Forage Res., **42** (1) : pp. 30-35 (2016) **http://forageresearch.in**

CHROMIUM TOXICITY AFFECTS ANTIOXIDANT ENZYME ACTIVITY IN *SORGHUM BICOLOR* (L.)

SWEETY SIHAG*, NEHA WADHWA AND U. N. JOSHI

Department of Chemistry & Biochemistry
CCS Haryana Agricultural University,
Hisar-125 004 (Haryana), India
**(e-mail : sweetypoonia2011@gmail.com)*

(Received : 10 June 2016; Accepted : 27 June 2016)

SUMMARY

A pot experiment was conducted to determine the effects of varying Cr (VI) levels [0.0-4.0 mg Cr (VI)/kg soil in the form of potassium dichromate] on the activity of antioxidant enzymes and their metabolites in sorghum. The present investigation showed that the enzyme activity of peroxidase, ascorbate peroxidase and their metabolites in leaves and stem of the plant at different growth stages i. e. 35, 70 and 90 days after sowing (DAS) were adversely affected with an increase in Cr (VI) levels from 0.0 to 4.0 mg Cr (VI)/kg soil. A significant decrease in the peroxidase activity and increase in ascorbate peroxidase activity was observed with increase in chromium concentration in the soil. The increase in activity of peroxidase consumed hydrogen peroxide and thus it resulted in decrease of hydrogen peroxide content, while due to decrease in activity of ascorbate peroxidase, ascorbate content get increased by Cr (VI) application.

Key words : Antioxidant enzymes, chromium, metal toxicity, *Sorghum bicolor*

Metals are necessary components of all ecosystems and occur naturally in the earth's crust. They appear in a wide range of oxidative states influencing their chemical characteristics and thus their bioavailability and toxicity (Pinto *et al.*, 2003). Certain metals such as iron (Fe), copper (Cu) and zinc (Zn) are considered as essential nutrients to plants and are needed for photosynthesis and as cofactors for many enzymes (Kovacik *et al.*, 2010; Shanmugam *et al.*, 2011). Chromium exists in several oxidation states and the impact of its contamination on the physiology of plants depends on the metal speciation, which is responsible for its mobilization, subsequent uptake and resultant toxicity in the plant system (Shanker *et al.*, 2005). The most stable and common forms of chromium are Cr (III) and Cr (VI) species. Cr toxicity in plants depends on its valence state. Cr (VI) being highly mobile is toxic, while Cr (III) is less mobile and is less toxic (Oliveira, 2012). Chromium is found in all phases of the environment, including air, water and soil. Naturally occurring in soil, chromium ranges from 10 to 50 mg/kg depending on the parental material. Chromium (VI) is a strong oxidant with a high redox potential in the range of 1.33-1.38 eV accounting for a rapid and high generation of reactive oxygen species (ROS) and resultant toxicity (Shanker *et al.*, 2004). Chromium toxicity in plants is observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis. There are many studies on Cr toxicity in crop plants. Chromium significantly affects the metabolism of plants such as barley (*Hordeum vulgare*) (Ali *et al*., 2004) and sorghum.

Sorghum [*Sorghum bicolor* (L.) Moench] is the world's fifth most important cereal crop, after rice, corn, wheat and barley; and the third leading crop in USA. Sorghum is cultivated for food, feed, fodder and the production of alcoholic beverage, but extensively grown for fodder in north India during **kharif** season due to its greater adaptability, high fodder yield, better palatability, quality and digestibility.

MATERIALS AND METHODS

Soil

A nutrient deficient loamy sand soil from Regional Research Station, Gangwa block of Hisar district was used in the present study. Its characteristics were : pH (1:2) 8.50, organic carbon 0.22 per cent; EC 1.5; N 4.0 mg/kg soil; P 13.0 mg/kg soil; K 163 mg/kg

soil; Zn^{2+} 0.61 mg/kg soil; Fe^{2+} 0.9 mg/kg soil; Cu^{2+} 0.18 mg/kg soil; Mn^{2+} 3.65 mg/kg soil; and Cr^{2+} 0.01 mg/kg soil.

