Pak. J. Bot., 43(6): 2853-2859, 2011.

ECOPHYSIOLOGICAL RESPONSES OF RICE (*ORYZA SATIVA* L.) TO HEXAVALENT CHROMIUM

MAQSOOD AHMAD[1], A. WAHID[2]*, SHEIKH SAEED AHMAD[3], ZAHID ALI BUTT[4] AND MARYUM TARIQ[1]

[1]*Sustainable Development Study Centre, GC University Lahore, Pakistan*
[2]*Department of Environmental Sciences, BZ University, Multan, Pakistan*
[3]*Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan*
[4]*Department of Botany, GC University, Lahore, Pakistan*

Abstract

The effects of hexavalent chromium (Cr) were studied in rice plants by applying its different concentrations ranging from 50-500 mg/kg of soil. Cr significantly altered growth of rice plants and reduced dry weights of shoot (7-58%) and roots (7-73%) in different treatments. Cr impact was remarkably high on photosynthetic rate (21-62%), transpiration rate (5-59%), and stomatal conductance (21-66%). Chlorophyll *a* and *b* and carotenoid contents were also reduced in Cr-treatment plants by 17-47%, 12-43%, 31-50%, respectively. Highly pronounced reductions were recorded in nitrogen (23-82%), phosphorous (4-37%), and potassium (6-42%) content of treated plant leaves. Cr accumulation was extremely higher in shoots (3575-19150%), roots (1023-5869%), and seeds (21-249%) of treated plants compared with control. Present investigation has reported injurious effects of Cr^{6+} on different aspect of rice plants. Cr accumulation in threshold amounts in plant parts and seeds is a matter of serious concern to human health as it causes cardiovascular diseases, kidney failure and cancer.

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 in environment are wastewater irrigation, sludge applications, solid waste disposal, automobiles 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). Heavy metals effects on crops and humans caused functional disorder in their body organs due to exposure of low dose over a long 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 (III) and Cr (VI), may be taken up by plants. Uptake of Cr (III) is considered passive, while that of Cr (VI) 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 Pakistan are facing problems of contamination of soil and water with Cr from different sources including steel industries, electroplating, tanning industries, oxidative dyeing, cooling water towers, refractory materials production, metallurgy, pigments and mining (Oktor *et al*., 2008) and its concentration above 0.05 mg/L considered to be toxic (Suwalsky *et al*., 2008).

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 Cr, cadmium (Cd), and mercury (Hg) according to Calba *et al*., (2004). When Cr entered into plant body, it can 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 (III) is included in micronutrients; on the other hand, Cr (VI) 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 (Anon., 1990). The toxic and carcinogenic properties of Cr (VI) 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(VI) to Cr(III) inside the cell (O'Brien *et al*., 2003). Levels of metal particles and 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 orthopaedic implants have been observed (Landon *et al*., 2004).

Heavy metals taken up by plants from contaminated soil and water are toxic to growth performance of plants and posses a hidden threat to consumers (Stobrawa *et al*., 2008).

Rice (*Oryza sativa* L.) is an important cereal crop around the globe including Pakistan due to its consumption as food by humans. This research program was envisaged to assess the effects and accumulation of Cr(VI) in different plant parts that may be transferred to humans through food chain, particularly in the contaminated areas.

*Corresponding address E-mail: drsaeed@fjwu.edu.pk; Tel: 00 92 321 5167726

Materials and Methods

Rice (*Oryza sativa* L.) cultivar 'Basmati-Super' was chosen for the present research work and potassium dichromate as the source of "hexavalent chromium" (Cr^{6+}). Salt was mixed with the sieved soil according to different treatments viz., 50 mg/kg (**T1**), 100 mg/kg (**T2**), 150 mg/kg (**T3**), 200 mg/kg (**T4**), 300 mg/kg (**T5**), 400 mg/kg (**T6**) and 500 mg/kg (**T7**). Pots filled with field soil without any addition of salt served as control (**T0**). Photosynthetic rate, transpiration rate, and stomatal conductance was carried out after 45 days of transplantation with the help of the Infra Red Gas Analyzer (IRGA). For this purpose, 3 leaves of similar age were randomly selected within same treatment to conduct the study of physiological parameters by enclosing them in the leaves chamber (Vernay *et al*., 2008). For the determination of dry biomass, the plants were put in labeled paper bags and placed in an oven at 80°C for 48 hours. The extraction procedures of pigment were carried out under dim light and in glassware wrapped with aluminum foil. The method consisted of acetone extraction repeated until reaching a colourless residue with pestle and mortar and filtered over a cotton pad. The extracts were made up to 50 ml with acetone. The concentration of carotenoids was measured at 440.5 nm, chlorophyll a at 662 nm, chlorophyll *b* at 644nm (Wettstein, 1957).

