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By
P.K. Tandon and Akash Vikram

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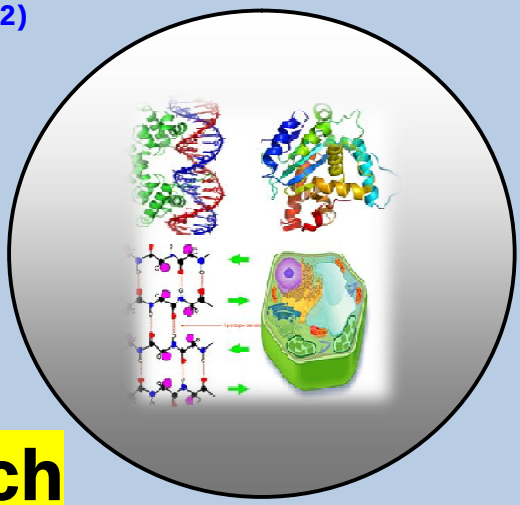

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Dr. A. Vikram Dr. P.K. Tandon
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

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Toxic Effects of Chromium on Growth and Metabolism of *Oryza sativa* (Rice) Plants

P.K. Tandon and Akash Vikram
Department of Botany, University of Lucknow, Lucknow – 226007, U.P., India

ABSTRACT

Heavy metal pollution in environment is of global concern. Chromium has been found to be highly toxic heavy metal, which is found in all the phases of the environment including air, water and soil. It has multifarious industrial uses. The leather industry is the major cause for the influx of chromium to the biosphere. The present study was conducted to evaluate effect of Cr (VI) on growth and metabolism of Oryza sativa (Rice) plants. Growth and water content of rice plants was found to be decreased at increasing levels of chromium. Photosynthetic pigments viz. chlorophyll a, b and carotenoids and contents of protein and sugar were found to be decreased at increasing doses of chromium. Lower doses of chromium caused stimulation of catalase activity while higher doses of this metal were proved to be toxic for this iron enzyme in rice plants. The activity of other anti oxidative enzyme i.e. peroxidase was found to be dose dependent i.e. with increasing doses its activity was found to be increased.
Key words: Chromium, Rice, Chlorophyll, Enzymes, Sugar and Protein.

INTRODUCTION

The increasing levels of heavy metals in the eco-system and biological tissues in recent past as revealed by environmental monitoring and epidemiological surveys has made the metal exposure a global concern. The metal contamination of the environment includes both natural and anthropogenic sources. The most important source of metal contamination is industrial activities. Among the pollutant elements chromium has been found to be highly toxic heavy metal. Chromium is found in all the phases of the environment including air, water and soil. It enters into the biotic components of ecosystem in many ways. Through its discharges from several industrial areas it continuously enters into the water sources whose water is consumed by human beings and animals, endangering their growth and health (Freedman and Hutchinson 1981; Sigel 1986).

In many areas these discharges are used by local farmers for irrigating their crops, thus introducing these pollutants to the crops (Warning et al, 1996).

Chromium and its compounds have number of industrial uses. They are extensively employed in leather processing and finishing (Nriagu 1988), in the production of refractory steel, drilling muds, electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals. Hexavalent chromium compounds are used in industry for metal plating, cooling tower water treatment, hide tanning and until recently, wood preservation. These anthropogenic activities have led to the wide spread contamination that chromium shows in the environment and increased its bio-availability and biomobilty. The leather industry is the major cause for the influx of chromium to the biosphere, accounting for the 40% of the total industrial use (Barnhart 1997). In India about 2000 to 3200, tons of elemental chromium annually escapes into the environment from tanning industries.

Chromium compounds are highly toxic to plants and are detrimental to their growth and development. Although some crops are not affected by low chromium concentration, chromium is toxic to higher plants. Keeping all this in view, this study was made to investigate the effect of different doses of chromium on growth and metabolism of *Oryza sativa* (Rice) plants.

