

EVALUATION OF DRASTIC EFFECTS OF CHROMIUM STRESS ON *BRASSICA CAMPESTRIS* L.

***Raja Usman Sharif, Khizar Hayat Bhatti*, Khalid Nawaz, Khalid Hussain, Ejaz Hussain
Siddiqi and Sehrish Anwar***

Department of Botany, Hafiz Hayat campus, University of Gujrat-Gujrat-50700, Pakistan

**Corresponding author's email: khizar.hayat@uog.edu.pk*

Received Date: 12-04-2022; Accepted Date: 02-06-2022; Published Date: 30-06-2022

ABSTRACT

Heavy metals including Chromium (Cr) are important source of pollution in water and soil. These heavy metals negatively affect the growth and other parameters of plants. The main objective of current study was to evaluate the effect Cr on rapeseed. For the said purpose, pot experiments were performed using completely randomized design (CRD). Therefore, two Brassica varieties (*Brassica olearaceae* var. *capitata* and *Brassica olearaceae* var. *botritis*) were used in the experiment to study its effects. When the seedlings were 21 days old, Cr treatments were applied as chromium oxide at conc. ($T_0=0$, $T_1=40$, $T_2=60$) pp. There were 5 replicates for each treatment of both the varieties. The treatments of Cr negatively affected the morphological, biochemical, physiological parameters in rapeseed plants. So, Cr is a toxic heavy metal which has detrimental effects on Brassica plant.

INTRODUCTION

Brassicaceae is a large family with approximately 380 genera and about 3350 species. It has a cosmopolitan distribution. It is especially found in temperate regions of the north hemisphere (Hedge *et al.*, 1976).

Rapeseed (*Brassica campestris* L.) commonly known as mustard. It is a cool season crop. It is also a thermo sensitive as well as photosensitive crop (Ghosh and Chatterjee, 1988).

There is a great scope of increasing yield of mustard by selecting high yielding varieties and improving management practices. Time of sowing is very important for rapeseed mustard production (Rahman *et al.*, 1998). It is economically most important genus in this family (Gomez-Campo, 1980).

Heavy metals make heterogeneous group of elements. These heterogeneous groups of elements largely differ in their chemical properties. These elements also differ in biological functions (Holleman and Wiberg., 1985). Heavy metal comes under environmental pollutant due to their toxic effects in plants animals. Some of these heavy metals i.e. chromium, arsenic, cadmium, lead, are cumulative poison. These heavy metals accumulate in the living organism and not metabolized in intermediate compounds. These heavy metals do not easily breakdown in environment. Some heavy metals such as Cd, Ni, As, Pb cause a number of dangerous effects to humans and many other animals, also in plants.

Chromium is a blue- gray metal that can be plated to get a high shine. It is also very brittle. Cr is a fairly active metal. While it does not react with water. It can react with most acids. It reacts with oxygen at room temperature to form Cr (III) oxide. Its melting point is 2180 K and boiling point 2944 K. Its specific heat capacity 23.35 J mol⁻¹K⁻¹. Cr forms over 20 different isotopes. Three of them are considered stable while nineteen are considered to be radioactive, and many have half lives shorter than 24 hours. Cr is highly toxic metal. It is non-essential element for microorganism and plants (Cervantes *et al.*, 2001).

The source of Cr in environment are natural and anthropogenic source. Natural source include burning of oil and coal, petroleum gas, from Ferro chromate refractory material, Cr steels, pigments oxidants, catalyst and fertilizers. Cr is also used in metal plating tannin industries and oil well drilling (Abbasi *et al.*, 2003). Fertilizers are also the prominent source of Cr. Cr is not suggested to be essential for plant growth and development. Some evidences have indicated that Cr stimulates plant growth at low concentrations (1µM) (Bonet *et al.*, 1991). Cr is a naturally occurring element. It found in rocks, plants, soil, animals and in volcanic dust and gases. Cr also found in the environment in several different forms with more common forms as Cr (III) and chromium (VI). Population increases is the major cause of the increased environmental pollution. The major factors which are responsible for environmental pollution and other type of environmental degradation in any area are effluents and technology (Meadows *et al.*, 1992). It has been found that *Brassica juncea* is an important accumulator plant for Cr in soils. Cd, Ni, Zn and Cu are also accumulated by *Brassica juncea* (Kumar *et al.*, 1995). Cr greatly affects the plants growth and development. Excess amount of cobalt also cause the toxic effect to the plants. Cr and copper also had an adverse effect on biomass, concentration of iron,

chlorophyll “a” and “b” protein and catalase activity in cauliflower (Chatterjee and Chatterjee, 2000).

Excess amount of Cr may have negatively affected the translocation of iron in the leaf of brassica plants. Earlier also several workers have reported inhibition of chlorophyll biosynthesis by metal in higher plants (Baszinsky and Prasad, 1980).

The amount of chlorophyll was reduced in *Brassica oleracea* var. *botrytis*. The presence of toxic compounds, such as heavy metals, is one important factor that can cause damage to plants by altering major plant physiological and metabolic processes (Chatterjee and Chatterjee., 2000).

Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma *et al.*, 1995). There are many studies on Cr toxicity in crop plants. Chromium significantly affects the metabolism of plants such as Citrullus (Dube *et al.*, 2003), cauliflower (Chatterjee and Chatterjee., 2000), vegetable crops (Zayed and terry., 2003), wheat (Sharma *et al.*, 1997) and maize (Sharma and Pant, 1997).

Chromium has its effect on certain enzymes such as Iron containing enzymes. It has been reported that Cr stimulates the catalase activity in barley (Agarwala and Kumar., 1962) Excessive toxicity of Cr was found with respect to photosynthetic pigment, photosynthesis, nitrate reductase activity and protein content of some alga (Rai *et al.*, 1992). The direct interaction of metal with cellular components can initiate variety of metabolic responses finally leading to a shift in the development of the plant (Assche *et al.*, 1993). Cr toxicity produces chlorosis and necrosis in plants (Cervantes *et al.*, 2001).

The contamination of soil and water by chromium (Cr) is of great concern(Azmat and Khanum., 2005). Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition’s stress is one of the imperative factors that influence photosynthesis and respiration in plants (Assche *et al.*, 1993). Plants that growing in contaminated environment can get heavy metals at higher concentration that causes the hazard to

human health. Moreover, heavy metals are dangerous because they have the ability to accumulate in living systems and in food chain and causing injurious effects (Alloway, 1990). Similar effects of cadmium and lead on plants have been reported elsewhere (Akinola *et al.*, 2006). The aims of research was to study the effect of chromium on the *Brassica oleraceae* var. *capitata* and *Brassica oleraceae* var. *botrytis*.

MATERIALS AND METHODS

Two Brassica varieties (*Brassica oleraceae* var. *capitata* and *Brassica oleraceae* var. *botrytis*) were used in the experiment to study the effects of Cr and role of foliar application of GB. Experiment was done in Botany lab of University of Gujrat, Hafiz Hayat Campus, Gujrat-Pakistan. Seeds of each variety were sown in sand in plastic pots.

15 pots for each variety were used. Irrigation was done immediately after sowing seeds. After 7 days of sowing Hoagland solution was given for better germination. Hoagland solution contain many essential nutrients require for the plants for better germination. After 21 days of old seedling, Cr treatment was given in the form of chromium oxide $T_0=0$ ppm, $T_1=40$ ppm and $T_2=60$ ppm. There were 5 replicates for each treatment of both varieties. After 15 days of treatment. Following parameters were measured.

Determination of growth parameters

Root /shoot lengths were measured. Root and shoot fresh weights were also measured with help of balance. Then, they were oven-dried for one week and their dry weights were recorded. Numbers of leaves per plant were also counted.

Determination of chlorophyll contents

Chlorophyll "a", chlorophyll "b", and carotenoid were determined according to Arnon method (1949) 0.5g fresh weight of leaf was taken. It was grinded with 2ml alcohol, then 5 ml more alcohol was added and after keeping overnight in test tubes, OD (optical density) was measured with the help of spectrophotometer.

Chlorophyll 'a', Chlorophyll "b" and Carotenoid were measured by following formulas:

Chlorophyll "a": $12.7 \times OD(663) - 2.69 \times OD(645)$ mM

Chlorophyll “b”: $22.9 \times \text{OD} (645) - 4.68 \times \text{OD} (663)$ mM

Carotenoid: $\text{OD} (480) + 0.114 \times \text{OD} (663) - (0.638) \times \text{OD} (645)$ mM

Determination of Na⁺ and K⁺

Samples were taken after digestion and got the reading from flame photometer (Jenway). Standard solution with different grades were made in testing nutrients (Na⁺, K⁺). Grades were made e.g. 50, 100, 150, 200, 250 ppm. Reading of standards from flame photometer was taken. Graph of standards was made by taking reading on x-axis and solution concentration on y-axis. The sample reading was multiplied with correction factor. After multiplying with CF and sample reading, value on graph paper were read and got the reading of desired nutrients

Statistical analysis:

Statistical analyses were carried out by analysis of variance (ANOVA) and Microsoft office 2003.

RESULTS

Heavy metal cause toxic effects on plants. Cr is a heavy metal which has detrimental effects on plant growth. A pot experiment was done to observe that how foliar application of GB compensate the adverse effects of Cr metal on Brassica varieties.

Shoot length (cm)

Data showed that Cr negatively affects the shoot length of both Brassica varieties at both treatments (40 and 60 ppm) as compared to control. As, the Cr concentration increases the shoot length decreases. Cr at 60 ppm decreased significantly ($p=0.001$) the shoot length in both varieties but it affected relatively more V1 (Fig. 1).

