Pol. J. Environ. Stud. Vol. 24, No. 2 (2015), 899-904

Short Communication

Chromium Accumulation and Toxicity in Corn (*Zea mays* L.) Seedlings

Sadia Naseem[1], Muhammad Yasin[2], Ambreen Ahmed[1]*, Muhammad Faisal[2]

[1]Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan
[2]Department of Microbiology and Molecular Genetics, University of the Punjab
Quaid-e-Azam Campus, Lahore-54590, Pakistan

Received: 18 September 2013
Accepted: 25 September 2014

Abstract

Extensive use of chromate compounds in the last few decades has resulted in contamination of our environment. In the present study we have investigated the effects of two different concentrations (10, 20 μg ml^{-1}) of chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) on the growth of *Zea mays* L. As concentrations of chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) increased, there was a significant decrease in seed germination (10-24%), shoot length (6-29%), root length (11-33%), seedling length (16-24%), fresh weight of seedlings (17-67%) and increase in dry weight per seedling (3-15%), chromium content, acid phosphatases content (215-707%), and peroxidases activity (129-200%) of *Zea mays* plants compared to control treatment. In all treatments, the effect of hexavalent salts (K_2CrO_4 and $K_2Cr_2O_7$) was more severe on plant growth compared to trivalent Cr salts ($CrCl_3$). *Zea mays* plants have the ability to accumulate various chromium salts in their tissues and thus help to remediate the polluted soil.

Keywords: heavy metals, maize, acid phosphatase, peroxidase, phytoremediation

Introduction

Heavy metals like chromium, arsenic, cadmium, barium, zinc, lead, etc., can cause severe damage to the environment [1, 2] and show toxic effects by modifying the active conformation of biological processes [3]. These metals when accumulated in the food chain can be harmful for humans, animals, and plants [4]. Chromium (atomic number 24 and average atomic mass 51.998g) is a group VI-B transition metal that is commonly found in the industrial effluents. The effluents from leather tanneries and textile manufacturing factories and applications of insecticides and fertilizers for agricultural purposes are major sources for the addition of chromium and other heavy metals in water [5]. Chromium exists in different oxidation states ranging from Cr^{+2} to Cr^{+6}. The hexavalent (VI) and trivalent (III) oxidation states of Cr are the most abundant forms and are of biological significance. In a terrestrial environment, Cr (III) is less toxic and less mobile, while Cr (VI) is highly toxic, carcinogenic, and mutagenic [6]. Hexavalent chromium causes inflammation of the skin and increased incidences of lung cancer.

Now numerous technologies are available to remediate soils contaminated by heavy metals (chromium, arsenic, lead, mercury, etc.). However, many of these technologies are costly. Phytoremediation is the application of green plants to reduce the toxicity of environmental contaminants [7]. Phytoremediation is a cost-effective and long-lasting solution for remediation of polluted sites. Many plants have been identified, which can remove hazardous and toxic heavy metals from the environment. *Typha angustifolia*, *Convolvulus arvensis,* and *Prosopis* sp., *Brassica* spp., *Zea mays,* and *Helianthus annuus* can efficiently remove chromate from metal-polluted soil [8, 9].

Keeping in view the metal accumulative potential of maize plants, the impact of various chromium salts ($CrCl_3$, K_2CrO_4, and $K_2Cr_2O_7$), on seed germination

*e-mail: ambreenahmed1@hotmail.com

and early growth of corn (*Zea mays*) were investigated. Many recent investigations have proven that the use of phytoaccumulator plant species have substantially improved soil contaminated with heavy metals [10]. High concentrations of chromium content were observed in various tissues of plants growing in chromium-polluted areas [11]. Roots have higher chromium concentrations than shoots and leaves. So the present investigation is a step towards the reclamation of a chromium-polluted environment by *Zea mays* seedlings.