MATERIAL AND METHODS

A pot experiment was conducted to study the effect of chromium on the growth and metabolism of *Oryza sativa* (Rice) plants at four different levels viz. 0.25mM, 0.5mM and 1.0mM of chromium along with control. Plants were raised from the seeds of *Oryza sativa* L. (verna. Paddy, Rice, Chawal) family- Gramineae (Poaceae). The deionized water was used for irrigating plants. For analytical purpose, glass distilled water (G.D.W.) was used. The glasswares used for the analytical work, were routinely acid washed and dried in oven. For experiments, virgin soil was collected from a patch of agricultural land on Lucknow- Sitapur road, Uttar Pradesh, India. About ten kilograms of fertilizer mixed soil was filled in each plastic pot. Plastic pots containing such soil were made wet with deionised water. The Earthen pots were labeled and arranged in random manner on the wooden benches, in a wire house which was pest free. The plant samples were collected carefully.

Observations with regard to various growth and biochemical parameters were recorded at an interval of seven days after first treatment supplied on the following parameters.

Growth parameters

(a) Height of Plant: Height of five plants for each treatment including control was measured. Height of each plant from the upper surface of the soil in each pot upto the tip of that particular plant was measured in cms. Mean value of the height for a particular population and percent increase/decrease in growth of plant in respect to control was calculated.

(b) Number of Leaves: Total number of leaves per plant was counted in replicates. Each replicate consisted of five plants.

(c) Number of Flowers: Total number of flowers per plant were counted including control and replicates.

(d) Number of Fruits: Total number of fruits produced by each plant of control and replicates were counted.

(e) Fresh weight, Dry weight and Total Biomass: Plants harvested were taken out of the pots and washed in running tap water. The plant was rinsed properly with tap water and finally was washed with Glass distilled water. The washed plant material was then blotted dry and separated into many parts. These parts were weighed on electronic weighing balance for determining fresh weight.

After taking fresh weight, the material was finally kept in paper bags and dried in oven at 70^{o}C for forty eight hours. The oven dried material was transferred to desiccators and after allowing it to cool to room temperature, it was weighed accurately on an electronic balance. Total Biomass was calculated.

Biochemical parameters

The various biochemical parameters except chloroplastic pigments were assayed in crude leaf extracts made in glass-distilled water. Finally chopped de-veined leaf material obtained from young leaf was ground with chilled acid washed silica sand in an ice chilled pestle and mortar kept in an ice bath. One gram of leaf tissue was extracted in 10 ml of glass-distilled water. The tissue extract was filtered through a double fold muslin cloth and kept at 20-40C in refrigerator till the time of various assays. Chlorophyll concentration was determined by the method of Arnon (1949). Catalase activity was assayed by the modified method of Bisht (1972). Peroxidase was assayed by the modified method of Luck (1963). Protein and total carbohydrate were estimated by the method of Lowry et. al. (1951) and Dubias et al. (1956) respectively.

Statistical Analysis: All estimations have been made in replicates and the data were analysed using analysis of variance (ANOVA) and compared for the level of significance by Dunncanns test using Sigma stat software. L.S.D. was calculated by Fisher test. All treatment values were in relation to control.

RESULTS

Vegetative Growth

A pot experiment was conducted to study the effect of different doses of chromium viz. 0.25mM, 0.5mM and 1.0mM along with control on the growth and metabolism of *Oryza sativa* (Rice) plants. The experiment was terminated after 120 days. The results indicated that the plant height decreased significantly with the increase in chromium concentration (Table 1). The decrease in plant height was 10.67%, 15.58% and 39.50% at 0.25mM, 0.5mM and 1mM chromium respectively (Table 1). The decrease in number of leaves was 10.86%, 19.56% and 26.08% at 0.25mM, 0.5mM and 1mM chromium respectively. The decrease in number of tillers was 11.11%, 22.22% and 44.44% at 0.25mM, 0.5mM and 1mM chromium respectively.

Fresh weight, dry weight and water content

The fresh weight of the *Oryza sativa* (Rice) plant decreased significantly with the increase in chromium concentration (Table 2). The decrease in fresh weight was 25.43%, 37.53% and 39.80% at 0.25mM, 0.5mM and 1mM chromium respectively. The dry weight decreased significantly with the increase in chromium concentration (Table 2).