Potassium (K) was determined by using the flame photometer. For making stock solution (1000 ppm K), about 3 g potassium chloride was dried in an oven at 120°C for 1.5 hours, cooled and stored in tightly stoppered bottle. Dissolved 1.907 g dried potassium chloride was put in distilled water to one litre volume. Series of standard solutions were prepared from the stock solution for calibration curve. The standard solutions and samples were feeded to the flame photometer and reading in ppm was recorded from the display of the equipment (Ryan *el al*., 2001).

Nitrogen was determined by Kjeldahl method by using automatic distillation plant. The samples were first digested in digestion flask of Behr Inkjel M Nitrogen Assembly. About one gram of dried plant material of each sample was then added with 15 ml of concentrated H_2SO_4 along with half tablet of catalyst (Selenium mixture). The digestion flasks were placed in the digester for about 90 minutes. After cooling, the final volume of the digested material was made up to 100 ml and the sample was run in distillation assembly. This was completed by raising the pH with 40% NaOH, to convert ammonium (NH_4^+) to ammonia (NH_3). Then 4% boric acid was taken in conical flask for trapping the ammonia during distillation. After completion of distillation, the boric acid was titrated against 7 N HCl by using mixed indicator (mixture of Bromocresol green and methyl red). The end point was violet colour. A reading of titration for blank was also obtained by the same procedure, necessary for calculation (Alvarado, 1988). N contents was calculated by using the following formula:

$$\% \text{ N} = \frac{\text{(A-B) x Normality of acid x 14.01 x 10 x 100}}{\text{Volume of sample}}$$

where: A = HCl used for sample; B = HCl used for blank; 14.01 is the atomic mass of nitrogen.

For the determination of phosphorus, dry plant samples were crushed and about one gram of each sample was filled in crucibles, and kept in Muffle furnace at 650°C for 2.5 hours (Panichev *et al.*, 2005). The sample was mixed with 10 ml of 0.7 N H_2SO_4 reagent were added. After one hour sample was then filtered in 50 ml volumetirc flask by using Whatman No. 42 filter paper. The volume of the filtrate was made up to 50 ml using distilled water.

For estimation, stock solution of phosphorous was made by dissolving the oven dried 0.4393 g of KH_2PO_4 in 1000 ml distilled water. From the stock solution, different standards were formed i.e., 2, 4, 6, 8, 10, 20, 30, 40 and 50 ppm. Then 5 ml of each standard was taken and 10 ml of Ammonium vanadomolybdate was added and the final volume was raised up to 50 ml by adding distilled water. Similarly, 5 ml of each plant extract was taken and 10 ml of Ammonium vanadomolybdate was added, and final volume was raised up to 50 ml by adding distilled water. The absorbance value of the standards and the samples were measured by spectrophotometer at 410 nm. A graph of the absorbance values of standards was plotted. Phosphorous values of samples were noted by placing their absorbance values in the graph (Ryan *et al*., 2001).

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 mixture of diluted nitric acid and hydrochloric acid and the solution was transferred to a 25.0 ml volumetric flask and made to volume with distilled water. A Perkin-Elmer atomic absorption spectrometer was used for all measurements. Amount of Cr was observed by using the Cr 357.9 nm resonance line. (Panichev *et al.*, 2005).

Statistical analysis: Data obtained for various parameters were subjected to one-way ANOVA followed by Duncan's Multiple Range Test in order to reach a certain conclusion.

Results

Dry weight of shoot and root (g): Rice plants grown in pots were healthier and lush green in control (T0) and then their health showed gradual and successive decline in different treatments till the highest Cr-treatment (T7). Plants in control and other treatments have correspondingly higher number of tillers, leaves and roots relative of various Cr-treatment plants, and thus were heavier in weight. Dry weight of shoots and roots in various treatments are shown in Fig. 1 that depicted 7-58% decrease in shoot weight than that 7-73% in root weight compared to control plants in T1 to T7 treatments depicting that roots were more sensitive to chromium. Higher concentrations of chromium proved more lethal than that of lower Cr levels.

Physiological attributes: Results of the physiological attributes are presented in Fig. 2 that depicted decreases in photosynthetic rate (21-62%), transpiration rate (5-59%), and stomatal conductance (21-66%) compared to control. Results clearly showed that the physiological parameters decreased as Cr treatment levels (T1 to T7) were increased and were statistically significant. Chlorophyll *a*, Chlorophyll *b*, and carotenoids are very important pigments for photosynthesis. Cr showed negative effects on the formation and persistence of these chemical compounds in the leaves. Data shown in Figure 3 for chlorophyll *a, b* and carotenoids depicted that the contents of these pigments gradually decreased as concentration of Cr treatments increased in the rice plants.