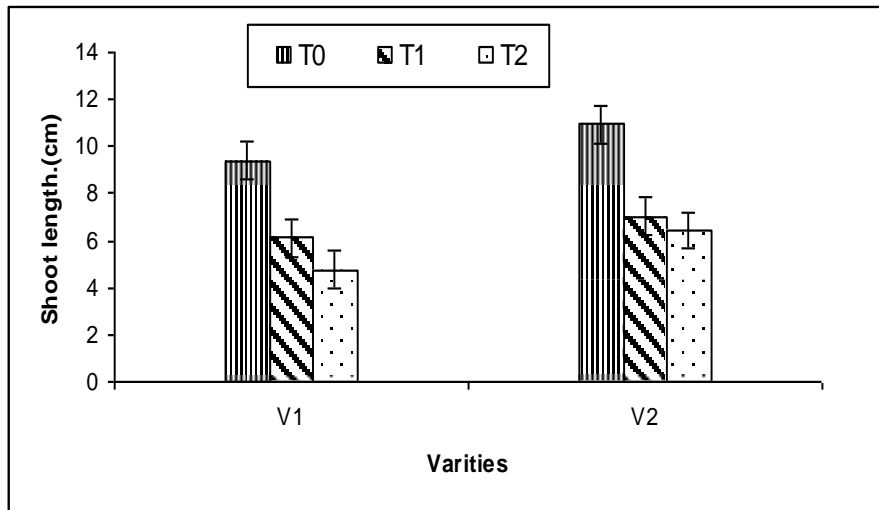


Fig.1 Shoot length of Brassica varieties under Cr stress when plants were 37 days old.

Where, T0=0 ppm, T1=40 and T2=60 ppm.

Root length (cm)

Results showed negative effect of chromium on two Brassica varieties is highly significant ($p=0.001$). And the interaction between chromium and varieties is non significant. As the concentration of chromium increases in Brassica varieties, root length decrease (Fig .2). But varieties comparison shows that V2 variety was less effective than V1.

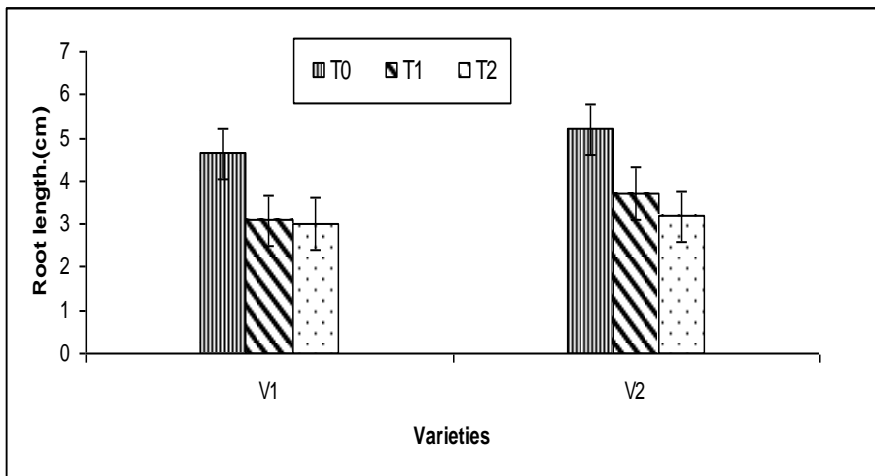


Fig.2 Root length of two Brassica varieties under Cr stress when plants were 37 days old.

Where, (T0=0, T1=40 and T2=60) ppm.

Number of leaves

Results showed that effect of Cr on number of leaves in both varieties is non significant (ns). V2 has relatively more number of leaves as compared to V1. Cr decreases the number of leaves in both varieties (Fig. 3).

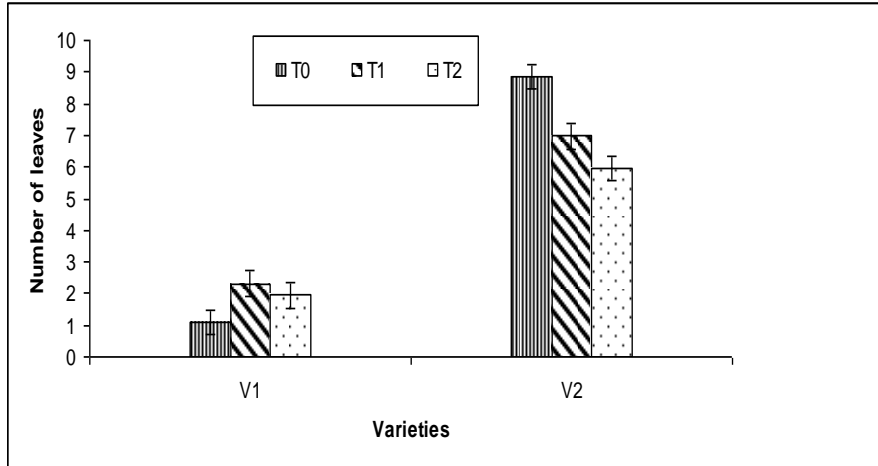


Fig. 3 Number of leaves of Brassica varieties under Cr stress when plants were 38 days old.

Where, (T0=0, T1=40 and T2=60ppm)

Chlorophyll “a” contents

Negative effect of Cr in chl “a” contents is highly significantly (0.001). It also shows that the interaction between Cr and variety is non-significant. As the chromium concentration increase chlorophyll “a” contents decrease in both varieties (Fig. 4).