The decrease in dry weight was 33.66%, 34.73% and 47.12% at 0.25mM, 0.5mM and 1mM chromium respectively. The significant decrease in water content was observed on increasing chromium concentration than the control. However it was found to be slightly higher in 1.0mM as compared to 0.5mM chromium concentration (Table 2). The decrease in water content was 18.53%, 39.88% and 33.67% at 0.25mM, 0.5mM and 1.0mM chromium respectively.

Photosynthetic Pigments

Effect of different concentrations of chromium were studied on the photosynthetic pigments viz. chlorophyll 'a', chlorophyll 'b' and carotenoids on *Oryza sativa* (Rice). Results indicated that chlorophyll 'a' was significantly decreased on increasing the chromium concentration. Further at 0.5mM and 1.0mM Cr, chlorophyll 'a' decreased significantly (Table 3). The decrease in chlorophyll 'a' was 4.85%, 13.17% and 27.45% at 0.25mM, 0.5mM and 1.0mM chromium respectively as compared to control. Chlorophyll 'b' decreased significantly with the increase in chromium concentration (Table 3). The decrease in chlorophyll 'b' was 5.37%, 18.33% and 32.75% at 0.25mM, 0.5mM and 1.0mM chromium respectively. There was increase in chlorophyll 'a' and chlorophyll 'b' ratio which was non-significant (Table 3). The increase in chlorophyll 'a' and chlorophyll 'b' ratio was 0.55%, 6.30% and 7.87% at 0.25mM, 0.5mM and 1.0mM chromium respectively.

The total chlorophyll decreased significantly with the increase in chromium concentration (Table 4). The decrease in total chlorophyll was 4.99%, 14.61% and 28.93% at 0.25mM, 0.5mM and 1mM chromium respectively.

There was significant decrease in carotenoid content with the increase in chromium concentration (Table 4). The decrease in carotenoid content was 4.97%, 18.27% and 28.36% at 0.25mM, 0.5mM and 1.0mM chromium respectively. The carotenoid chlorophyll ratio increase was 1.67%, 5.29% and 0.584% at 0.25mM, 0.5mM and 1mM Cr respectively (Table 4).

Metabolic activities

Effect on anti-oxidative enzymes viz. catalase (CAT) and peroxidase (POD): The catalase activity was significantly increased on application of 0.25mM and 0.5mM chromium concentration but at 1.0mM chromium concentration, catalase activity was significantly decreased than control (Table 5). The increase in catalase activity was 11.11% and 7.40% at 0.25mM Cr. and 0.5mM Cr while at 1.0mM chromium, it was 15.55% decrease as compared to control. The activity of enzyme peroxidase, increased non-significantly at 0.25mM and 0.5mM Cr. concentration. There was significant increase in peroxidase at 1.0mM Cr. concentration. The increase in peroxidase activity was 3.56%, 3.85% and 8.19% at 0.25mM, 0.5mM and 1.0mM chromium respectively.

Protein and sugar contents

Results indicated that on application of 0.25mM Cr. in *Oryza sativa* (Rice) plants there was a non-significant decrease in protein content in leaves of the plants. Further on increasing the concentration of chromium at 0.5mM and 1.0mM concentration, it decreased significantly (Table 6). The decrease in protein content was 28.57%, 42.86% and 71.43% at 0.25mM, 0.5mM and 1mM chromium respectively as compared to control. As the concentration of chromium increased, sugar content was found to be decreased significantly in the leaves of rice plants (Table 6).

The decrease in sugar content was 43.87%, 51.35% and 58.84% at 0.25mM, 0.5mM and 1.0mM chromium respectively.