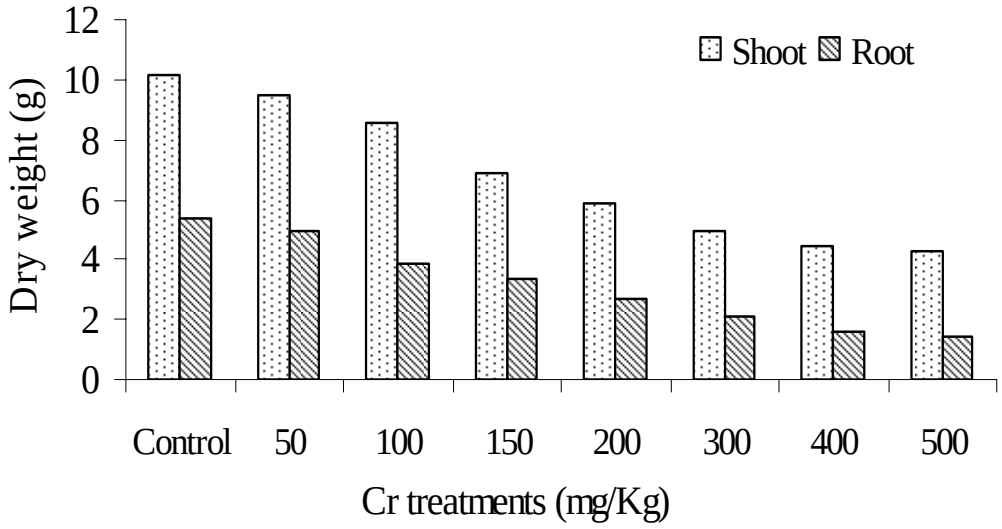

Fig. 1. Effects of chromium on shoot and root dry biomass of rice

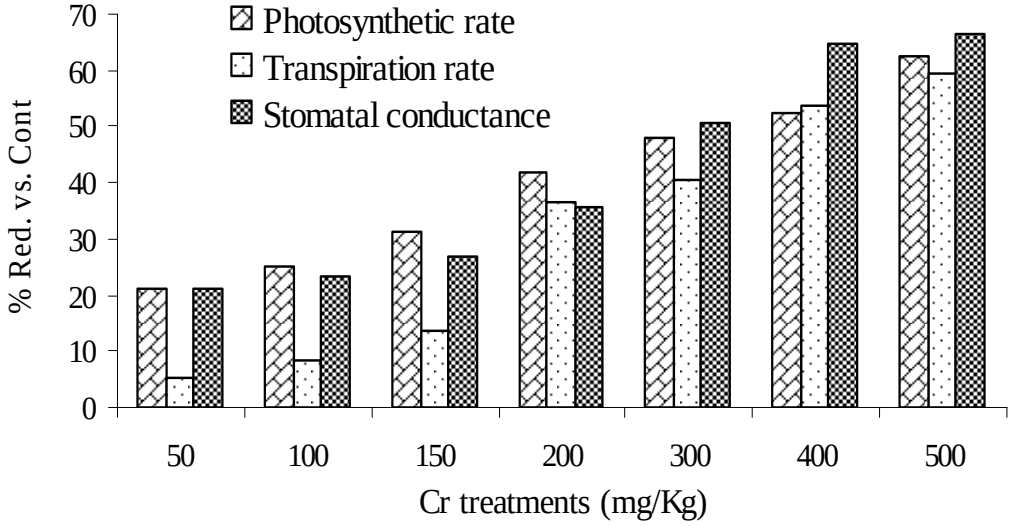

Fig. 2. Effects of chromium on some physiological attributes of rice.

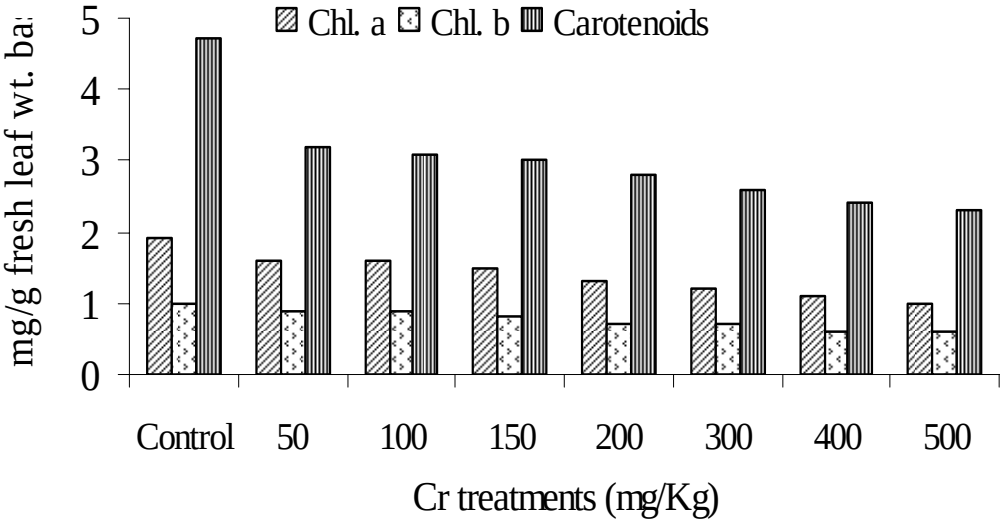

Fig. 3. Effects of chromium on chlorophyll and carotenoids contents of rice.