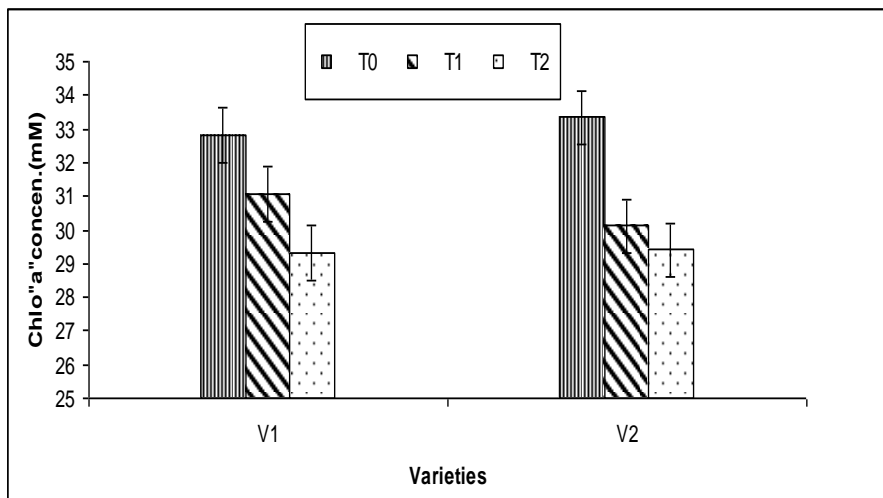


Fig.4 Chl ‘a’ contents of Brassica varieties under Cr stress when plants were 38 days old
 Where (T0=0, T1=40 and T2=60) ppm.

Chlorophyll “b” contents

Results showed that effect of Cr is highly significant on chl ‘b’ in both varieties of Brassica. The interaction of Cr between two varieties is non significant. The Cr concentration decreases the chl ‘b’ content in both cultivars (Fig. 5).

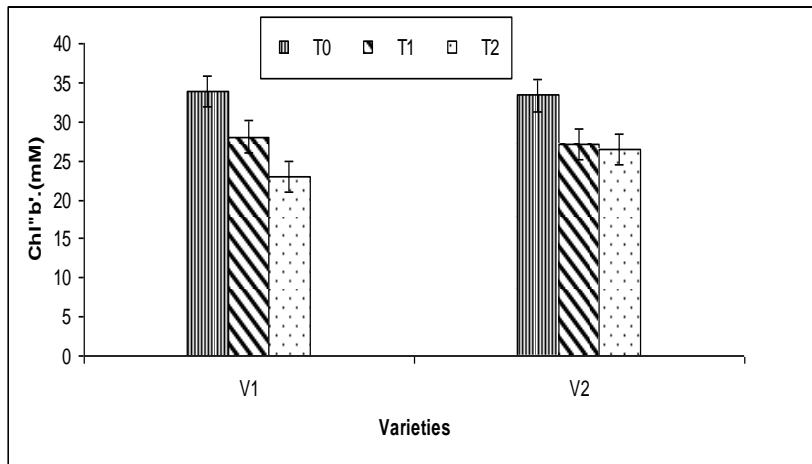


Fig.5 Chl b contents under Cr stress in old Brassica varieties when plants were 38 dayold.

Where, (T0=0, T1=40 and T2=60)ppm.

Carotene contents

Effect of Cr on Brassica varieties is highly significant. The interaction between Cr and Brassica varieties is highly significant. The effect of chromium on *Brassica olearaceae* var. *capitata* and *Brassica olearaceae* var. *botrytis* is highly significant.

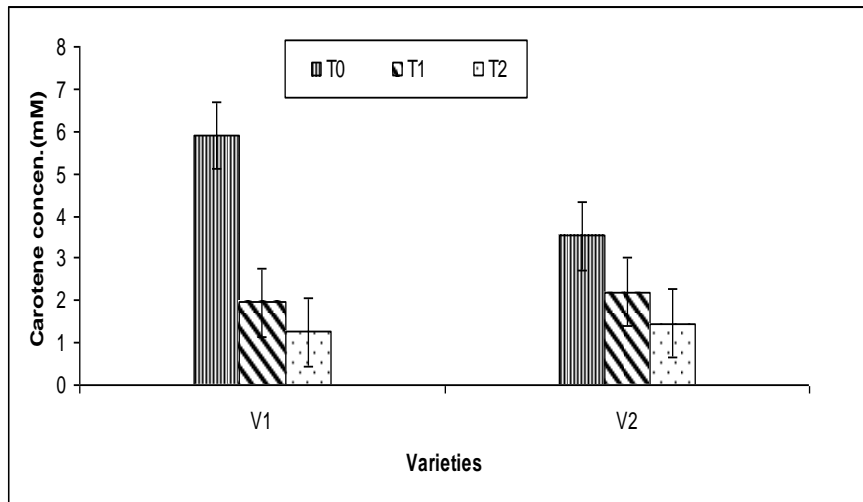


Fig. 6 Carotene contents under Cr stress in both varieties of Brassica when plants were 38 days old. Where (T₁=40 and T₂=60)ppm.

Root fresh weight

Effect of Cr on root fresh weight of Brassica varieties is highly significant (p=0.001). Cr concentration decreases the root fresh weight in Brassica varieties (Fig. 7).

