Collection of plant materials

The seeds of *Sesbania cannabina* were collected from the Plant Genetics and Breeding Department, Orissa University

of Agriculture and Technology (OUAT), Bhubaneswar, Odisha. *Sesamum orientale* and *Ricinus communis* seeds were procured from the National Seed Corporation, Bhubaneswar, Odisha. These three seeds were used for all the experiments done in this study.

Treatment of Seed and Germination study

The experimental seeds were surface sterilized with 70% ethanol for 5 min followed by treatment with 0.01% HgCl_2 for 10 minutes then washed with sterilized distilled water three times [17]. 20 numbers of sterilized seeds were kept on filter paper containing 10 ml of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution of different concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm). Controls (0 ppm) were kept on filter paper with 10 ml distilled water. In the culture room i.e. under dark controlled condition the seeds were kept for five days. For each Cr^{+6} treatment three replicates were taken into consideration which were repeated three times. After 7 days of treatments radicle length was measured. Metal tolerance index [18], Seedling vigour [19] and percentage of phytotoxicity were calculated.

Estimation of Chlorophyll content

0.5 gm of fresh leaves were taken and homogenized in chilled 80% acetone with the help of a pre-chilled mortar and pestle. The homogenized samples were centrifuged at 10,000 rpm for 10 minutes then the supernatant was collected from the sample and this process was carried out in absence of light [20]. The collected supernatant was checked the absorbance at 470nm, 646.8nm and 663.2nm using UV-Visible double beam spectrophotometer.

Estimation of Protein content

0.5 gm of fresh leaf sample was taken and homogenized in 10% ice cold TCA through a pre-chilled mortar and pestle incubated overnight at 40°C. The mixture was centrifuged at 10,000 rpm for 10 minutes, successively washed with 80% ethanol, diethyl ether (to remove phenolic compounds). Pellet was washed suspended in a known volume of 0.1N NaOH. Then protein was estimated by Lowry's method [21].

Estimation of Proline content

0.5 gm of fresh leaves were taken and homogenized with sulfosalicylic acid then filtered through Whatman No-2 filter paper. 2ml of the filtrates was taken to which 2ml ninhydrin and 2ml glacial acetic acid was added. The mixture was then incubated at 100°C for 1hr. After, the reaction was stopped rapidly by placing the test tubes in ice chamber. In the solution 4ml of toluene was added and for 15-20 seconds the mixture was vigorously shaken. The aqueous toluene layer was separated. Then at absorbance 520 nm the red color aliquot was measured. Estimation of proline was done through Bates *et al* (1973) method [22].

Estimation of Total sugar

0.1 gm of freshly chopped leaves were kept in a test tube with 5ml of 2.5N HCl and boiled in water bath for about 1 hr. By adding solid sodium carbonate in the sample was then neutralized till effervescence ceases. The volume was made up to 10 ml and centrifuged. After the supernatant was collected, 0.5 ml of the aliquots was taken for analysis. The

volume was made up to 1ml by adding distilled water. Then anthrone reagent (4ml) was added and heated for 8 minutes in boiling water bath. Cooled rapidly and analyzed at 630nm [23].

Estimation of Starch

0.1 gm of fresh leaves was homogenized in hot 80% ethanol using a mortar and pestle. To retain the residue the homogenate was centrifuged. The residue was repeatedly washed with hot 80% ethanol. After discarding the supernatant the residue was dried well over a water bath. In the dried residue 5 ml distilled water and 6.5 ml 52% perchloric acid were added and centrifuged for 20 minutes and the supernatant was collected. 4ml of anthrone reagent was added and the tubes were heated for 8 minutes in a boiling water bath. After cooling immediately the absorbance was measured at 630 nm [23, 24].

Statistical Analysis

All the treatments were conducted in triplicates and data presented in the tables are mean \pm SEM (Standard error of the mean of triplicates). To compare the means of data of different treatments one way analysis of variance (ANOVA) was carried out [25].