Nitrogen, phosphorous and potassium contents: Data for the nitrogen, phosphorous and potassium are given in Table 1 which demonstrated that NPK contents in the leaves were higher in the control followed by successive reductions in different Cr-treated plants. Nitrogen contents were reduced by 23-82% in various Cr-treatment plants, while reductions in phosphorous and potassium were 4-37% and 6-42% compared to counterparts grown in control.

Table 1. Effects of chromium on nitrogen, phosphorous and potassium contents of leaves of rice plants.

Treatments (ppm)	Nitrogen (%)	Phosphorous (ppm)	Potassium (ppm)
T0 (Control)	0.52a ± 0.01	31.55a ± 0.32	174.1a ± 1.10
T1 (50)	0.4b ± 0.003	30.25b ± 0.14	163.9b ± 2.11
T2 (100)	0.2c ± 0.001	28.85c ± 0.11	154.6c ± 2.02
T3 (150)	0.16c ± 0.01	28.1d ± 0.17	147.7d ± 1.8
T4 (200)	0.16c ± 0.003	26.6e ± 0.35	136.6e ± 0.66
T5 (300)	0.11c ± 0.002	23.5f ± 0.37	131.6e ± 0.66
T6 (400)	0.1c ± 0.01	22.55g ± 0.26	116.0f ± 1.15
T7 (500)	0.09c ± 0.003	19.8h ± 0.28	101.2g ± 1.64

Treatment means followed by different letters in each column are significantly different at p=0.05 according to Duncan's Multiple Range Test

Accumulation of chromium in shoots, roots and seeds: Data shown in Table 2 depicted that chromium was accumulated in the shoots, root and seeds of rice plants. There were least concentrations of Cr found in the control plants as compared to successive increases recorded in shoots, roots and seeds of Cr-treated plants. The differences between treatment means when analyzed were statistically highly significant. The magnitude of Cr uptake in shoots roots and seeds were alarming. In shoots, Cr accumulation was 3575% (T1), 5350% (T2), 5415% (T3), 6310% (T4), 12065% (T5), 16025% (T6), and 19150% (T7), while in roots, it remained 1023% in T1, 1755% in T2, 2204% in T3, 2644% in T4, 3762% in T5, 4669% in T6, and 5869% in T7. There was highly pronounced increase in Cr uptake in seeds with 21% in T1, 33% in T2, 47% in T3, 76% in T4, 120% in T5, 178% in T6, and 249% in T7 compared to control.

Table 2. Accumulation of Cr in roots, shoots and seeds of rice plants.

Treatments (ppm)	Cr accumulation (ppm)		
	Roots	Shoots	Seeds
T0 (Control)	3.25 ± 0.29	0.20f ± 0.04	0.107h ± 0.01
T1 (50)	36.5 ± 1.06	7.35e ± 1.89	0.129g ± 0.02
T2 (100)	60.28 ± 3.08	10.90de ± 1.25	0.142f ± 0.14
T3 (150)	74.90e ± 1.26	11.03de ± 1.06	0.156e ± 0.11
T4 (200)	89.20d ± 0.59	12.82d ± 1.03	0.188d ± 0.12
T5 (300)	125.50c ± 3.66	24.33c ± 1.25	0.235c ± 0.03
T6 (400)	155.00b ± 1.22	32.25b ± 1.38	0.297b ± 0.01
T7 (500)	194.00a ± 1.49	38.00a ± 1.38	0.372a ± 0.04

Treatment means followed by different letters in each column are significantly different at p=0.05 according to Duncan's Multiple Range Test

Discussion

Cr-treatment plants showed stunted growth and produced less number of tillers and leaves compared to counterparts grown in control. Cr-treated plants also showed earlier leaf senescence depicting the slower rate of synthesis of carbohydrates as compared to control plants in which number of green leaves remained alive for a longer period of time. Upon harvests, plants from Cr-treatments showed reduced fresh weights of shoot and roots (data not shown) along with corresponding reduced dry biomass of shoots and roots (Fig. 1). According to Anderson (1972), there was about 11, 22 and 41 % reduction in plant height, respectively, over control after addition of concentration of 2, 10 and 25 ppm Cr to nutrient solutions in sand cultures in oats, while Huffman & Allaway (1973 a&b) reported that Cr compounds were highly toxic to agricultural plants and were detrimental to their growth and development. However, some crops were not affected by low Cr concentration (3.8×10^{-4} µM). McGrath (1984) studied that tri and hexavalent Cr, both absorbed by oat and were equally toxic when supplied at 2 to 200 μM in nutrient solution.

Barcelo *et al*., (1985) found that primary and trifoliate leaves of bush bean plants grown under Cr stress showed a marked decrease in leaf area. Dry leaf biomass of bush bean plants was found to decrease up to 45% when 100 ppm of Cr(VI) was added to soil. Barcelo *et al*., (1986) reported that general response of decreased root growth due to Cr toxicity could be due to inhibition of root cell division/root elongation or to the extension of cell cycle in the roots. Under high concentrations of both the Cr species (trivalent and hexavalent) combination, the reduction in root growth could be due to the direct contact of seedlings roots with Cr in the medium causing a collapse and subsequent inability of the roots to absorb water from the medium.

