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Effect of hexavalent chromium on paddy crops (*Oryza sativa*)

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Abstract
Increasing concentration of Cr(VI) in the soil of chromite mining area drastically affect the plant productivity. The high concentration of hexavalent Cr (39.46PPM) in the paddy field soil of Sukinda mining area is now the matter of serious concern which significantly impact on the growth and productivity of the rice plants. Hence an experiment has carried out to study the effect of Cr(VI) on different morpho-physiological properties of rice plants in each 30 days interval throughout the experiment through pot culture method. The germination percentage was 89% in controls and 68% in treated plants. The highest shoot length was 97.4cm and 78.3cm in control and treated plants respectively. The dry biomass and the weight of 1000 grains of the treated plants were found to be lower than the control. The Cr(VI) deposition in root, stem, leaf and seeds of the treated plants was 11.50, 0.289, 0.119 and 0.06PPM respectively.

Keywords: Cr(VI), Cr accumulation, *Oryza sativa*, germination, morpho-physiological properties

Introduction
Rice (*Oryza sativa*) is the most important cereal crop for its consumption as a major food component by the human [2]. Hence, it is treated as the staple food for two third population of the whole world. India is an agriculture based country and the cultivation of rice crop mostly depends upon the natural environment [21] and rain fall here. Any variation in the climatic condition or the soil will affect the growth and productivity of the plant. Moreover, the contamination of the soil with heavy metals has a lot of negative impacts on soil health and ultimately on the plant growth [20]. The accumulation of large amount of chromium through soil run off in the rice fields around the mining area badly affects the rice crop. Sukinda Valley of Jajpur district in Odisha is one of the well known chromium (Cr) reserve containing about 98% of the total chromium deposits [16] of the whole country. There are several numbers of opencast chromium mines are located in this region that leads to the deposition of excess chromium in the surrounding paddy fields soil. Although Cr (VI) and Cr (III) are the two stable forms of chromium [19] but the contamination of soil with Cr (VI) is more harmful than Cr (III). The presence of Cr (VI) in the crop field is taken by the root of rice plant and then translocated to different plant parts. The deposition of Cr (VI) in the rice grain transfers to humans through food chain and create several threats for the consumers [18]. It may cause damage to dermatitis, liver and kidney circulation, nerve tissue damage, and even death [4, 15]. Generally, plants experience oxidative stress due to the exposure of heavy metals which leads to cellular damage and disturbance in cell's ionic homeostasis and disruption in the morphology, physiology and productivity of plants [5, 17]. According to Bhalerao and Sharma, 2015, uptake of surplus amount of chromium poses many problems such as reduced germination percentage, shoot and root length, shoot and root weight, yield, photosynthetic rate, mineral nutrient uptake and enzyme activities (nitrate reductase, ferric reductase, H^+ ATPase and antioxidant enzymes[6]. Hence, here, an attempt has envisaged for the study Cr (VI) accumulation effects of in different parts of rice plants.

Materials and Methods
A comparative study was carried out to study the effect of hexavalent chromium treated and controlled paddy crop's growth and yield. Lalata variety was chosen for this study. This crop matures in about 120-130 days. Rice seeds were collected from the Department of Plant breeding and Genetics. Initially the seeds were disinfected with 95% ethanol for 10 min before soaking it in water for 48 h in darkness. During the first phase of study, the seeds were taken in two different germination tray, among which one was containing normal garden soil (control) and the other one was having the hexavalent chromium contaminated soil collected from the rice fields of Sukinda mining area (treated).

Before the preparation of nursery bed some physiological parameters (pH, concentration of Cr^{6+}, nitrogen, phosphorous and potassium, electrical conductivity) of the soils were observed. The nursery beds were watered once in a day. The growth of seedlings was also measured. After 30 days, 45 healthy seedlings of similar height were picked up from the nursery bed and transplanted at the rate of three seedlings per pot. The experiment was laid out in 12 pots with six replications for each soil type. Among the 12 pots, six were having garden soil and the rest six were containing chromium contaminated rice field soil. The effect of hexavalent chromium treatment was examined on the growth parameters such as germination parameters, length of the root and shoot, seed vigour index, dry weight of root and shoot, number of leaves and tillers, weight of 1000 grains and yield of paddy crop. All the measurements were taken in every 30 days after transplantation (DAT).

Effect of Cr (VI) on germination percentage estimation

Seed germination percentage was calculated on the basis of primary root emergence from the seeds and the results were expressed in percentage following the equation mentioned below:

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated x 100}}{\text{Total number of seeds}}$$

Shoot and root length

The shoot height was measured using a meter scale from the base of the plant to tip of the terminal bud. Similarly, the root height was measured from the base of the plant to the end of the root. Measurement of height was taken at an interval of 30 days and expressed in centimetres. For this 3 plants from each treatment were taken each time and the average was calculated.