DISCUSSION AND CONCLUSION

In the present work, effect of chromium was studied on *Oryza sativa* (Rice). Results indicated that on application of chromium (VI) in rice, plant growth was significantly decreased, on increasing the concentration of the chromium. The toxic effects in terms of growth were severe on the rice crop supplied with Cr. The same trend was observed earlier by other workers (Akinci and Akinci 2010; Nath et. al., 2009; Aziz Eman et. al., 2007; Tandon and Gupta 2002; Chatterjee and Chatterjee, 2000, Hunter and Vergnano, 1953, Daniels et. al., 1972). Typical phytotoxic symptoms of chromium and cobalt include chlorosis first in younger and then in older leaves and also reduced plant growth. Similar finding was observed in wheat with excess chromium by Sharma et.al., (1995). According to Anderson et. al., (1972); when Cr was added at 2, 10 and 25 ppm to nutrient solution in sand cultures in oats, it was 11%, 22% and 41% reduction in plant height respectively over control which is in conformity with the finding of the present study. Plant height was found to be decreased by 10.6%, 15.5% and 39.5% than the control on application of 0.25mM, 0.5 mM and 1.0mM Cr respectively. The reduction in plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoot contributing to the reduction in plant height (Shanker et. al., 2005). Sharma and Sharma (1993) reported that after 32 and 96 days growth, plant height reduced significantly in wheat cv. UP 2003 in a glasshouse trails when sown in sand with 0.5µM sodium dichromate. There was a significant reduction in plant height in *Sinapsis alba* when Cr was supplied at the rate of 200 or 400 mg/ kg soil along with N, P, K and S fertilizers (Hanus and Tomas 1993). The typical phytotoxicity symptoms of heavy metals (Cr, Co and Cd) include chlorosis first in younger and then in older leaves and also reduced plant growth. The chlorotic response of plant growth has been attributed to the interference in iron metabolism. Heavy metals used to produce visible symptoms in plants, some of which were resembled with the symptoms of iron deficiency, while others are somewhat specific to particular heavy metals (Agarwala et. al., 1977). Reduced growth in plants might be due to abnormal transport of essential nutrients including zinc. As heavy metal interfere in iron metabolism and reduce the transport of essential nutrients like K, Fe to meristematic (foliar and bud) regions of plants. Absence of same essential nutrients in the meristematic regions of plants may also be a cause of reduced plant growth. Lack of growth might be due to the deficiency of zinc, which helps in the synthesis of auxin. Some essential nutrients are also known to be constituents of cytoplasm and enzymes (Singh 2004). Zornoza et. al., (2002) and Wu and Zhang (2002); reported that addition of cadmium to nutrient solution reduces Zn, Cu and Fe in the shoots of white lupin and barley plants respectively. Stunted growth of plant due to excess amount of chromium and cobalt was reported by different workers (Bisht, 1972; Vazquez et. al., 1987; Bisht and Mehrotra 1989; Tripathi and Tripathi 1999; Gopal et. al., 2003).