Sharma & Mehrotra (1993) reported that the effect of Cr on the plant processes during early growth and development culminates in 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 dry matter was essentially an indirect effect of Cr on plants. The overall adverse effect of Cr on growth and development of plants could be serious impairment of uptake of mineral nutrients and water leading to deficiency in the shoot. Chen *et al*., (2001) reported that the total root weight and root length of wheat was affected by 20 mg Cr (VI)/kg soil as $K_2Cr_2O_7$ as compare to control plants. Vajpayee *et al*., (2001) studied that the Cr accumulation and toxicity in relation to biomass production found that dry matter

production was severely affected by Cr concentrations above 2.5 g/ ml in nutrient medium of *Vallisneria spiralis.* Singh (2001) in a study on the effect of Cr(III) and Cr(VI) on spinach, reported that Cr applied at 60 mg kg^{-1} and higher levels reduced the leaf size, caused burning of leaf tips or margin and slowed leaf growth rate resulting in reduced dry biomass.

Vernay *et al*., (2008) reported that *D. innoxia* plants grown in presence of Cr(VI) showed reduced growth leading to reduction in root and shoot biomass. The addition of Cr(III) in the nutrient solution restricted the shoot and root growth but at a lesser level than Cr(VI). The decrease of shoot growth was observed from 0.05 and 0.5 mM for Cr(VI) and Cr(III), respectively. The highest decrease, 73% for the shoot and 67% for the roots, was obtained from 0.5 mM for Cr(VI). The highest decrease for Cr(III) was obtained from a 1 mM concentration with 47% and 55% for shoot and root, respectively. The ability of *D. innoxia* to accumulate Cr varied highly regardless of its oxidation state. Cr accumulation in plant roots was higher than in leaves for Cr(III) and Cr(VI), indicating that Cr was not easily translocated within plants. The accumulation of Cr(VI) in root tissues was higher than the accumulation of Cr(III). A maximum of 10469 µg g^{-1} dry weight, was found in the roots of the plants exposed to 2 mM Cr(VI), whereas only 1546 µg g^{-1} dry weight were taken up when Cr was supplied in the form of Cr(III). In spite of the high Cr concentration in roots, the content in Cr leaves was less. They are respectively 83 and 54 µg g^{-1} dry weights for the Cr(VI) and Cr(III) at 2 mM in nutrient media.

Different Cr levels also remarkably affected growth physiology of the plants. Photosynthetic rate showed 21-62% reduction as shown in Fig. 2, while transpiration rate was reduced by 5-59%. There were also reductions of 21-66% in stomatal conductance of rice crop treated with Cr treatments compared with control. Cr affected all parameters of physiology in Cr-treated plants of rice than that of control depicting the cause of their poor vegetative growth.

Van Assche & Clijsters (1983) noticed the disorganization of the chloroplast ultrastructure and inhibition of electron transport processes due to Cr and a diversion of electrons from the electron-donating side of PS I to Cr(VI) was a possible explanation for Cr-induced decrease in photosynthetic rate. It was further noted that electrons produced by the photochemical process were not necessarily used for carbon fixation as evidenced by low photosynthetic rate of the Cr stressed plants. Due to the known oxidative potential of Cr(VI), it was possible that alternative sinks for electrons could have been enhanced by reduction of molecular oxygen which in part explained the oxidative stress brought about by Cr(VI). The overall effect of Cr ions on photosynthesis and excitation energy transferred could also be due to Cr(VI)-induced abnormalities in the chloroplast ultrastructure like a poorly developed lamellar system with widely spaced thylakoid and fewer grana.

Liu *et al*., (2008) found that photosynthetic rate when compared to the controls, the net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration were changed slightly in the treated plants with 10^{-6} M Cr(VI) and decreased markedly at 10^{-5} M Cr(VI) and 10^{-4} M Cr(VI) treatments. At 10^{-4} M Cr(VI) treatment for 20 days, the net photosynthetic rate and the intercellular CO_2 concentration were reduced by 78.4% and 45.2%, separately. Subrahmanyam (2008) reported that Cr(VI) significantly reduced rates of net photosynthesis and transpiration and of stomatal conductance.

Chlorophyll is the key chemical compound present in the plants and Cr affected the chlorophyll *a* (reduced in the range from 17-47% than control), chlorophyll *b* (reduced in the range from 12-43% than control), and carotenoids (reduced by 31-50% than control). Data for these parameters have been shown in Fig. 3. Carotenoids are accessory pigments needed in the process of photosynthesis. Leaf nitrogen contents were reduced by 23-82%. Phosphorous is regarded as the energy currency of the cell, and it was reduced (ranged from 4-37%) due to different Cr-treatments in rice plants while potassium that is needed in various metabolic activities was also reduced (ranged from 6-42%) in rice treated with various Cr levels (Table 1).