Results and Discussion

Effect of Cr^{+6} on rate of germination

The germination rate of three plant seeds responded differently at different concentrations of hexavalent chromium. Germination rate decreased with increasing concentration of Cr^{+6} and extremely declined at the 100 ppm as compared to 20 ppm as mentioned in Table 1. The germination percentage was observed to be 99.5% (20 ppm), 62.74% (100 ppm) for *Sesbania cannabina*, 97.2% (20 ppm), 32.5% (100 ppm) for *Sesamum orientale* and 84.1% (20 ppm), 30.54% (100 ppm) for *Ricinus communis*. The radicle length of all 3 plants decreased with increased in Cr (VI) concentrations i.e. 8.5 cm (20 ppm), 1.69 cm (100 ppm) for *Sesbania cannabina*, 7.4 cm (20 ppm), 0.21 cm (100 ppm) of *Sesamum orientale* and for *Ricinus communis* 4 cm (20 ppm), 0.08 cm (100 ppm). Seedling vigour index in *Sesbania cannabina* was 699.51 mg/g, 88.69 mg/g for 20 ppm and 100 ppm, in *Sesamum orientale* was 653.58 mg/g, 98.07 mg/g for 20 ppm and 100 ppm and in *Ricinus communis* was 174.25 mg/g, 21.096 mg/g for 20 ppm and 100 ppm respectively. Metal tolerance index in *Sesbania cannabina* was 59.42, 2.9 for 20 ppm and 100 ppm, in *Sesamum orientale* was 55.32, 1.26 for 20 ppm and 100 ppm and in *Ricinus communis* was 50.11, 3.11 for 20 ppm and 100 ppm respectively. Percentage of phytotoxicity recorded for *Sesbania cannabina* was 45.279% (20 ppm), 95.853% (100 ppm), for *Sesamum orientale* was 43.753% (20 ppm), 94.83% (100 ppm) and for *Ricinus communis* the phytotoxicity percentage was 33.265% (20 ppm), 90.007% (100 ppm) respectively. With the increased Cr^{+6} concentrations, the seedling vigour index and metal tolerance index decreased whereas the percentage of phytotoxicity increased with increasing concentration of Cr^{+6} . Therefore, the finding of the experiment revealed that Cr^{+6} has a negative impact on the rate of germination and growth of all the mentioned three plants.

Table 1: Effect of hexavalent chromium on seed germination of three plant species after 7 days of treatment.

Variety	Treatments	Germination percentage	Radicle length (cm)	Seedling vigour index	Metal tolerance index	Phytotoxicity (%)
<i>Sesbania cannabina</i> (Retz.)Pers.	Control	99.7 ± 0.198	9.9 ± 0.06	867.13	100	0
	20 ppm	99.5 ± 0.201	8.5 ± 0.07	699.51	59.42	45.279
	40 ppm	99.4 ± 0.236	5.95 ± 0.05	473.65	42.75	58.69
	60 ppm	98.9 ± 0.114	3.55 ± 0.05	216.47	21.30	71.73
	80 ppm	88.7 ± 0.211	1.95 ± 0.15	101.65	10.26	86.11
	100 ppm	62.74 ± 0.326	1.69 ± 0.12	88.69	2.9	95.853
<i>Sesamum orientale</i> L.	Control	99.5 ± 0.286	9.4 ± 0.03	714	100	0
	20 ppm	97.2 ± 1.082	7.4 ± 0.12	653.58	55.32	43.753
	40 ppm	92.8 ± 1.327	4.55 ± 0.025	381.11	39	55.097
	60 ppm	89.7 ± 1.684	1.05 ± 0.04	205.37	33.92	75.43
	80 ppm	51.43 ± 2.311	0.55 ± 0.03	111.12	17.97	83.16
	100 ppm	32.77 ± 0.532	0.21 ± 0.05	98.07	1.26	94.83
<i>Ricinus communis</i> L.	Control	97.2 ± 0.475	8.5 ± 0.05	396.75	100	0
	20 ppm	84.1 ± 0.755	4 ± 0.17	174.25	50.11	33.265
	40 ppm	80.42 ± 1.065	1.85 ± 0.01	103.25	34.62	47.53
	60 ppm	69.83 ± 1.119	0.78 ± 0.04	99.75	20.84	74.07
	80 ppm	45.1 ± 1.673	0.33 ± 0.016	53.265	10.03	81.53
	100 ppm	30.54 ± 2.632	0.08 ± 0.018	21.096	3.11	90.007

Values of 3 replicate ± SEM

Effect of Cr⁺⁶ on total chlorophyll pigment in 21 days old plants

In total chlrophyll content (TCC) there was a significant decrease observed increasing concentrations of Cr⁺⁶. The TCC for *Sesbania cannabina* at 20 ppm was 605.22 µg/g and 383.11 µg/g at 100 ppm correspondingly. In the *Sesamum orientale* the TCC at 20 ppm has been shown as 331.08 µg/g and 107.25 µg/g at 100 ppm. Similarly the TCC

of *Ricinus communis* at 20 ppm has been observed 227.18 µg/g and 121.66 µg/g at 100 ppm respectively. Due to increased hexavalent chromium toxicity, the chloroplast is disrupted for which photosynthetic pigments was reduced and reradiated [26] of all the three test plants when they exposed to different concentrations of Cr⁺⁶. The changes in total chlorophyll content of all three plants were shown in the Table 2.