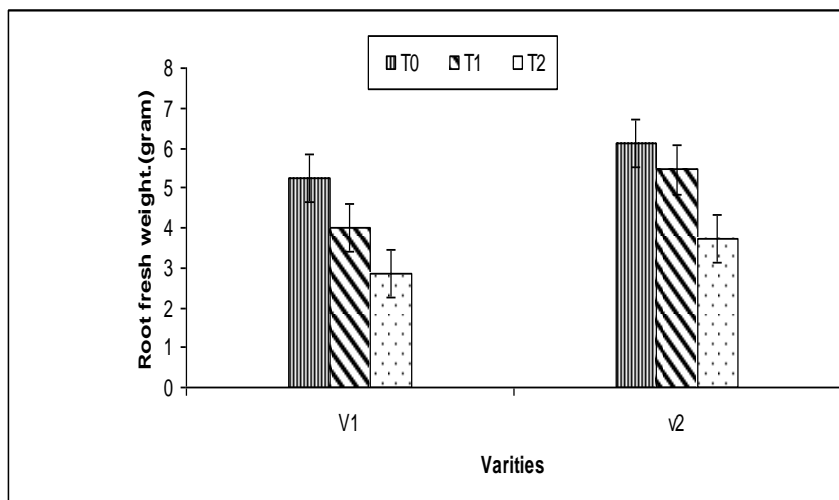


Fig. 7. Root fresh weight under Cr stress chromium when plants were 37 days old.

V1=*Brassica olearaceae* var. *capitata*, V2=*Brassica olearaceae* var. *botrytis*

Where, (T₀=0, T₁=40 and T₂=60) ppm.

Root dry weight

Effect of Cr on root dry weight of Brassica varieties is highly significant ($p=0.001$). Cr causes the reduction in root dry weight (Fig. 8).

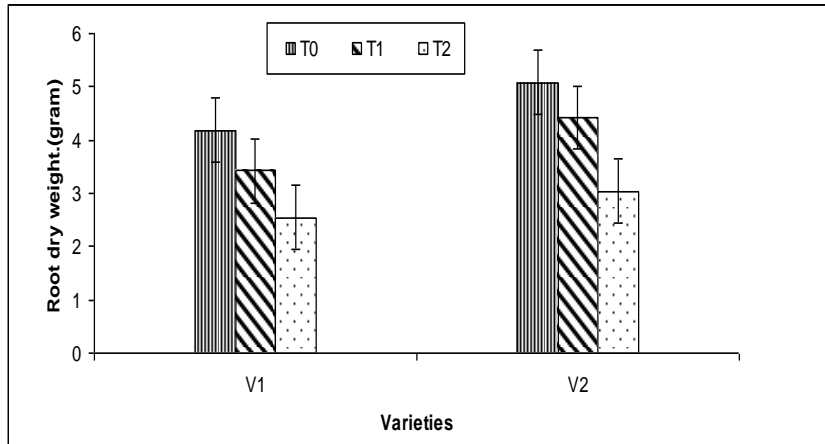


Fig. 8. Root dry weight under Cr stress in two Brassica cultivars when plants were 39 days old. V1=*Brassica olearaceae var. capitata* V2=*Brassica olearaceae var. botrytis*.

Where (T0=0, T1=40 and T2=60)ppm.

Shoot fresh weight

Results showed that Cr decreases the shoot fresh weight significantly. There is no significant variation in both varieties regarding shoot fresh weight.

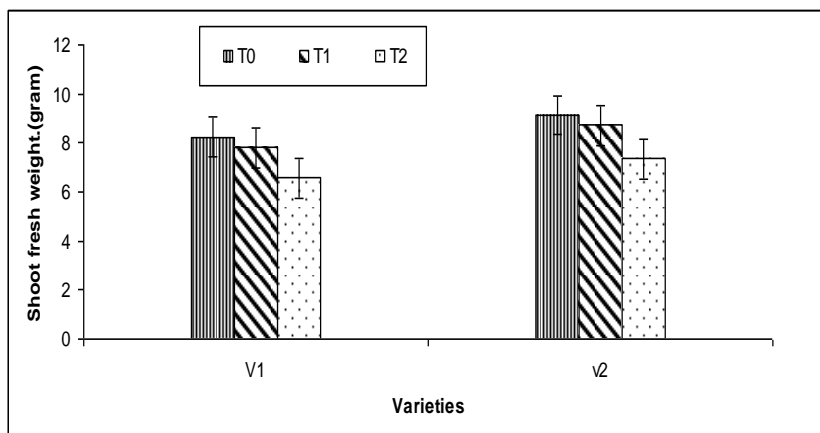


Fig. 9 shoot fresh weight of Brassica varieties when plants were 39 days old after exogenous application of GBV1=*Brassica olearaceae var. capitata* V2=*Brassica olearaceae var. botrytis*.

Where, T1=0mM, T2=50mM, and T2=100mM.

Shoot dry weight

Cr stress decreases the shoot dry weight least significantly ($p=0.05$). There is no significant variation in both varieties regarding shoot dry weight.