Bera *et al*., (1999) studied the effect of Cr present in tannery effluents on chloroplast pigment content in mungbean and reported that irrespective of concentration, chlorophyll *a*, chlorophyll *b* and total chlorophyll decreased in 6-day-old mungbean seedlings as compared to control. Appenroth *et al*., (2001) demonstrated the effect of different chromate concentrations over both growth rate and yield. He reported gradual inhibition by the heavy metal that finally resulted in almost completely destroyed fronds by the highest concentration (200 µM, 10 days). Chlorophyll contents were decreased by 70 % after 5 days and 90 % after 10 days in presence of 200 µM chromate. They also found decreased rate of photosynthetic O_2 evolution and rate of respiration. Boonyapookana *et al*., (2002) reported that Cr stress can induce three possible types of metabolic modification in plants, one was alteration in the production of pigments which were involved in the life sustenance of plants (eg., chlorophyll, anthocyanin), second was increased production of metabolites (eg., glutathione, ascorbic acid) as a direct response to Cr stress which may cause damage to the plants, and third was alterations in the metabolic pool to channelise the production of new biochemically related metabolites which may confer resistance or tolerance to Cr stress (eg., phytochelatins, histidine).

Singh & Agrawal (2007) found that the higher accumulation of heavy metals in plants led to reduced photosynthetic rate and chlorophyll pigments, disturbed photochemical light quenching, increased lipid peroxidation and proline and protein contents and stunted growth. Rai & Mehrotra (2008) used *P. amarus* seedlings of approximately same height and weight to grow under Cr stress in experimental garden. The plants were harvested after 30 days, chlorophyll, protein, sugar, starch and secondary metabolites concentration were estimated in these plants. Besides ultramorphological variations in leaves and Cr accumulation in roots and aerial parts were also checked. The study revealed that Cr caused significant decrease in fresh and dry weight, length of root and shoot, protein, sugar, chlorophyll and carotenoids in *P. amarus*. Besides, ultramorphological changes viz., wide stomatal opening and less wax deposition on both the surfaces of leaves were also observed. Kumara & Joshi (2008) studied sorghum (*Sorghum bicolor* L.) and

found that Cr (VI) application adversely affected the nitrogen metabolism by inhibiting the activity of NR, GS, GDH and Urease. Further, Cr(VI) inhibits both GS/GOGAT and GDH pathway for ammonium assimilation. Cr (VI) treatments adversely affected the growth of forage sorghum as a result of its interference with photosynthetic pigments and key enzymes of nitrogen metabolism.

Effects of Cr was alarming on all aspects of plant growth, and the impact was further worrying when Cr was found in rice shoots (ranged from 3575-19150 % higher in different Cr-treated plants than that of control), roots (ranged from 1023-5869% higher in different Cr-treated plants compared with control), and rice seeds (ranged from 21-249% higher in Cr-treated plants compared with control).

Sauerbeck (1991) reported that comparatively lower concentration of Cr in the grains compared to roots was probably due to reduction of Cr(VI) to Cr(III), which reduced its mobility from roots to shoots. Bahmanyar (2008) reported that accumulation of Cr in rice root was more than in whole shoot and grain, but in agricultural land influenced by industrial wastewater, the contents of Cr in root, whole shoot, and grain were increased two times. Khanjani *et al*., (2008) also reported that watermelon irrigated by wastewater has high concentration of heavy metals like Ni, Cr, Pb, Zn and Mn in fruit, stem and root. Concentrations of most heavy metals were in descending order from roots, stems and fruits showing a filtration processes from roots to fruits.

Conclusions

Present study clearly demonstrated that hexavalent Cr is injurious to rice plants and it severely affected the growth and biomass. The study was probed into the underlying causes of the harmful effects of Cr on plant's growth and development by extensively investigating the physiological parameters. It was known that adverse effects of Cr were due to the negative impacts on physiological parameters, for instance, photosynthesis, transpiration rate and stomatal conductance. It is worth mentioning that some of the main attributes of metabolic activity like chlorophyll, carotene, nitrogen, phosphorous and potassium uptake was seriously affected by high concentrations of Cr in the soil. The study has reported that threshold amounts of Cr were found in the shoot, root and even in the seeds of plants which when consumed can cause serious illness to humans through accumulation in the body organs (Jarup 2003), causing functional disorders or even exposure of low doses of Cr over a long period of time could also cause serious diseases like cancer in humans (Jianjie *et al*., 2008). The results are of serious concern as the tanneries wastewaters and other industrial waters containing Cr are chanellized into water bodies without any treatment which is used for agricultural production in a developing country like Pakistan.

Acknowledgements

Authors are thankful to field staff of Botanic Garden & SDSC, GC University, Lahore and PCSIR Labs, Lahore for their help during the course of experimentation.