Plant Growth Conditions

Seeds of *Sorghum bicolor* (cv. HJS-541) were procured from Forage Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar and raised in earthen pots filled with 5 kg of chromium free sandy loam soil in a naturally lit net house. The soil in each pot was treated with different levels of chromium (VI) (0.0, 1.0, 2.0 and 4.0 mg Cr (VI)/kg soil) in the form of potassium dichromate. At weekly intervals, the plants were supplied with equal quantities (250 ml) of nutrient solution. The plant samples from each treatment were collected at different stages viz., vegetative (35 DAS), flowering (70-75 DAS), and grain filling (90-100 DAS) stages. The plants were treated with following Cr (VI) concentrations :

T_1 = Control (No treatment)
T_2 = 1.0 mg Cr (VI)/kg soil
T_3 = 2.0 mg Cr (VI)/kg soil
T_4 = 4.0 mg Cr (VI)/kg soil

Enzyme Assays

One gram of plant tissue was hand homogenized in 10 ml 0.1 M phosphate buffer (pH 7.0) in a previously chilled mortar using glass beads as abrasive. The homogenate, thus, obtained, was then centrifuged at 10,000 rpm for 20 min in a refrigerated centrifuge at 0-4°C. The supernatant thus obtained was referred as crude extract and stored in a refrigerator for enzyme assays and total soluble protein estimation. The crude extract was used on the same day for enzyme assay.

Peroxidase (POX) Activity (E. C. 1.11.1.7)

Peroxidase was assayed according to the method of Shannon *et al.* (1966). The enzyme specific activity was expressed in units and one unit = change in 0.01 absorbance/min/mg protein.

Ascorbate Peroxidase (APX) Activity (E. C. 1.11.1.11)

Ascorbate peroxidase was assayed by the method of Nakano and Asada (1981). One enzyme unit was expressed as amount of enzyme required to oxidize one nmol of ascorbate/min/ml.

Metabolites of Antioxidant Enzyme

The amount of hydrogen peroxide was estimated by the method of Sinha (1972), and the content of ascorbic acid was estimated with slight modifications of the method described by Roe (1964).

Soluble Protein

Soluble protein in enzyme extracts was precipitated with 20 per cent trichloroacetic acid and determined by the method of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

Metal stress leads to generation of highly reactive, ROS species, and if not scavenged, they cause oxidative damage to biomolecules (Apel and Hirt, 2004). Thus, plants have evolved a well-organized and intricate antioxidant mechanism to scavenge excess ROS and protect cells from oxidative damage (Mittler, 2002). In addition to plant defense mechanism, certain essential ions particularly Fe may also affect uptake and toxicity of heavy metals in plants and algae (Volland *et al.*, 2014). It has been reported that the toxic property of Cr (VI) originates from the formation of ROS i. e. superoxide radical, hydrogen peroxide and hydroxyl radical, and in higher concentrations, these ROS produce cytotoxic effects due to their ability to oxidize lipids, proteins and nucleic acids (Shanker *et al.*, 2004; Pandey *et al.*, 2009). The defense mechanism consisted of various antioxidant enzymes such as peroxidase (POX) and ascorbate peroxidase (APX); and non-enzymatic antioxidants such as hydrogen peroxide and ascorbic acid (Apel and Hirt, 2004).

Effect of Cr (VI) on Peroxidase Activity (T_1, T_2, T_3 and T_4)

Peroxidase (POX) activity increased with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI)/kg soil in leaves and stem of sorghum plants at all the stages of growth. Maximum increase in activity was observed in 4.0 mg Cr (VI)/kg soil treated plants.

The POX activity was observed to be maximum at 70 DAS, after which it declined at 90 DAS. At 70 DAS, the POX activity increased from 3.12 to 3.98 units in leaves (Fig. 1) and 3.16 to 4.37 units in stem (Fig. 1) with the increase in concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI)/kg soil. The POX activity was generally highest in stem followed by leaves except at 90 DAS. Sen *et al.* (1994) also observed an increase in peroxidase activity at concentrations above 10 mg/l Cr (VI), whereas the enzyme activities were least affected at lower concentration of Cr (VI). Sinha *et al*. (2005) also observed increase in peroxidase activity by application of Cr (VI) in spinach leaves. The increase in antioxidant enzyme activity observed might have been in direct response to the generation of superoxide radical by Cr induced blockage of the electron transport chain in the mitochondria. The increase in the activity as the concentration of the external Cr increased might be due to the stimulatory effect of Cr ions on the enzyme itself (Shanker *et al.*, 2005).