Experimental Procedures

Plant Growth Experiment

Certified seeds of *Zea mays* (EV-90) were taken from Punjab Seed Corporation, Lahore, Pakistan. Glass petriplates of 120 mm diameter were washed and oven dried. Two layers of Whatman filter paper No. 1 were placed on each plate. Plates were properly labeled with different concentrations of working solutions, i.e., ($CrCl_3$; 10 μg ml^{-1} and 20 μg ml^{-1}), (K_2CrO_4; 10 μg ml^{-1} and 20 μg ml^{-1}), and ($K_2Cr_2O_7$; 10 μg ml^{-1} and 20 μg ml^{-1}), and control (containing distilled water). Ten ml of respective salt concentration ($CrCl_3$, K_2CrO_4, and $K_2Cr_2O_7$) was supplied to each respective plate so that the filter papers were well moistened. *Zea mays* seeds (25 in each petriplate) were uniformly spread on the moistened filter paper. All petriplates were kept in the dark at 25 + 2ºC for 3 days. After 3 days, germination of seeds was noted. After germination, germinated seedlings were transferred to the labeled pots, each containing 140 gm sieved soil and stress solution (10 and 20 μg ml^{-1}) of chromium salts ($CrCl_3$, K_2CrO_4 and $K_2Cr_2O_7$) were given to the respective pots. The pots were shifted to light (10 Klux, 16 hours duration) at 25 + 2ºC. Seedlings were grown for 30 days and after 30 days the plants were harvested and various growth and biochemical parameters were measured. The experiment was repeated thrice.

Estimation of Chromium Uptake, Peroxidases and Acid Phosphatases Content

For chromium content analysis, oven dried plant material was digested in 10 ml of HNO_3 and 2 ml of $HClO_4$ in flasks and heated on a sand bath. When solution became clear, volume was made up to 15 ml with distilled water. Samples were now ready for chromium estimation. Chromium content estimation was done following Rand et al. [12]. 2-3 drops of methyl orange were added in 1ml of digested plant material. Then NH_4OH was added until the pink color of the solution turned to yellow. Now 1:1 H_2SO_4 was added dropwise and again pink color appeared. When pink colour reappeared, 1ml of 1:1 H_2SO_4 was added in excess. After that, volume was made up to 40 ml with distilled water. Flasks were heated till boiling of the solution. In the boiling solution, 2-3 drops of $KMnO_4$ were added. If the red/purple color persisted then 1 ml of sodium azide was added to each flask and boiled for two minutes until the solution became colorless. After cooling, 0.25 ml of H_3PO_4 was added. Final volume was made up to 100 ml with distilled water. 2 ml of diphenyl carbazide was added to each flask. Flasks were kept in the dark for 30 minutes. Purple color developed due to the reaction of chromium with diphenyl a carbazide. Optical density was measured at 510 nm on a spectrophotometer (200-D).

Moreover, the David and Murray [13] method was followed for the quantitative estimation of peroxidases. Weighed and frozen plant material was crushed in phosphate buffer (0.1 M, pH 7.0) in a cold pestle and mortar in the ratio of 1:4 (w/v, one gram of plant material, 4 ml of phosphate buffer). The samples were centrifuged at 10,000 rpm for 10 minutes at 4ºC. The supernatant was used for estimation of peroxidase enzyme. The two sets of test tubes were labeled, one for test and the other for control reactions. To all the test tubes (both sets), 0.2 ml of enzyme extract, and 2.5 ml of phosphate buffer (0.1 M, pH 7.0) was added and mixed. For test reactions, 0.2 ml of 1% Guaiacol also was added and mixed. Both sets were left at room temperature for 15-20 minutes. After that, 0.1 ml of 0.3% H_2O_2 solution was added to both the sets, stirred to mix. For blank, 0.2 ml glass distilled water, 2.5 ml of phosphate buffer, and 0.1ml of 0.3% H_2O_2 solution were mixed. Optical density of both sets were observed against blank at 470 nm on D-2 spectrophotometer in order to determine the amount of peroxidases.

For the estimation of acid phosphatase content, the Iqbal and Rafique [14] method was used. Weighed and frozen plant material (shoots) was crushed in a cold pestle and mortar with cold 0.1 M Tris HCl buffer (pH 6.5). The ratio of buffer to plant material was 4:1 (v/w). The crushed samples were centrifuged at 14,000 rpm for 10 minutes. The supernatant thus obtained was used for the estimation of enzyme acid phosphatase. As a result of hydrolysis of acid phosphatase, phenol was released from the substrate phenyl phosphate under specific conditions of time, temperature, and pH. For the activity of acid phosphatase enzyme, the time duration was one hour, temperature was 37ºC, and pH was 4.9. For the quantitative estimation of enzyme, series of reactions, i.e., test, control, standard, and blank were carried out. For test reaction, 1ml of citrate buffer pH 4.9 was mixed with 1ml of substrate phenyl in a properly labelled set of test tubes. Set was placed in water bath at 37ºC for three minutes. After incubation, 0.2 ml of enzyme extract was added and incubated again at 37ºC for one hour. Then 1ml of 0.5 N NaOH was added. For control reaction, 1ml of citrate buffer pH 4.9 was mixed with 1 ml of substrate phenyl in a labelled set of test tubes. The samples were placed at 37ºC for one hour in water bath. After that 1 ml of 0.5 N NaOH was added followed by 0.2 ml of enzyme extract in each tube and mixed thoroughly. For standard reaction 1.2 ml of citrate buffer (pH 4.9), 1 ml of phenol standard and 1ml of 0.5 N NaOH were mixed together in a test tube. For the blank, 1.2 ml of citrate buffer (pH 4.9), 1 ml of distilled water, and 1 ml of 0.5 N NaOH were mixed together in a test tube for blank