Seed vigour index

Seed vigour index (SVI) helps to understand the potential for the emergence and development of seedlings in field conditions. SVI is considered as a sensitive and important component of germination studies as it provides a better understanding of seed damage and deterioration in response to stress. Seed germination percentage, root length, and shoot length were taken in to account to calculate the seed vigour index.

Seed vigour index= (Mean root length +Mean shoot length) x Percentage of germination

Shoot and root dry weight

For the determination of dry biomass, the shoot and root were put in labelled paper bags and placed in an oven at 80°C for 48 hours. Then the weight was measured using an electronic weighing balance.

Weight of 1000 seeds

Thousand healthy grains are randomly selected from the treated and control plants, weighed by the help of an electronic weighing balance.

Analysis of chromium in different plant parts

Dry plant material was weighed in crucible and placed in a Muffle furnace at 650 °C. The samples were ashed for approximately 2.5 h, until a whitish gray ash residue was obtained. After cooling, the residue was dissolved in a di-acid mixture (nitric acid and hydrochloric acid) and the solution was transferred to a volumetric flask and the volume was made up to 100 ml with distilled water. Inductively coupled plasma- optical emission spectroscopy was used for the measurement of total chromium at 357.9 nm resonance line.

Statistical analysis

The Statistic 10 software (version 10, USA) was used to analyze the data including the analysis of variance (ANOVA). Mean value of the samples were compared using least significant difference (LSD) at $P<0.05$.

Result and Discussion:

Physio-chemical parameters of soils

The physiochemical parameters (pH, EC, concentration of N, P, K and Cr^{6+}) of the two types of soil (control and treated rice plants) were observed before the treatment and presented in Table No.1. The physicochemical parameters have shown that the control soil had all the normal values of pH, EC, N, P, K and Cr (VI) concentration but in case of the treated soil the requirements were not in the required levels. Some were found in higher or lesser amount making the unfavourable for plant growth. Similar trend of pH, EC and Cr (VI) concentration were also observed by some workers who have already selected Sukinda as their study sites [22, 23].

Table 1: Physiochemical parameters of soil (control and treated rice plants)

Name of the sample	pH	EC (dSm-1)	Organic carbon (%)	Mineralizable nitrogen (kg/ha)	Available phosphorus (kg/ha)	Available potassium (kg/ha)	Available hexavalent chromium (PPM)
Control rice plants	7.79	2.5	0.65	305.56	66.78	238.15	0.001±0.01
Cr (VI) treated rice plants	6.01	6.8	0.66	162.5	18.5	157.2	39.46±0.97

Germination percentage estimation

The germination percentage of the selected rice seeds were severely affected by the higher concentration of chromium as compared to control. The germination percentage was found to be lower (68%) in the rice seeds of chromium contaminated soil as compared to control (89%). The reduction in germination percentage of seeds under Cr stress is due to the negative impact of hexavalent Cr upon the transport of carbohydrates to the embryo axis. Enhancement of the protease activity caused by excessive amount of chromium is also contributed towards the reduction of germination rate [3].

Shoot and root length

In every 30 days interval, the observation indicated highest shoot and root length in control but the lowest values were noticed in the chromium treated plants. The seedling length of control and treated samples (7 days of germination) were 12.59± 1.78cm and 9.35± 1.09cm respectively presented in Table No.2.

Table 2: Seedling length of control and Cr (VI) treated rice plants

Name of the sample	Seedling length (cm)
Control rice plants	12.59± 1.78
Cr (VI) treated rice plants	9.35± 1.09

The shoot and root lengths of the plants were drastically affected in soils contaminated with Cr (VI) (Table No. 3). According to Peralta *et al*., 2001, at higher doses of hexavalent Cr (20 and 40 mg L^{-1}) there was a dose-inhibition effect on plants for which the shoot and root length became affected [24]. Increase in Cr (VI) concentrations up to 200 mg L^{-1} exhibited a decrease in the growth of rice plants (*Oriza sativa* L.) [6, 7].

Table 3: Shoot and root lengths of control and Cr (VI) treated rice plants

Days after transplantation	Shoot length (cm)		Root length (cm)	
	Control rice plants	Cr (VI) treated rice plants	Control rice plants	Cr (VI) treated rice plants
30	35.4±1.45	27.5±1.29	13.3±1.98	09.13±1.43
60	59.8±1.76	48.01±1.87	21.9±1.67	15. 9±1.09
90	87.6±1.56	71.3±1.03	39.8± 1.87	31.8±1.56
120	97.4±1.43	78.3±1.73	52.9±64	48.8±76

Seed vigour index

The seed vigour index was presented in the Table no. 4 which depicted the reduced seed vigour index in case of hexavalent chromium treated rice plants. The adverse effect of Cr^{6+} appeared in the growth of rice plants which ultimately results in lower seed vigour index. The seed vigour index of the control rice plant was 160.67 while a minimum seed vigour index (108.03) was observed in the treated rice plants. Joshi *et al*., 2019 has also found a significant decrease in the seed vigour index with increase in the concentration of chromium [3]. With increase in the concentration of chromium from 0 to 500 ppm, the SVI was reduced from 1034.5 to 0.