The stimulation in the activity of peroxidase in excess Cr conditions might result either from peroxidative damages of the thylakoid membrane or lower auxin and protein content or high phenols in tissue inhibiting the growth of plant. Similar results were reported in chromium treated radish (Jayakumar *et al.*, 2007). Conversely, Bhattacharjee (1998) reported a gradual decline in activity of peroxidase in cadmium and lead treated *Amaranthus* over untreated control.

Effect of Cr (VI) on Ascorbate Peroxidase Activity (T_1, T_2, T_3 and T_4)

In the present study, a gradual but significant reduction in activity of ascorbate peroxidase was observed in different plant parts of sorghum with increasing concentration of Cr (VI), which is in agreement to the work conducted by Subrahmanyam (2008) that indicates the susceptibility of this enzyme to Cr (VI) toxicity. Maximum reduction in its activity was observed at 4 mg Cr (VI)/kg soil. At 70 DAS, its activity decreased from 12.55 to 5.11 and 15.66 to 9.06 with increasing concentration of Cr (VI) from 0 to 4 mg Cr (VI)/kg soil in leaves and stem, respectively (Fig. 2). Stem, in general, had higher activity of ascorbate peroxidase than leaves. Advancement of age of plants resulted in increase in its activity till 70 DAS and then decreased at 90 DAS. The results obtained are in agreement to the work conducted in pea (*Pisum sativm* L.) seedling (Surekha and Duhan, 2012), in which the effect of chromium stress was studied on peroxidase, ascorbate peroxidase and acid invertase, that resulted in the increase of peroxidase activity and decrease in ascorbate peroxidase activity at higher concentration of Cr (VI) treatments.