References

Alloway, B.J. 1995. *Heavy metals in soils*. 2nd edition. USA: Blackie Academic & Professional.

Alvarado, J., M. Marquez and L.E. Leon. 1988. Determination of organic nitrogen by the Kjeldahl method using microwave acid digestion. *Analytical Letters*, 21: 357-365.

Anderson, A.J., D.R. Meyer and F.K. Mayer. 1972. Heavy metal toxicities levels of nickel, cobalt and chromium in the soil and plants associated with visual symptoms and variation in growth of an oat crop. *Australian Journal of Agriculture Research*, 24: 557-571.

Appenroth, K.J., J. Stockel, A. Srivastava and R.J. Strasser. 2001. Multiple effects of chromate on the photosynthetic apparatus of *Spirodela plyrhiza* as probed by OJIP chlorophyll a fluorescence measurements. *Environmental Pollution*, 115: 49-64.

Arun, K., T. Shanker, C. Cervantes, H. Loza-Tavera and S. Avudainayagam. 2005. Chromium toxicity in plants. *Environment International*, 31: 739-753.

Bahmanyar, M.A. 2008. Cadmium, Nickel, Chromium, and Lead Levels in Soils and Vegetables under Long-Term Irrigation with Industrial Wastewater. *Communications in Soil Science and Plant Analysis*, 39: 2068-2079.

Barcelo, J., C. Poschenrieder and B. Gunse. 1986. Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris* L. cv. Contender) under both normal and water stress conditions. *Journal of Experimental Botany*, 37: 178-187.

Barcelo, J., C. Poschenriender, A. Ruano and B. Gunse. 1985. Leaf water potential in Cr(VI) treated bean plants (*Phaseolus vulgaris* L.). *Plant Physiology Supplement*, 77: 163-174.

Barnhart, J. 1997. Occurrences, uses, and properties of chromium. *Regulatory Toxicology and Pharmacology*, 26: 53-57.

Bera, A.K., A.K. Kanta-Bokaria and K. Bokaria. 1999. Effect of tannery effluent on seed germination, seedling growth and chloroplast pigment content in mungbean (*Vigna radiata* L. Wilczek). *Environmental Ecology*, 17(4): 958-961.

Boonyapookana, B., E.S. Upatham, M. Kruatrachue, P. Pokethitiyook and S. Singhakaew. 2002. Phytoaccumulation and phytotoxicity of cadmium and chromium in duckweed Wolffia globosa. *International Journal of Phytoremediation*, 4: 87-100.

Buerge, I.J. and S.J. Hug. 1997. Kinetics and pH dependence of Cr^{6+} reduction by Fe^{2+}. *Environmental Science and Technology*, 31: 1426-1432.

Calba, H., P. Cazevieille, R. Poss and B. Jaillard. 2004. The dynamics of protons, aluminium and calcium in the rhizosphere of maize cultivated in tropical acid soils, experimental study and modeling. *Plant Soil*, 260: 33-46.

Case, C.P., V.G. Langkamer, C. James, M.R. Palmer, A.J. Kemp, P.F. Heap and L. Solomon. 1994. Widespread dissemination of metal debris from implants. *Journal of Bone and Joint Surgery*, (Br.) 76-B: 701-412.

Chen, N.C., S. Kanazawa and T. Horiguchi. 2001. Effect of chromium on some enzyme activities in the wheat rhizosphere. *Soil Microorganism*, 55: 3-10.

Gunaratnam, M. and G.M. Helen. 2008. Cr(VI) inhibits DNA, RNA, and protein synthesis in hepatocytes: involvement of glutathione reductase, reduced glutathione and DT-diaphorase. *Toxicology In Vitro*, 22: 879-886.

Hinsinger, P., C. Plassard, C. Tang, and B. Jaillard. 2003.Origions of root-Indused pH changes in the rhizosphere and their responses to environment constraints, a review. *Plant Soil*, 248: 43-59.

Huffman, Jr. and H.W. Allaway. 1973a. Chromium in plants: distribution in tissues, organelles, and extracts and availability of bean leaf Cr to animals. *Journal of Agriculture and Food Chemistry*, 21: 982-986.

Huffman, Jr. and W.H. Allaway. 1973b. Growth of plants in solution culture containing low levels of chromium. *Plant Physiology*, 52: 72-75.

IARC Lyon. 1990. International Agency for Research on Cancer (IARC), IARC Monographs of the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Chromium, Nickel and Welding, p. 49.

Jarup, L. 2003. Hazards of heavy metals contamination. *British Medicine Bulletin*, 68: 167-182.

Jianjie, Fu., Z. Qunfang, L. Jiemin, L. Wei, W. Thanh, Z. Qinghua and J. Guibin. 2008. 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: 1269-1275.

Khanjani, M.J., A.A. Maghsoudi moud, V.R. Saffari, S.M. Hashamipor and M. Soltanizadeh. 2008. Statistical analysis of heavy metals concentration in watermelon plants irrigated by wastewater. CP971, International Conference on Mathematical Biology -ICMB07, edited by K. A. Mohd. Atan, American Institute of Physics 978-0-7354-0489.