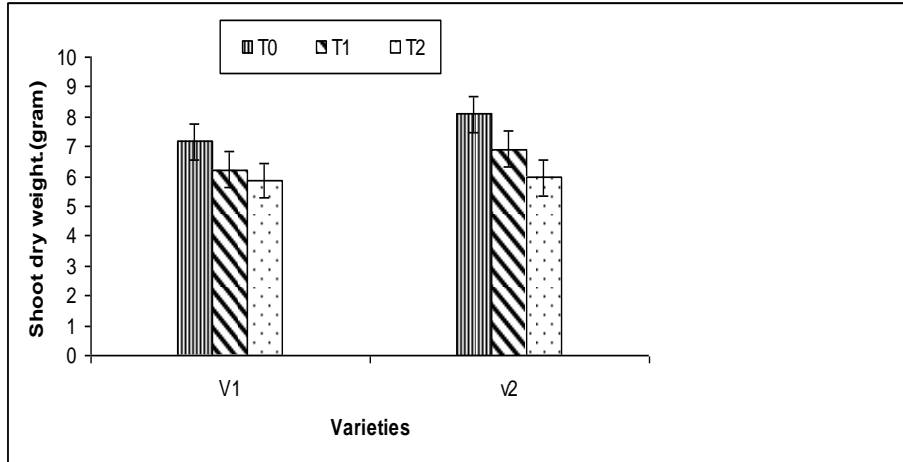


Fig. 10 Shoot dry weight of two Brassica varieties under Chromium stress show the effect of Chromium in concentration when plants were 49 days old with GB application.

Where (T0=0, T1=40, T2=60) ppm, GB= (T0=0, T1=50, T2=100)mM.

K⁺ uptake in Root

Cr reduces the K⁺ ion uptake significantly ($p=0.001$). Figure 11 showed that as chromium concentration increase in Brassica varieties K⁺ ion uptake decrease.

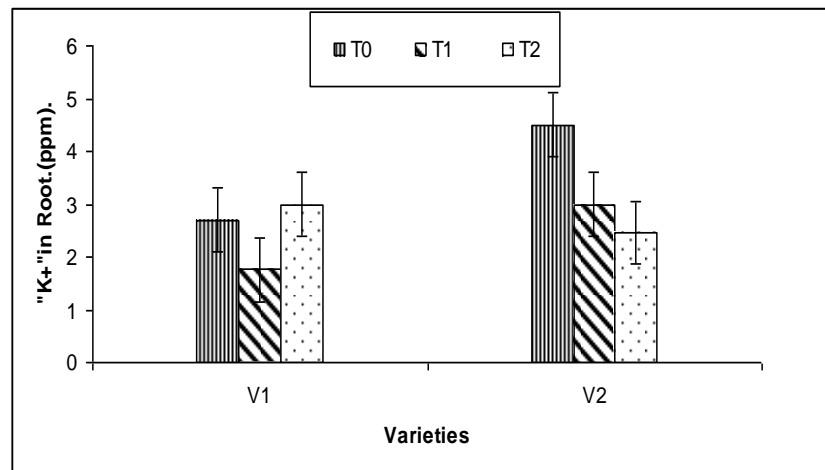


Fig. 11 K⁺ ion contents in root when plants were 37 days old under chromium stress.

V1=*Brassica olearaceae* var. *capitata*, V2=*Brassica olearaceae* var. *botrytis*.

Where, (T1=0 T2=40 and T2=60)ppm.

K⁺ uptake in shoot

Results showed that Cr decreases the K⁺ ion contents in shoot significantly (p=0.001) at both treatments (40ppm, 60ppm).

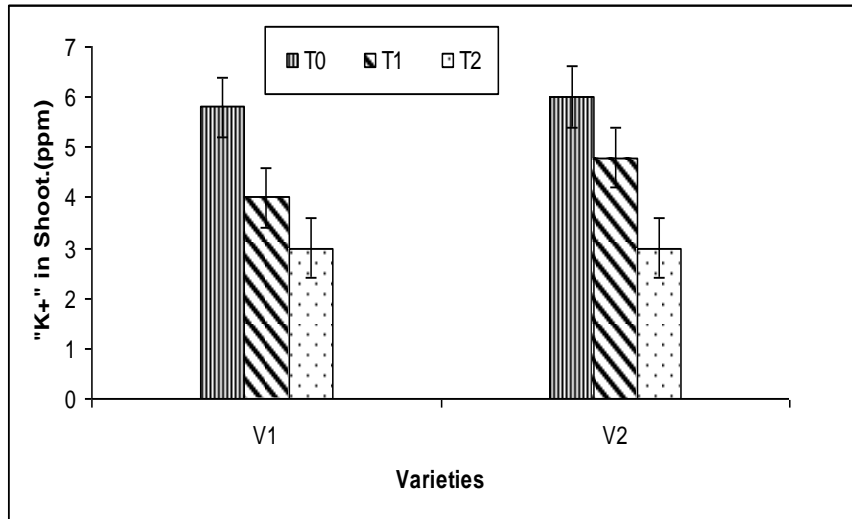


Fig. 12 K⁺ ion contents in shoot when plants were 43 days old under chromium stress
V1=Brassica olearaceae var. capitata, V2=Brassica olearaceae var. botrytis; Where (T1=0
T2=40 and T2=60) ppm.

Na⁺ uptake in root:

Cr stress decreases the Na⁺ contents in root significantly at both treatments 40 and 60 ppm, but 60 ppm has more reducing effect on Na⁺ contents. V2 has more Na⁺ ion as compared to V1 (fig 13).