Leaf growth and total leaf number decisively determine the yield of crops. The leaf number per plant in rice crops was reduced by 10.8%, 19.5% and 26.% at 0.25 mM, 0.5 mM and 1.0 mM Cr respectively over control. The similar findings was observed by Sharma and Sharma (1993), who had reported that leaf number per plant reduced by 50% in Wheat. When 0.5mM Cr was added in nutrient solution, leaf area and biomass of *Albeizia lebbek* seedling was severely affected by a high concentration (200 ppm) of Cr (VI) (Tripathi et. al., 1999). These workers noted that leaf growth traits might serve as suitable bio-indicators of heavy metal pollution and in the selection of resistant species. Karunyal et al. (1994) reported that all the concentrations of tannery effluent decreased leaf area and leaf dry weight in *Oryza Sativa*, *Acacia*, *Leucaena*. In a study on effect of Cr (III) and Cr (VI) on Spinach, Singh (2001) reported that chromium applied at 60 mg kg^{-1} and higher levels reduced the leaf size, caused burning of leaf tips or margins and slowed leaf growth rate. Jain et. al., (2000) observed leaf chlorosis at 40ppm chromium that turned to necrosis at 80ppm. In a study with several heavy metals, Pedreno et al. (1997) found that Cr had an adverse effect on leaf growth and preferentially affected young leaves in tomato plants. Reduction in leaf biomass was correlated with the oxalate acid extractable Cr in *P. vulgrais* by Poschenrieder et. al., (1993). According to Chatterjee and Chatterjee (2000) excess Cu after 10-12 days of supply, restricted the growth of cauliflower and young leaves exhibited interveinal chlorosis towards the apex and gradually spread downward. Decrease in the size of lateral appendages of pea plants might be due to reduced cell size and area of vascular tissues due to water stress. Plant showed water stress like condition due to certain heavy metals this is somewhat in accordance with the studies made by Pandey and Sharma (2002). The water content was also decreased on increasing the dozens of chromium in rice. This finding was in conformity with Nath et. al., (2009). The effect of heavy metals on plants water relations are quite complex and are dependent on the type of metal and its concentration, plant species, genotype and exposure time. There is a report according to which heavy metal can influence water relation by causing changes in photo-assimilate partitioning or increase the photosynthesis which bring about increased turgor of leaves, stomatal opening and closing (Barcelo and Poschenrieder, 1990). The dry matter production was decreased on increasing the concentration of Cr in rice. This finding was in conformity with the finding of Nath et. al., (2008) in which he reported maximum dry weight in controlled conditions. The fresh and dry weight of cowpea seedling was decreased significantly with the increase in concentration of chromium and tannery effluent. According to Vajpayee et. al., (2001) dry matter production was severely affected by Cr (VI) concentrations above 2.5 mg/ml in nutrient medium. Zurayk et. al., (2001); reported that salinity and Cr (VI) interaction caused a significant decreased in dry biomass accumulation of *Portulaca oleracea*. Excess of chromium caused restricted dry biomass in cauliflower (Chatterjee and Chatterjee, 2000). A number of physiological and biochemical processes are severely affected by Cr, and consequently, the yield and productivity of crops are affected (Barcelo et al. 1993). The effect of Cr on the plant processes during early growth and development culminates in reduction of yield and total dry matter as a consequence of poor production, translocation and partitioning of assimilates to the economic parts of the plant.

The negative effect on yield and dry matter is possibly an indirect effect of Cr on plants. The overall adverse effects of Cr on growth and development of plants could be seriously an impairment of uptake of mineral nutrients and water leading to deficiency in shoot. In addition, the normal mechanism of selective inorganic nutrient uptake may have been destroyed by oxidative damage thus permitting large quantities of Cr (VI) to enter the roots passively and further translocation of Cr (VI) to shoot causing oxidative damage to the photosynthetic and mitochondrial apparatus eventually reflecting the poor growth.

In the present study, it was observed that, photosynthetic pigments (chlorophyll 'a', 'b' and carotenoid contents) were found to be decreased in rice on increasing the concentration of Cr. The increase in chlorophyll content of moong (*Phaseolus radiate*s L.) at various doses of cobalt was earlier reported by Tandon et. al., (2000), which could be due to heavy metals in low quantity favourably influencing iron metabolism. Chromium stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, photophosphorylation and enzyme activities (Clijsters and Van Assche 1985; and Chatterjee and Chatterjee 2000). The decrease in concentrations of chlorophyll 'a' and 'b' in rice leaves exposed to excess supply of chromium is similar to work of Chatterjee and Chatterjee (2000) on cauliflower. This finding was also in conformity with Singh et. al., (2006) in which he reported reduced chlorophyll content at increasing doses of chromium in paddy (*Oryza sativa* L.) plants. Nath et. al., (2008) reported reduction in chlorophyll content of cowpea (*Vigna sinensis* L. *Savix Hassk*) seedling with increase in concentration of chromium and tannery effluent. These finding were also similar to the effects of other pollutant elements (Agarwala et. al., 1977; Vazquez et. al., 1987; Lee et. al., 1993). The decrease in chlorophyll concentration may be result of an inhibited photosynthetic electron transport (Bohner et. al., 1980) and decomposition of the chloroplast membrane with excess copper. The adverse effects of excess heavy metals in rice may be because of chromium interfere in transformation of chlorophyll either through the direct inhibition of an enzymatic step or through the induced iron deficiency (Van Assche and Clijstrs 1990). In higher plants and trees, the effect of chromium on photosynthesis is well documented (Foy et. al., 1978; Van Assche and Clijstrs 1983). However it is not well understood to what extent chromium inhibition of photosynthesis is due to disorganization of chloroplast ultrastructure (Vazquez et. al., 1987), inhibition of electron transport or the influence of chromium on the enzymes of Calvin cycle. Chromate is used as Hill reagent by isolated chloroplast (Desmet et. al., 1975). The more pronounced effect of chromium (VI) on PS I than PS II activity in isolated chloroplasts has been reported by Bishnoi et. al., (1993 a, b) in peas. Zeid (2001) observed in peas that chromium at the highest concentration tested (10^{-2}M) decreased photosynthesis drastically. It was reported by Bishnoi et. al., (1993 a) that the 40% inhibition of whole plant photosynthesis in 52 day old plants at 0.1mM Cr (VI) was further enhanced to 65% to 95% after 76 and 89 days of growth respectively. Disorganization of the chloroplast ultrastructure and inhibition of electron transport process due to Cr and a diversion of electrons from electron donating side of PS I to Cr (VI) is a possible explanation for Cr induced decrease in photosynthetic rate. Bioaccumulation of Cr and its toxicity to photosynthetic pigments in various crops and trees is well documented (Barcelo et al., 1986; Sharma and Sharma, 1996; Vajpayee et al., 1999).