Table 4: Seed vigour index of control and Cr (VI) treated rice plants

Name of the sample (rice plants)	Seed vigour index
Control rice plants	160.67
Cr (VI) treated rice plants	108.03

Shoot and root dry weight

The dry biomass of the shoot and root were significantly increased in each 30 days interval. But the dry weight of chromium treated plant parts were found to lower than that of the control. Dry weight of shoots and roots in 30 days interval were given in Table No.5. It depicted that roots were more sensitive to chromium. Hence higher concentrations of chromium proved to be more lethal.

Table 5: Dry weight of shoots and roots of control and Cr (VI) treated rice plants

Days after transplantation	Shoot weight (gm)		Root weight (gm)	
	Control rice plants	Cr (VI) treated rice plants	Control rice plants	Cr (VI) treated rice plants
30	3.45	3.14	0.98	0.75
60	9.19	5.46	6.39	2.31
90	13.39	8.07	10.89	4.87
120	15.87	10.31	11.76	5.69

The decrease in plant height was due to the reduced growth of root which directly influences the absorption and transport of water and nutrients to the upper parts of the plant. The decrease in root growth due to heavy metals is well-documented in several and crops [8]. Cr toxicity caused inhibition of root cell division, root elongation and the extension of cell cycle in the roots. High Cr concentrations affect the seedling roots by causing collapses and hence, stop ability of the roots to absorb water from the medium [9]. Reports are also available suggesting the heavy metals are more lethal for root growth as they can easily accumulate on root part of the plant and retard the cell elongation and cell division cycle [10]. Accumulation of heavy metals including Cr in soil increases the risk of crop uptake and food chain contamination [11].

Weight of 1000 seeds

The total weight of 1000 numbers grains was found to 28.94gm in case of control plants. In other hand, the total weight of 1000 numbers of grains became so less in hexavalent chromium treated rice plants. (Table no. 6). The study indicated that yield was decreased with increase in hexavalent chromium concentration. Plant yield depends upon the leaf growth, leaf area, and number which is directly linked with the available nitrogen. But nitrogen uptake is hampered due to the reduced root growth which significantly affects the productivity of the plant [7, 12]. Moreover, Cr affects most of the morpho-physiological properties in plants and hence, productivity and yield were also affected [6]. A similar trend of finding was also observed by [7]. They have observed that the highest weight of 1000 seeds (24.78 g/plant) were recorded in the control plants while the lowest seed weight (11.0 g/plant) were recorded in the paddy irrigated with higher amount of chromium (200 mg/l). Around 56.21% of reduction was observed in the present study. Bishnoi *et al*., 1993 has also observed that the yield was reduced about 70 to 80% while treated with 0.2mM Cr^{6+} in case of peas [13].

Table 6: Total weight of 1000 numbers of grains of control and Cr (VI) treated rice plants

Name of the sample	weight of 1000 seeds (gm/1000 seeds)
Control rice plants	28.94
Cr (VI) treated rice plants	12.67

Analysis of chromium in different plant parts

Accumulation of chromium was analysed and given in Table no.7. As per the analysis it has been observed that the chromium treated plant parts like the root, stem, leaf and grains contained some amount of hexavalent chromium which is beyond the permissible limits. In that regards the control plant parts also had some amount of hexavalent chromium but in minute concentrations within the permissible limits. The hexavalent chromium treated plant contained high amount of chromium (11.50 ppm) in its root while 0.06 ppm of Cr^{6+} was present in the grains. Hence, the findings showed that the magnitudes of chromium in those chromium treated plants are alarming. The least concentrations of Cr^{6+} were recorded in the control plant parts. The difference between the Cr^{6+} content in the different parts of chromium treated and controlled rice plants were highly significant. Bahmanyar, 2008 has reported that high amount of chromium was accumulated in the root region of the rice plant than the other parts like grain and whole shoot [1]. Sauerbeck, 1991 found that a lower concentration of Cr in the grains than that of the roots might be due to the reduced mobility of chromium from roots to shoots [14].

Table 7: Accumulation of chromium in different plant parts of control and Cr (VI) treated rice plants

Different plant parts	Rice plants of Cr contaminated soil (PPM)	Rice plants of normal garden soil (PPM)
Root	11.50	0.035
Stem	0.289	0.057
Leaf	0.119	0.0 28
grain	0.06	0.005

Conclusion

The findings of the present study clarified that the hexavalent Cr contaminated rice field soil of Sukinda mining area drastically affected the growth and yield of rice plants. The accumulation of chromium is more in the root, shoot and grains of the treated plants than the control. Particularly the level of chromium deposition in the grains of rice plants is above the permissible limit which will affect the food chain and human health. So the bioremediation of the Cr (VI) contaminated soil of Sukinda mining area is advisable.

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