Table 1. Effects of different chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) on seed germination, and shoot and root lengths of *Zea mays* seedlings. Data shows SE±mean (n=3), and different alphabets in the same column show a significant difference ($p \leq 0.05$).

Cr salt (μg ml^{-1})	Plant treatments	Seed germination (%)	Shoot length (cm)	Root length (cm)
0	Control	84 ± 1^{d}	17 ± 0.2^{bc}	$9\pm0.2b^{c}$
10 ($CrCl_3$)	M1	76 ± 2^{c}	19 ± 0.3^{cd}	10 ± 0.2^{cd}
20 ($CrCl_3$)	M2	75 ± 2^{c}	19 ± 0.3^{e}	10 ± 0.1^{d}
10 (K_2CrO_4)	M3	$84\pm^{d}$	16 ± 0.6^{b}	9 ± 0.2^{b}
20 (K_2CrO_4)	M4	64 ± 1^{a}	13 ± 1.4^{a}	8 ± 0.2^{b}
10 ($K_2Cr_2O_7$)	M5	72 ± 1^{c}	16 ± 0.3^{b}	8 ± 0.3^{b}
20 ($K_2Cr_2O_7$)	M6	68 ± 1^{b}	12 ± 0.3^{a}	6 ± 0.4^{a}

reaction. To all the test tubes (test, control, standard, and blank) 1 ml of 0.5 N $NaHCO_3$ was added followed by the addition of 1 ml of 4-aminoantipyrin solution and 1 ml of potassium ferri-cyanide solution. The test tubes were shaken to mix the solutions and the reading was taken immediately against water at 510 nm on a Beckman D-2 spectrophotometer.

Statistical Analysis

Data obtained was analyzed statistically using SPSS v20. Analysis of Variance for multiple means was followed by Post Hoc-Tukey test ($\alpha = 0.05$).

Results

In this study the effects of various stress concentrations (10, 20 μg ml^{-1}) of different chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) on the growth of *Zea mays* var. EV-609, were observed. The seeds were allowed to germinate and grow under chromium stresses. Growth parameters were studied after 10 days of shifting to light. Later on, acid phosphatase and peroxidase activity was checked and chromium content of seedlings also was estimated.

Seed Germination

Percentage germination was checked at three concentrations of trivalent ($CrCl_3$) and hexavalent (K_2CrO_4, $K_2Cr_2O_7$) salts, i.e., 0,10 and 20 μg ml^{-1}. Maximum seed germination was observed in control treatment but as concentration increased upto 20 μg ml^{-1} ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$), there was a significant decrease (10-24%) in percentage germination, except $K_2CrO_4$10 *μg ml^{-1}*, of *Zea mays* seeds over control treatment (Table 1).

Shoot Length

Shoot length increased in treatment with 10 and 20 μg ml^{-1} $CrCl_3$ while in the case of hexavalent salts (K_2CrO_4, $K_2Cr_2O_7$) increase in salt concentration of 20 μg ml^{-1} caused significant reduction (6-29%) in shoot length when compared with control. Lower concentrations of hexavalent salt (10 μg ml^{-1}) did not show a significant effect on shoot length (Table 1).

Root Length and Seedling Length

Trivalent salt and $CrCl_3$ in concentration of 20 μg ml^{-1} showed an increase in root length and seedling length

Table 2. Effects of different chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) on seedling length, fresh weight per seedling, and dry weight per seedling of *Zea mays*. Data shows SE±mean (n=3), and different alphabets in the same column show a significant difference ($p \leq 0.05$).