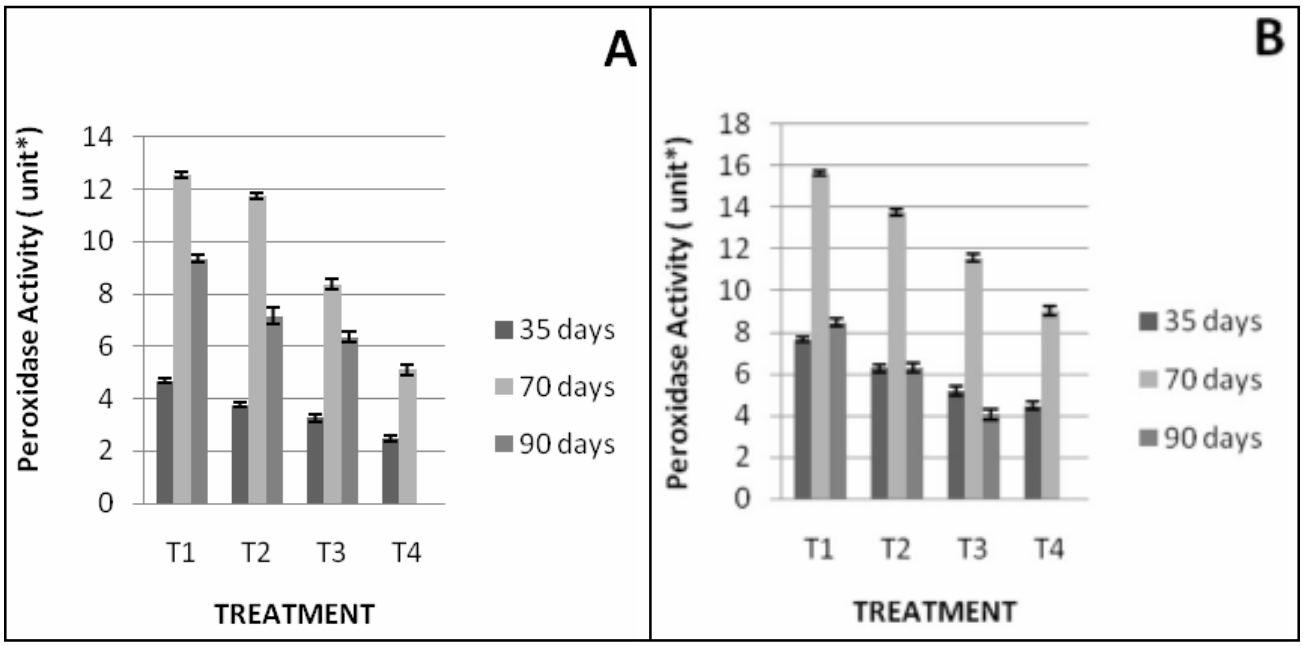

Fig. 1. Effect of different treatments of chromium on activity of peroxidase enzyme A (leaves) and B (shoots) of sorghum plant at three stages. The bars denote±standard error. 1 unit* = Change in 0.01 absorbance/min/mg protein.

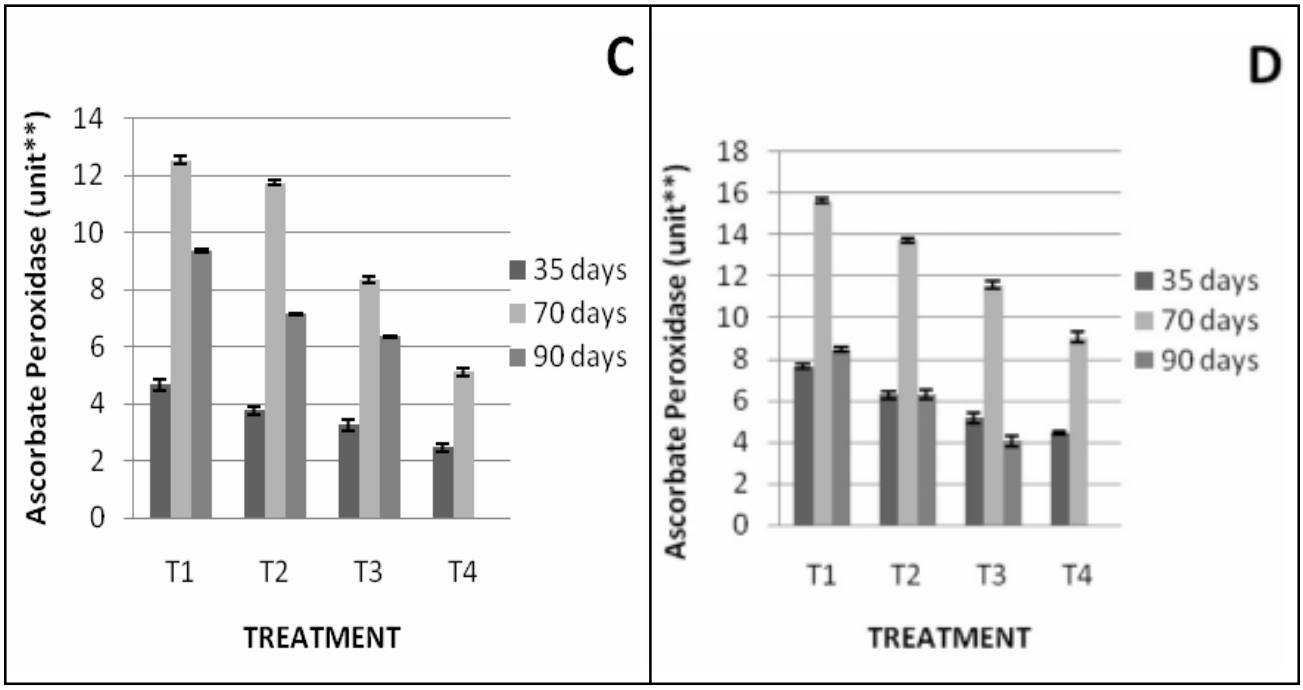

Fig. 2. Effect of different treatments of chromium on activity of ascorbate peroxidase enzyme C (leaves) and D (shoots) of sorghum plant at three stages. The bars denote±standard error. 1 unit** = one nmol of ascorbate/min/ml.