Kumara, S. and U.N. Joshi. 2008. Nitrogen metabolism as affected by hexavalent chromium in sorghum (*Sorghum bicolor* L.). *Environmental and Experimental Botany*, 64: 135-144.

Landon, D., A. Doherty, R. Newson, J. Turner, M. Bhamra and C.P Case. 2004. Changes in metal levels and chromosomal aberrations in the peripheral blood of patients after metal-on-metal arthroplasty. *Journal of Arthorplasty*, 19: 78-83.

Liu, D., J. Zou, M. Wang, and W. Jiang. 2008. Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L. *Bioresource Technology*, 99: 2628-2636.

McGrath, S. P. 1984. The uptake and translocation of tri and Hexa-valent chromium and effects on the growth of oat in flowing Nutrient solution and in soil. *New Phytol*ogist, 92: 381-390.

O'Brien, T.J., S. Ceryak and S.R. Patierno. 2003. Review, Complexities of Chromium Carcinogenesis: Role of Cellular Response, Repair and Recovery Mechanisms, *Mutation Research*. pp. 3-36.

Oktor, K., S. Yilmaz, G. Turker and E. Erkus. 2008. Speciative determination of Cr(III) and Cr(VI) in dyeing waste water of Dil Creek discharge to lzmit Gulf (Izmit-Kocaeli, Turkey) by ICP-AES. *Environmental Assessment*, 141: 97-103.

Panda S.K. and H.K. Patra. 2000. Nitrate and ammonium ions effect on the chromium toxicity in developing wheat seedlings. *Plant National Academy of Science of India*, B70: 75-80.

Panichev, N., K. Mandiwana, M. Kataeva and S. Siebert. 2005. Determination of Cr(VI) in plants by electrothermal atomic absorption spectrometry after leaching with sodium carbonate. *Spectrochimica Acta*, Part B 60: 699-703.

Rai, V. and S. Mehrotra. 2008. Chromium-induced changes in ultramorphology and secondary metabolites of *Phyllanthus amarus* Schum & Thonn. - An hepatoprotective Plant. *Environmental Monitoring and Assessment*, 147: 307-315.

Rouphael, Y., M. Cardarelli, E. Reab and G. Colla. 2008. Grafting of cucumber as a means to minimize copper toxicity. *Environment And Experimental Botany*, 63: 49-58.

Ryan, J., G. Estfan and A. Rashid. 2001. Soil and Plant Analysis Laboratory Manual. Second edition. pp 87-89.

Sauerbeck, D.R. 1991. Plant, element and soils properties governing uptake and availability of heavy metals derived from sewage sludge. *Water Air Soil Pollut*ion, 57-58: 227-237.

Sharma, D.C. and S.C. Mehrotra. 1993. Chromium toxicity effects on wheat (*Triticum aestivum* L. cv. HD 2204). *Indian Journal of Environmental Health*, 35: 330-342.

Shi, Z., 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. 2005. Contamination of rivers in Tianjin, China by polycyclic aromatic hydrocarbons. *Environmental Pollution*, 134: 97-111.

Singh, A.K. 2001. Effect of trivalent and hexavalent chromium on spinach (*Spinacea oleracea* L.). *Environmental Ecology*, 19: 807-810.

Singh, R.P. and M. Agrawal. 2007. Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants. *Chemosphere*, 67: 2229-2240.

Stobrawa, K. and P.G. Lorenc. 2008. 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: 86-96.

Subrahmanyam, D. 2008. Effects of chromium toxicity on leaf photosynthetic characteristics and oxidative changes in wheat (*Triticum aestivum* L.). *Photosynthetica*, 46 (3): 339-345.

Suwalsky, M., R. Castro, F. Villena and C.P. Sotomayor. 2008. Cr (III) exerts stronger structural effectsthan Cr (VI) on human erythrocyte membrane and molecular modes. *Journal of Inorganic Biochemistry*, 102: 842-849.

Vajpayee, P., U.N. Rai, M.B. Ali, R.D. Tripathi, V. Yadav and S. Sinha. 2001. Chromium induced physiological changes in *Vallisneria spiralis* L. and its role in phytoremediation of tannery effluent. *Bulletin of Environmental Contamination Toxicology*, 67: 246-56.

Van Assche, F. and H. Clijsters. 1983. Multiple effects of heavy metals on photosynthesis. In Marcelle R, editor. Effects of stress on photosynthesis. *The Hague Nijhoff/Junk.*, 371-382.

Vernay, P., C. Gauthier-Moussard, L. Jean, F.O. Bordas, F. Faure, G. Ledoigt and A. Hitmi. 2008. Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia*. *Chemosphere*, 72: 763-771.

Wettstein, D. 1957. Chlorophyll-letale und der Submikroskopische Formwechsel der Plastiden. *Experimental Cell Research*, 12: 427-506.

(Received for publication 24 December 2009)