Table 2: Effect of hexavalent chromium on total chlorophyll content (TCC) of three plants after 21 days of treatment.

Treatments (Conc. of Cr ⁺⁶)	Total chlorophyll content (µg/g fresh wt.)		
	<i>Sesbania cannabina</i> (Retz.)Pers.	<i>Sesamum orientale</i> L.	<i>Ricinus communis</i> L.
Control (0 ppm)	653.24 ± 0.042	306.85 ± 0.047	289.35 ± 0.028
20 ppm	605.22 ± 0.057	331.08 ± 0.009	227.18 ± 0.013
40 ppm	541.84 ± 0.011	277.05 ± 0.032	201.77 ± 0.091
60 ppm	486.31 ± 0.013	201.1 ± 0.058	193.72 ± 0.1
80 ppm	407.92 ± 0.062	139.42 ± 0.08	142.08 ± 0.034
100 ppm	383.11 ± 0.021	107.25 ± 0.013	121.66 ± 0.039

Values of 3 replicate ± SEM

Effect of Cr⁺⁶ on protein content in 21 days old plants

There was a significant decrease observed in protein content with increasing concentrations of Cr⁺⁶. The protein content of *Sesbania cannabina* plant was 605.221 µg/g and 383.11 µg/g at 20 ppm and 100 ppm respectively. In the plant *Sesamum orientale* the protein content recorded at 20 ppm and 100 ppm was 331.08 µg/g and 107.25 µg/g respectively.

Similarly the protein content of *Ricinus communis* plant at 20 ppm was 227.18 µg/g and 121.66 µg/g at 100 ppm respectively. During Cr⁺⁶ toxicity in plants protein degradation result in inhibition of nitrate reductase activity [27]; and also correlated with reduced photosynthetic activity. The changes in total soluble protein content of all three plants were shown in the Table 3.

Table 3: Effect of hexavalent chromium on Protein content of three plants after 21 days of treatment.

Treatments (Conc. of Cr ⁺⁶)	Protein content (µg/g fresh wt.)		
	<i>Sesbania cannabina</i> (Retz.)Pers.	<i>Sesamum orientale</i> L.	<i>Ricinus communis</i> L.
Control (0 ppm)	2.004 ± 0.2	1.183 ± 0.007	3.142 ± 0.015
20 ppm	2.048 ± 0.83	1.042 ± 0.051	2.876 ± 0.058
40 ppm	1.817 ± 0.161	1.001 ± 0.057	2.214 ± 0.027
60 ppm	1.042 ± 0.091	0.893 ± 0.009	1.557 ± 0.1
80 ppm	0.992 ± 0.055	0.755 ± 0.077	1.283 ± 0.073
100 ppm	0.817 ± 0.037	0.374 ± 0.048	1.093 ± 0.033

Values of 3 replicate ± SEM

Effect of Cr⁺⁶ on proline content in 21 days old plants

The changes in proline content of *Sesbania cannabina*, *Sesamum orientale* and *Ricinus communis* plants were

shown in the Table 4. At 21 days of interval, the content of proline *Sesbania cannabina* plant was 7.851 mg/g and 15.685 mg/g for 20 ppm and 100 ppm respectively. The

proline content of *Sesamum orientale* plant was 2.429 mg/g and 8.004 mg/g for 20 ppm and 100 ppm; whereas the proline content of *Ricinus communis* plant was 3.853 mg/g and 8.069 mg/g for 20 ppm and 100 ppm respectively. The proline content was increased with increasing concentrations of Cr⁺⁶.

The increased proline content in the Cr⁺⁶ contaminated seedlings indicates a stress regulating factor which protects plants under heavy metal toxicity [28]. As a result, proline estimation in all the mentioned three Cr⁺⁶-stressed plants was much important to study whether increase in proline content can be a protection mechanism.

Table 4: Effect of hexavalent chromium on Proline content of three plants after 21 days of treatment.