Increase in the ratio of chlorophyll a/b in rice plants might have an outcome of decreased concentration of chlorophyll 'b' except for the presence of an aldehyde group instead of methyl group at the third position of porphyrin ring. The chlorophyll 'b' is identical to chlorophyll 'a' and the conversion of chlorophyll 'a' and 'b' has been shown to involve oxidation of methyl to aldehyde group (Ito et. al., 1996). This oxidation is proposed to be carried out enzymatically by an iron enzyme chlorophyll 'a' oxygenase (Tanaka et. al., 1998). In prolonged iron deficient condition, accumulation of chlorophyll 'a' takes place in wheat plants. Increased rate of chlorophyll a/b ratio was reported by Gil et. al., (1995); in tomato plants. However in mustard plants chromium showed stimulatory effect in chlorophyll at low concentration and decreased in chlorophyll a/b ratio. It appears that this otherwise toxic metal is beneficial for chlorophyll synthesis in mustard plants in low doses. Heavy metal stress varies from plant to plant and species to species. This increase in or decrease in chlorophyll concentration might have resulted due to genotypic difference in different plant species.

Table 1. Effect of Chromium on vegetative growth of *Oryza sativa* (Rice) plants.

Treatments	Plant height (cm)	Number of leaves	Number of tillers
Control	40.75 ± 1.061	23 ± 1.414	4.5 ± 0.707
0.25mM Cr.	36.4 ± 0.707	20.5 ± 0.707	4 ± 0.707
0.5mM Cr.	34.4 ± 0.566	18.5 ± 0.707	3.5 ± 0.707
1.0mM Cr.	24.65 ± 0.495	17 ± 1.414	2.5 ± 0.707
L.S.D. $_{\alpha=0.05}$	2.054	3.104	1.097

Table 2. Effect of chromium on fresh weight, dry weight and water content of *Oryza sativa* (Rice) plants.

Treatments	Fresh Wt. (gms)	Dry Wt. (gms)	Water Content
Control	39.229 ± 0.529	17.885 ± 0.297	21.344 ± 0.826
0.25mM Cr.	29.251 ± 2.044	11.863 ± 1.607	17.388 ± 0.437
0.5mM Cr.	24.502± 0.0106	11.672 ± 0.865	12.830 ± 0.855
1.0mM Cr.	23.612 ± 1.428	9.456 ± 1.069	14.156 ± 2.498
L.S.D. $_{\alpha=0.05}$	3.538	2.965	3.887

Anti oxidative enzymes are those enzymes, which activate and produce against heavy metal stress, damage to plasma membrane and generation of reactive oxygen species (ROS), H_2O_2. Chromium stress can induce three possible type of metabolic modification in plants. (i) Alteration in production of pigments which are involved in the life sustenance of plants (e.g. chlorophyll, anthocyanin) (Boonyapookana et. al., 2002). (ii) Increased production of metabolites (e.g. glutathione, ascorbic acid) as a direct response to chromium stress which may cause damage to plants (Shanker 2003) and (iii) Alteration in metabolic pool to channelize the production of new biochemically related metabolites which may confer resistance or tolerance to Cr stress (e.g. phytochelatins, histidine) (Schmoger 2001).