Effect of Cr (VI) on Hydrogen Peroxide Content (T_1, T_2, T_3 and T_4)

Levels of hydrogen peroxide decreased significantly and regularly with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI)/kg soil. At 70 DAS, its concentration decreased from 15.10 to 14.91 and 14.63 to 14.37 µmol/g fresh weight in leaves and stem (Fig 3) with increasing concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI)/kg soil, respectively. With growth, levels of hydrogen peroxide increased and became maximum at 70 DAS and then decreased at 90 DAS, in all plant parts. The increase in activity of peroxidase consumed hydrogen peroxide and thus it resulted in decrease in of hydrogen peroxide concentration in different parts of sorghum plant by Cr (VI) application.

Effect of Cr (VI) on Ascorbic Acid Content (T_1, T_2, T_3 and T_4)

Increase in the concentration of Cr (VI) from 0.0 to 4.0 mg Cr (VI)/kg soil resulted in increase in ascorbate concentration in leaves (Fig. 4) at 35 DAS. However, at 70 and 90 DAS, its concentration decreased at 2.0 and 4.0 mg Cr (VI)/kg soil treatment. In stem (Fig. 4), ascorbate concentration decreased from 13.87 to 12.80, 16.80 to 15.50 and 13.53 to 13.59 µmols/g fresh weight with increasing concentration of Cr (VI) from 0.0 to 2.0 mg Cr (VI)/kg soil at 35, 70 and 90 DAS, respectively. Ascorbate concentration was maximum in leaves followed by stem. Decrease in activity of ascorbate peroxidase, might be responsible for higher ascorbate content in different plant parts by Cr (VI) application.

CONCLUSION

It is, therefore, concluded that activity of antioxidant enzymes and their metabolites in leaves and shoot adversely affected with an increase in Cr (VI) concentration from 0.0 to 4.0 mg Cr (VI)/kg soil.

ACKNOWLEDGEMENT

The authors are highly grateful to Head, Department of Chemistry & Biochemistry; Head, Forage Section and Director of Research, CCS Haryana Agricultural University, Hisar for their cooperation and providing the necessary facilities for this investigation.