Treatments (Conc. of Cr ⁺⁶)	Proline content (mg/g fresh wt.)		
	<i>Sesbania cannabina</i> (Retz.)Pers.	<i>Sesamum orientale</i> L.	<i>Ricinus communis</i> L.
Control (0 ppm)	3.573 ± 0.521	0.052 ± 0.009	2.995 ± 0.093
20 ppm	7.851 ± 0.311	2.429 ± 0.012	3.853 ± 0.11
40 ppm	10.118 ± 0.438	3.105 ± 0.186	5.877 ± 0.093
60 ppm	12.074 ± 0.076	4.974 ± 1.005	6.904 ± 0.041
80 ppm	13.506 ± 1.004	6.822 ± 0.311	7.873 ± 0.32
100 ppm	15.685 ± 1.264	8.004 ± 0.32	8.069 ± 0.17

Values of 3 replicate ± SEM

Effect of Cr⁺⁶ on starch content in 21 days old plants

The changes in starch content of *Sesbania cannabina*, *Sesamum orientale* and *Ricinus communis* plants are shown in the Table 5. The starch content of *Sesbania cannabina* plant at 21st day of treatment has been observed 17.8 µg/g and 9.19 µg/g for 20 ppm and 100 ppm respectively. While

at 21 days of interval, the starch content of *Sesamum orientale* plant was 2.006 µg/g and 0.824 µg/g for 20 ppm and 100 ppm and in *Ricinus communis* plant, it was recorded as 1.937 µg/g and 0.482 µg/g for 20 ppm and 100 ppm respectively. In all the three plants the starch content decreased with increasing stress of Cr (VI).

Table 5: Effect of hexavalent chromium on Starch content of three plants after 21 days of treatment.

Treatments (Conc. of Cr ⁺⁶)	Starch content (µg/g fresh wt.)		
	<i>Sesbania cannabina</i> (Retz.) Pers.	<i>Sesamum orientale</i> L.	<i>Ricinus communis</i> L.
Control (0 ppm)	18.36 ± 0.004	2.174 ± 0.006	2.028 ± 0.02
20 ppm	17.8 ± 0.017	2.006 ± 0.013	1.937 ± 0.001
40 ppm	15.44 ± 0.007	1.913 ± 0.018	1.348 ± 0.052
60 ppm	12.09 ± 0.011	1.531 ± 0.003	1.002 ± 0.011
80 ppm	10.37 ± 0.023	1.067 ± 0.01	0.721 ± 0.037
100 ppm	9.19 ± 0.009	0.824 ± 0.015	0.482 ± 0.005

Values of 3 replicate ± SEM

Effect of Cr⁺⁶ on total sugar content in 21 days old plants

At 21st day of treatment, the content of total sugar in *Sesbania cannabina* plant was 3.769 mg/g and 2.622 mg/g for 20 ppm and 100 ppm respectively. The total sugar content of *Sesamum orientale* after 21 days of treatment was 1.809 mg/g and 0.614 mg/g for 20 ppm and 100 ppm; whereas in the plant *Ricinus communis* the content was

observed to be 2.215 mg/g and 0.938 mg/g for 20 ppm and 100 ppm respectively. The sugar content in a plant depends on the photosynthetic activity. As the photosynthetic activity was affected by the Cr (VI) toxicity and it was not efficient for the test three plants, for this there was a notable reduction observed in total sugar content with increasing concentrations of Cr⁺⁶ which mentioned in the Table 6.

Table 6: Effect of hexavalent chromium on total sugar content of three plants after 21 days of treatment.

Treatments (Conc. of Cr ⁺⁶)	Total sugar content (mg/g fresh wt.)		
	<i>Sesbania cannabina</i> (Retz.)Pers.	<i>Sesamum orientale</i> L.	<i>Ricinus communis</i> L.
Control (0 ppm)	3.903 ± 0.022	2.512 ± 0.024	2.793 ± 0.084
20 ppm	3.796 ± 0.031	1.809 ± 0.007	2.215 ± 0.031
40 ppm	3.434 ± 0.017	1.743 ± 0.012	1.903 ± 0.008
60 ppm	3.127 ± 0.053	1.6 ± 0.1	1.652 ± 0.023
80 ppm	2.902 ± 0.081	0.896 ± 0.058	1.117 ± 0.042
100 ppm	2.622 ± 0.007	0.614 ± 0.14	0.938 ± 0.005

Values of 3 replicate ± SEM

Conclusion

The germination rate, growth of root not only inhibited by the increasing Concentrations of hexavalent chromium but also the plants showed decrease value in biochemical as well as physiological activities.

Results from the above studies revealed that *Sesbania cannabina* (Retz.)Pers. showed higher resistance to Cr (VI) toxicity as compared to other two plants i.e. *Sesamum*

orientale L. and *Ricinus communis* L. This experiment may help to select the resistant plant species to execute further investigation in the field of phytoremediation strategy and efficiency.

Acknowledgement

The authors are thankful to Head of the Post Graduate Department of Botany, Utkal University Bhubaneswar,

India for providing necessary facilities to conduct the present investigation.

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