In the present study activity of antioxidative enzyme catalase was increased in rice, at 0.25mM and 0.5mM concentration of chromium but at 1.0mM concentration catalase activity was found to be decreased. This finding is in conformity with earlier work of Nath et. al., (2009) in which he reported increase in the catalase activity of *Phaseolus mungo* Roxb. With increasing concentration of chromium and tannery effluents.

Table 3. Effect of Chromium on photosynthetic pigments i.e, Chl 'a', Chl 'b' and Chl 'a'/ Chl 'b' ratio of *Oryza sativa* (Rice) plants.

Treatments	Chl 'a'(mg/g FW)	Chl 'b'(mg/g FW)	Chl 'a'/Chl 'b' ratio
Control	2.823 ± 0.00566	1.0885 ± 0.00778	2.593477 ± 0.00943
0.25 mM Cr	2.686 ± 0.110	1.03 ± 0.00283	2.607767 ±0.0707
0.5 mM Cr	2.451 ± 0.0283	0.889 ± 0.0099	2.75703 ± 0.0442
1.0 mM Cr	2.048 ± 0.00707	0.732 ± 0.0099	2.797814 ± 0.0199
L.S.D. $_{\alpha=0.05}$	0.159	0.0226	N.S.

Table 4. Effect of Chromium on photosynthetic pigments i.e, total chlorophyll and carotenoid chlorophyll ratio of *Oryza sativa* (Rice) plants.

Treatments	Total Chl (mg/g FW)	Carotenoid (mg/gFW)	Carotenoid/Chl ratio
Control	3.9115 ± 0.0134	1.5075 ± 0.00919	0.3854 ± 0.00103
0.25 mM Cr.	3.716 ± 0.113	1.4325 ± 0.0148	0.385613 ± 0.00774
0.5 mM Cr.	3.34 ± 0.0184	1.232 ± 0.0283	0.368845 ± 0.00644
1.0 mM Cr.	2.78 ± 0.017	1.08 ± 0.0127	0.388482 ± 0.00221
L.S.D. $_{\alpha=0.05}$	0.162	0.0494	N.S.

Table 5. Effect of Chromium on enzyme activities i.e., catalase and peroxidase of *Oryza sativa* (Rice) plants.

Treatments	Catalase (µ mole H_2O_2 split/ 100mg FW)	Peroxidase (▲ OD/ 100mg FW)
Control	675 ± 7.071	24.41 ± 0.0665
0.25mM Cr.	750 ± 0.00	25.28 ± 0.170
0.5mM Cr.	725 ± 7.071	25.35 ± 0.495
1.0mM Cr.	570 ± 0.00	26.41 ± 0.0707
L.S.D. $_{\alpha=0.05}$	13.882	1.178

Table 6. Effect of Chromium on protein and sugar content of *Oryza sativa* (Rice) plants.

Treatments	Protein (mg/g FW)	Sugar (mg/g FW)
Control	27.72525 ± 5.601	0.5345 ± 0.0488
0.25mM Cr.	19.80375 ± 5.601	0.3 ± 0.0141
0.5mM Cr.	15.843 ± 0.00	0.26 ± 0.0141
1.0mM Cr.	7.9215 ± 0.00	0.22 ± 0.0424
L.S.D. $_{\alpha=0.05}$	10.997	0.094