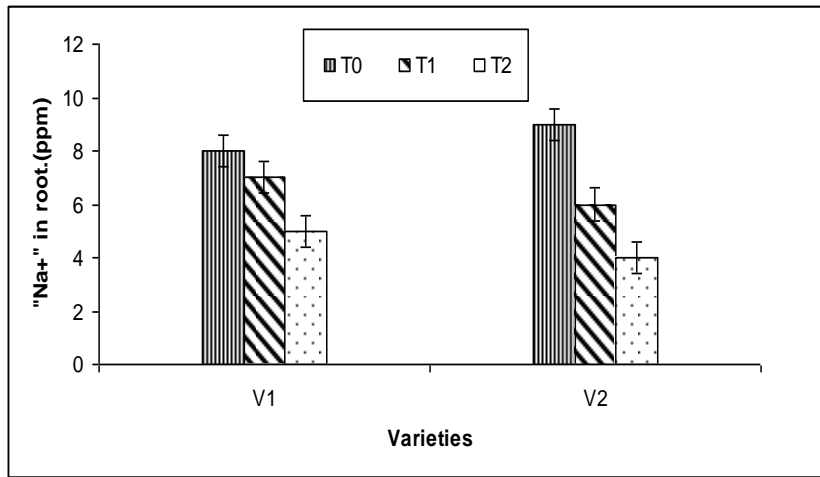


Fig. 13. Na⁺ contents in root by the effect of Chromium when plants were 43 days old in two varieties of Brassica (T0=0ppm, T1=40ppm, T2=60ppm).

Na⁺ uptake in shoot

Cr stress decreases the Na ion contents in shoot significantly ($p=0.001$) at both treatment 40 and 60 ppm as compared to control however 60 ppm solution has more reduced the Na ion contents (Fig. 14).

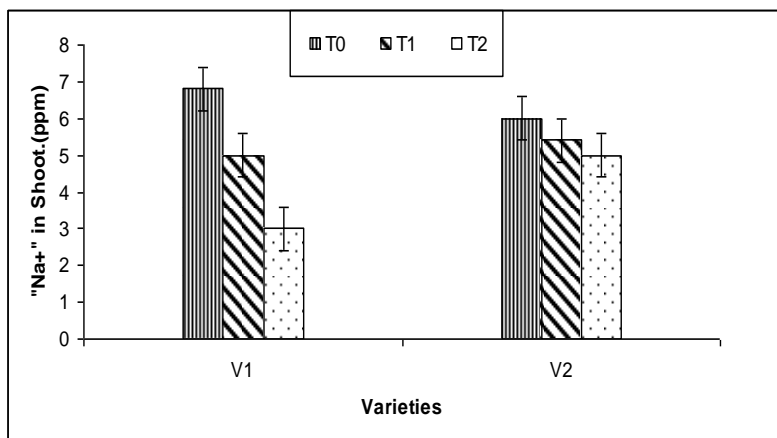


Fig 14 Na⁺ contents in shoot under chromium stress when plants were 43 days old

Where (T0=0 ppm, T1=40Ppm T2=60Ppm).

DISCUSSION

According to my result, Cr treatment on *Brassica olearaceae* var. *capitata* and *Brassica olearaceae* var. *botrytis* caused stunted growth of both varieties due to Cr toxicity. It was observed that number of leaves decrease in both varieties. Result of my experiment coincides with the Singh's result in which he studied the effect of Cr (III) and Cr (VI) on spinach.

In my pot experiment, when shoot length was measured then it was observed that 40 and 60 ppm treatment of Cr in both varieties cause the reduction of shoot length as compared to control plants. According to my result, Cr greatly influences the root length and root weight. Our result matches with Vernay *et al.*, (2009). He studied the effect of chromium on *D. innoxia* plants (Vernay *et al.*, 2009). Our data suggest that Cr caused the reduction in chl "a", chl "b" and carotene pigments. As the Cr concentration increase in both varieties, chlorophyll "a" contents decrease, ultimately leads to the yellowing of leaves. Same effect was seen with chlorophyll "b" and carotene. Data regarding to the reduction of chlorophyll 'a', chlorophyll 'b' and carotene has been shown in my results. My result coincides with the result of Chatterjee. He performed his experiment on Cauliflower (Chatterjee and Chatterjee., 2000). Na⁺ and K⁺ contents in root and shoot also decrease by increasing the concentration of chromium. This decrease is due to the reduction in root length, root weight. My results match with the previous study of Moral. Previous study showed that increasing chromium concentrations from 10- 40 ppm in the plants decreased the N, P, K, Na, Ca and Fe contents of *Brassica juncea* shoot system (Sharma *et al.*, 1995).

CONCLUSION

On the basis of our data, it is concluded that chromium is a potent toxic heavy metal release from leather industry that pollute soil and water. The Cr presence in the environment causes reduction in growth and development of plants. The treatments of Cr negatively affected the morphological, biochemical, physiological parameters in rapeseed plants.

REFERENCES

- Abbassi, Pilay and Dube. 2003. Effect of Chromium. *J. Physiol.*, 41: 55-157.
- Agarwala, S.C and A. Kumar. 1962, The effect of heavy metals and bicarbonate excess on sun flower plants grown in sand culture with special reference to catalase peroxidase. *J. Ind. Bot. Soc.*, 41: 72-77.