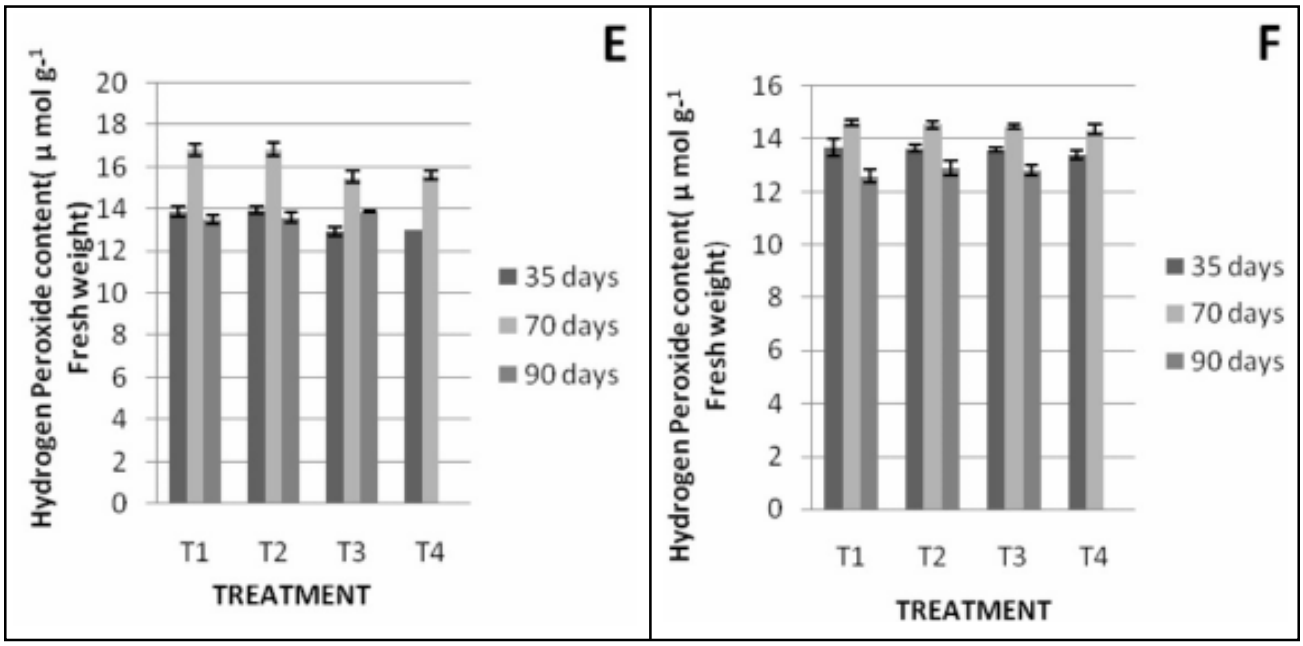

Fig. 3. Effect of different treatments of chromium on hydrogen peroxide content of E (leaves) and F (shoots) of sorghum plant at three stages. The bars denote±standard error.

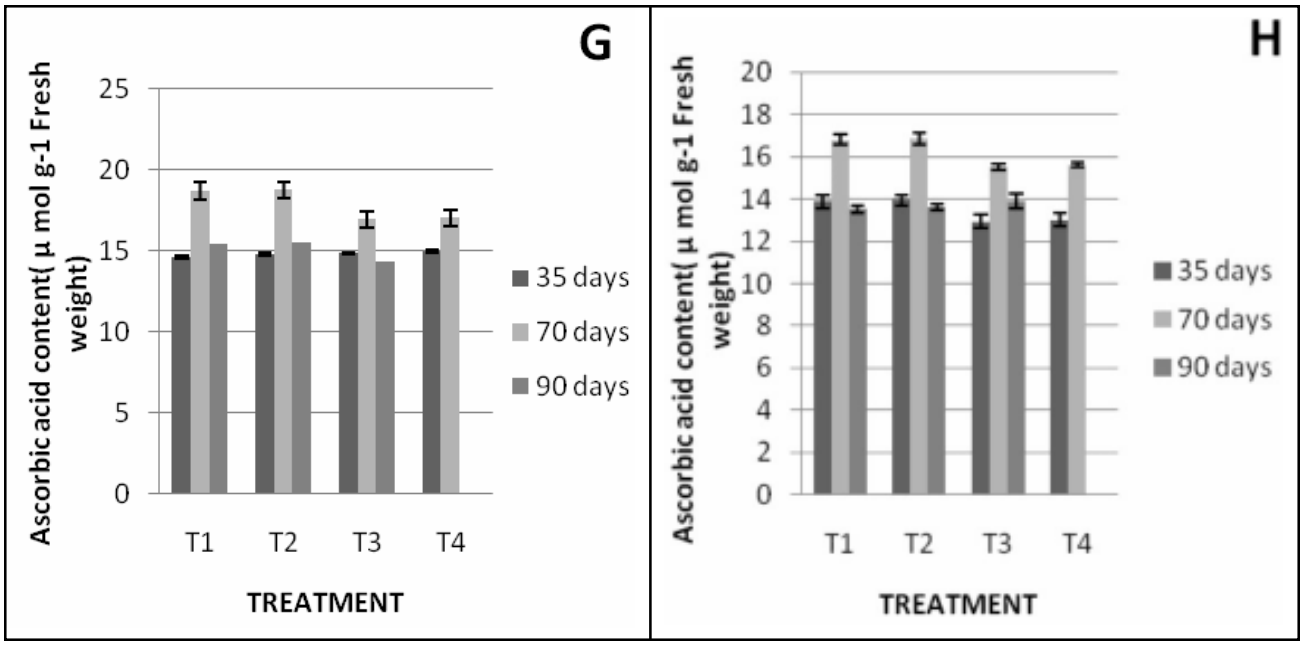

Fig. 4. Effect of different treatments of chromium on ascorbic acid content of G (leaves) and H (shoots) of sorghum plant at three stages. The bars denote±standard error.

REFERENCES

Ali, N. A., M. Ater, G. I. Sunahara, and Robidoux P. Y. 2004 : Phytotoxicity and bioaccumulation of copper and chromium using barley (*Hordeum vulgare* L.) in spiked artificial and natural forest soils, *Ecotoxicol. Environ. Safety*, **57** : 363-374.

Apel, K., and H. Hirt 2004 : Reactive oxygen species : metabolism, oxidative stress and signal transduction. *Ann. Rev. Plant Biol.*, **55** : 373-399.