The peroxidase activity was significantly increased on increasing the concentration of chromium in rice. Induction and activation of catalase is some of the major metal detoxification mechanisms in plants (Prasad 1998; Shanker et. al., 2003). Gwozdz et. al., (1997) reported increased activity of antioxidative enzymes at lower heavy metal concentration, while at higher concentrations; catalase activity was found to be decreased. This finding is in conformity with the present study. Pea plants exposed to environmentally relevant (20 µM) and acute (200 µM) concentrations of Cr (VI) for 7 days affected total SOD activity of root mitochondria differently. At 20 µM Cr (VI), SOD activity was found to increase by 29%, whereas 200 µM Cr (VI) produced a significant inhibition (Dixit et al., 2002). A decline in the specific activity of catalase with an increase in Cr concentration from 20 to 80 ppm was found (Jain et al., 2000). The low activity of the enzyme may be due to complete or partial displacement of iron from active sites or may be due to low iron in leaves decreasing the incorporation of iron in the prophyrin of the enzyme. In *E. colona* plants supplemented with Cr at 1.5 mg L^{-1}, activities of peroxidase and catalase were higher in tolerant calluses than in non-tolerant ones (Samantaray et al., 2001). Samantaray et al. (1999) used peroxidase and catalase activities as enzyme markers for identifying Cr tolerant mung bean cultivars. The application of 0.05-0.5 mM Cr caused decreased activities of both antioxidative enzymes in wheat cultivar cv. UP (Sharma and Sharma, 1996). Sen et al. (1994) observed a decrease in catalase activity and increase in peroxidase activity at concentrations above 10 μL^{-1} Cr (VI), whereas the enzyme activities were least affected by Cr (VI) at lower concentrations. The increase in antioxidant enzymes activity observed might have been an in direct response to the generation of superoxide radical by Cr induced blockage of the electron transport chain in the mitochondria. The higher increase noticed due to Cr (VI) indicated that Cr (VI) addition probably generates more singlet oxygen than Cr (III). The decrease in the activity of the enzyme as the concentration of the external Cr increased might be because of the inhibitory effect of Cr ions on the enzyme system itself (Shanker et. al., 2005).

Phytotoxic concentration of chromium caused decrease in protein content in rice. The restricted biomass of these crops in the presence of excess Cr might have resulted poor protein formation in such conditions. Disruption of nitrogen metabolism in these plants by pollutants has been observed with excess Co, Cu and Cr in cauliflower by Chaterjee and Chaterjee (2000), Sharma et. al., (1995); in wheat as well as in other plant species (Hunter and Vergnano 1953). Since nitrogen is one of the essential nutrients involved as a constituents of biomolecules such as nucleic acids, nitrogen bases, coenzymes and proteins, any deviation in these constituents would severely inhibit the growth and yield of plants. Metal induced inhibition of protein synthesis was earlier reported by Samantary (2002). The decrease in protein content in *Albizia lebbak* is either due to reduced de-novo synthesis or increased decomposition of protein into amino acid (Tripathi et. al., 1999).

In the present study, sugar concentration was decreased on increasing the concentration of chromium in rice. This finding is in conformity with Nath (2010) and Verma et. al., (2009) in which they reported reduction in sugar content with the increasing doses of chromium. Heavy metal such as chromium reduces leaf area and number of leaves in tomato and brinjal (Purohit et. al., 2003 and Tripathi et. al., 1999).

Closure and bending of leaves due to toxicity of cobalt was already reported by Rauser (1978). Chromium may also be responsible for depression in young leaves development and cause narrowing of lamina in *Citrullus vulgaris* plants (Gopal et. al., and Dubey et. al., 2003). Reduced sugar synthesis in plants by chromium might be due to lower synthesis caused by depression in young leaves development narrowing of synthesis areas or diversion of metabolites to other synthesis processes. Finding of this study is in conformity with some earlier reports on heavy metals (Tripathi et. al., 1999; Hemalatha et. al., 1997; Bazzaz et. al., 1975 and Prince et. al., 2002). Tandon and Gupta (2002), have also reported decreased sugar content at increasing doses of cobalt and lead.

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Corresponding author Dr. Akash Vikram, Department of Botany, University of Lucknow, Lucknow – 226007, U.P., India
Email: akashvikram05@gmail.com