- Akinola, Schonherr and T.A. Ekiyoyo. 2006. Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Ribila at Odo-nla area of Ikorodu, Lagos state, Nigeria. *J. Environ. Biol.*, 27(3): 597-599.
- Alloway. 1990. Heavy Metal in Soils. *Ind. J. of Soil Sci.*, 54: 311-339.
- Assche, F.V. H. Clijsters and Medows. 1990-1993, Effects of metals on enzyme activity in plants. *PlantCell Environ.*, 13: 195-206.
- Assche, F.V. H. Clijsters and Medows. 1990-1993, Effects of metals on enzyme activity in plants. *Plant Cell Environ.*, 13: 195-206.
- Azmat, R and R. Khanum. 2005. Effect of Chromium on uptakes of minerals in Bean plant. *Pak. J. Biol. Sci.*, 8(2): 281-283.
- Baszinsky, Prasad. 1980. Heavy metal toxicity. *J. Plant Physiol.*, 5: 123-127.
- Bonet, A., C. Poschenrieder and J. Barcelo. 1991. Chromium III-iron interaction in Fe-deficient and Fe-sufficient bean plants. I. Growth and nutrient content. *J. Plant Nutr.*, 14: 403-414.
- Cervantes, C. C., Garcia, Diaz-Zorita J. Debars, S.G. Corona, F. L.Tavera, H.C.T. Guzman and R. M.Sanchez. 2001. Interaction of chromium with microgenesis and plants. *FEMS Microbiol. Rev.*, 25: 335-347.
- Cervantes, C. C., Garcia, Diaz-Zorita J. Debars, S.G. Corona, F. L.Tavera, H.C.T. Guzman and R. M.Sanchez. 2001. Interaction of chromium with microgenesis and plants. *FEMS Microbiol. Rev.*, 25: 335-347.
- Chatterjee, J. and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium and copper in Cauliflower. *J. Environ. Pollut.*, 109:69-74.
- Chatterjee, J. and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium and copper in Cauliflower. *J. Environ. Pollut.*, 109:69-74.
- Chatterjee, J. and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium and copper in Cauliflower. *J. Environ. Pollut.*, 109:69-74.
- Chatterjee, J. and C. Chatterjee. 2000. Phytotoxicity of cobalt, chromium and copper in Cauliflower. *J. Environ. Pollut.*, 109:69-74.
- Dube, B.K., K. Tewari, J. Chatterjee, and C. Chaterejee. 2003. Excess chromium alters uptake and translocation of certain nutrients in citrullus. *Chemosphere*, 53: 1147-1153.
- Ghosh, R. K. and Chatterjee. 1988. Effect of dates of sowing on oil content and fatty acid profiles of Indian mustard. *Indian J. Oilseed Res.*, 5(2):144-149.



- Gomez-Campo, 1980. *J. Biotechnology in Agriculture and Forestry*, 54: 553-597.
- Hedge, J.G., A.J. MacLeod and Jones .1976. A systematic and geographical survey of the word Cruciferae. *J. Biol.*, 3: 1-45.
- Holleman, and Wiberg. 1985. *Lehebuchdu Anoranischen chemie*. Water de Gruyter, Berlin, pp-868.
- Kumar, P. B., V. Dushenkov, H. Motto and I. Raskin.1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ. Sci. Technol.*, 29: 1232-1238.
- Meadows, D.H., D. L. Meadows and Randers. 1992. Beyond the limits Earthscan publication, London, Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of *B. juncea* L. *Indi. J. Biol.*, (3)144-178.
- Rahman, M., M. Salam, M. Miah and M. S. Islam. 1988. Effect of sowing time on the performance of mustard (SS-75). *Bangladesh J. Agric. Res.*, 13(1): 47-51.
- Rai, U.N., R. D. Tripathi and N. Kumar. 1992. Bioaccumulation of chromium and toxicity on growth, photosynthetic pigments, photosynthesis *Chromosphere. Int. J. Biochem.*, 25:721-732.
- Sharma, D. C, C. Chatterjee, Moral and C. P. Sharma.1995. Chromium accumulation by barley seedlings (*Hordeum vulgare* L.). *J. Exp. Bot.*, 25: 241-251.
- Sharma, D. C, C. Chatterjee, Moral and C. P. Sharma.1995. Chromium accumulation by barley seedlings (*Hordeum vulgare* L.). *J. Exp. Bot.*, 25: 241-251.
- Sharma, D. C, C. Chatterjee, Moral and C. P. Sharma.1997. Chromium accumulation by barley seedlings (*Hordeum vulgare* L.). *J. Exp. Bot.*, 25: 241-251.
- Sharma, D.C. and R.C. Pant .1994. Chromium uptake its effects on certain plant nutrients in maize (*Zea mays* L. cv Ganga 5). *J. Environ. Sci. Health.*, 29: 941-948.
- Vernay, M., Ashraf and M. Shahbaz. 2009. *Pak. J. Bot.*, 41(3): 1291-1302.
- Zayed, A.M and N. Terry. 2003. Chromium in the environment: factors affecting biological remediation. *J. Pl. Soil*, 249: 139-156.