Bhattacharjee, S. 1998 : Membrane lipid peroxidation, free radical scavengers and ethylene evolution in

Amaranthus affected by lead and cadmium. *Bill. Plant.*, **40** : 131-135.

Jayakumar, K., C. A. Jaleel, and P. Vijayarengan. 2007 : Changes in growth, biochemical constituents and antioxidant potentials in-radish (*Rapthanus sativus* L.) under cobalt stress, *Turk. J. Biol.*, **31** : 127-136.

Kovacik, J., B. Klejdus, J. Hedbavny, and M. Backor. 2010 : Effect of copper and salicylic acid on phenolic metabolites and free amino acids in *Scenedesmus quadricauda* (Chloro-phyceae). *Plant Sci.*, **178** : 307-311.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951 : Protein measurement with the folin phenol reagent, *Biol. Chem.*, **193** : 265-275.

Mittler, R. 2002 : Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, **7** : 405-410.

Nakano, Y., and K. Asada. 1981 : Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, *Plant Cell Physiol.*, **22** : 867-880.

Oliveira, H. 2012 : Chromium as an environmental pollutant : insights on induced plant toxicity. *J. Bot.*, Article ID 375843, 8 pages. doi : 10.1155/2012/375843.

Pandey, V., V. Dixit, and R. Shyam. 2009 : Chromium effect on ROS generation and detoxification in pea (*Pisum sativum*) leaf chloroplasts. *Protoplasma*, **236** : 85-95.

Pinto, E., T. C. S. Sigaud-Kutner, M. A. S. Leitao, O. K. Okamoto, D. Morse, and P. Colepicolo, 2003: Heavy metal-induced oxidative stress in algae, *J. Phycol.*, **39** : 1008-1018.

Roe, J. H. 1964 : Chemical determination of ascorbic, dehydroascorbic and diketo gluconic acids. In : *Methods in Biochemical Analysis*, Glick, D. (ed.). Inter-Science, New York. pp. 115-139.

Sen, A. K., N. G. Mondal, and S. Mandal, 1994 : Toxic effects of chromium (VI) on the plant *Salvinia natans* L., *Environ. Ecol.*, **12** : 279-283.

Shanker, A. K., C. Cervantes, H. Loza-Tavera, and S. Avudainayagam. 2005 : Chromium toxicity in plants. *Environ. Int.*, **31** : 739-753.

Shanker, A. K., M. Djanaguiraman, R. Sudhagar, C. N. Chandrashekar, and G. Pathmanabhan. 2004 : Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in greengram [*Vigna radiata* (L). R. Wilczek] roots. *Plant Sci.*, **166** : 1035-1043.

Shanmugam, V., J. C. Lo, C. L. Wu, S. L. Wang, C. C. Lai and E. L. Connolly. 2011 : Differential expression and regulation of iron-regulated metal transporters in *Arabidopsis halleri* and *Arabidopsis thaliana*–the role in zinc tolerance. *New Phytol.*, **190** : 125-137.

Shannon, L. M., E. Key, and J. Y. Law. 1966 : Peroxidase isoenzymes from horse radish roots : Isolation and physical properties. *J. Biol. Chem.*, **241** : 2166-2172.

Sinha, A. K. 1972 : Colorimetric assay of catalase. *Anal. Biochem.*, **4**7 : 389-394.

Sinha, P., B. K. Dube, and C. Chatterjee. 2005 : Amelioration of chromium phytotoxicity in spinach by withdrawal of chromium or iron application through different modes. *Plant Sci.*, **169** : 641-646.

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

Surekha, and J. S. Duhan. 2012 : Chromium stress on peroxidase, ascorbate peroxidase and acid invertase in pea (*Pisum sativum* L.) seedling. *Int. J. Biotechnol. Mol. Biol. Res.*, **3** : 15-21.

Volland, S., E. Bayer, V. Baumgartner, A. Andosch, C. Lütz, E. Sima, and U. Lütz-Meindl. 2014 : Physiology rescue of heavy metal effects on cell physiology of the algal model system *Micrasterias* by divalent ions. *J. Plant Physiol.*, **171** : 154-163.