Cr salt (μg ml^{-1})	Plant treatments	Seedling length (cm)	Fresh weight per seedling (g)	Dry weight per seedling (mg)
0	Control	25.0 ± 0.1^{c}	0.6 ± 0.06^{c}	61 ± 0.6^{a}
10 ($CrCl_3$)	M1	27 ± 0.2^{d}	0.5 ± 0.02^{cd}	63 ± 0.8^{ab}
20 ($CrCl_3$)	M2	29.0 ± 0.6^{d}	0.5 ± 0.02^{de}	68 ± 0.7^{c}
10 (K_2CrO_4)	M3	25 ± 0.2^{c}	0.5 ± 0.02^{de}	64 ± 0.2^{c}
20 (K_2CrO_4)	M4	21 ± 0.3^{b}	0.4 ± 0.01^{b}	68 ± 0.3^{cd}
10 ($K_2Cr_2O_7$)	M5	25 ± 0.2^{c}	0.4 ± 0.2^{c}	64 ± 0.5^{c}
20 ($K_2Cr_2O_7$)	M6	19 ± 0.2^{a}	0.2 ± 0.2^{a}	70 ± 0.3^{d}

Table 3. Effects of different chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) on chromium uptake, acid phosphatases (units g^{-1}), peroxidases (units g^{-1}), and content of *Zea mays* seedlings. Data shows SE±mean (n=3), and different alphabets in the same column show a significant difference ($p \leq 0.05$).

Cr salt (μg ml^{-1})	Plant treatments	Chromium uptake (μg g^{-1})	Acid phosphatases (units g^{-1})	Peroxidases (units g^{-1})
0	Control	0.0^{a}	2.6 ± 0.5^{a}	1.4 ± 0.02^{a}
10 ($CrCl_{3)}$	M1	1.3 ± 0.03^{b}	3.0 ± 0.3^{a}	3.2 ± 0.1^{bc}
20 ($CrCl_{3)}$	M2	2.4 ± 0.05^{c}	8.2 ± 0.2^{b}	3.2 ± 0.2^{b}
10 ($K_2CrO_{4)}$	M3	14.6 ± 0.1^{d}	8.2 ± 0.03^{b}	3.8 ± 0.2^{cd}
20 ($K_2CrO_{4)}$	M4	16.9 ± 0.1^{g}	21.3 ± 0.3^{c}	4.1 ± 0.1^{de}
10 ($K_2Cr_2O_{7)}$	M5	15.2 ± 0.01^{e}	8.9 ± 0.1^{b}	4.0 ± 0.03^{de}
20 ($K_2Cr_2O_{7)}$	M6	16.3 ± 0.03^{f}	9.7 ± 0.4^{b}	4.2 ± 0.1^{e}

compared to control. Hexavalent salt (K_2CrO_4) showed no significant effect over root length and seedling length at lower concentrations of salt (10 μg ml^{-1}), but a higher concentration (20 μg ml^{-1}) showed reduction in root length (11-33%) and seedling length (16-24%) compared to control plants (Tables 1, 2). Compared to control, maximum and significant reductions in root length and seedling length was observed by $K_2Cr_2O_7$ (20 μg ml^{-1}).

Fresh and Dry Weight per Seedling

With increases in salt concentration of 20 μg ml^{-1}, a reduction in fresh weight (17-67%) of seedlings was observed in all treatments compared to control. A higher concentration (20 μg ml^{-1}) of hexavalent salts (K_2CrO_4, $K_2Cr_2O_7$) showed significant reduction in fresh weight of seedlings compared to control treatment (Table 2). Interestingly, an increase in salt concentrations (20 μg ml^{-1}) of different chromium salts ($CrCl_3$, K_2CrO_4, $K_2Cr_2O_7$) showed an increase in dry weight (3-15%) per seedling, and the most significant increase was shown by K_2CrO_4 and $K_2Cr_2O_7$ at higher concentrations of 20 μg ml^{-1} compared to control (Table 2).

Chromium Content

No chromium content was detected in control plants. Under $CrCl_3$ stress (20 μg ml^{-1}), an increase in salt stress caused a significant increase in chromium content of seedlings compared to control. Both K_2CrO_4 and $K_2Cr_2O_7$ salts (10-20 μg ml^{-1}) showed a significant increase in Cr contents compared to $CrCl_3$-treated plants as well as control treatment (Table 3).

Acid Phosphatases and Peroxidases Activity

A significant increase was observed in acid phosphatase contents (215-707%) and peroxidase activity (129-200%) at all concentrations of $CrCl_3$, K_2CrO_4, and $K_2Cr_2O_7$ compared to control (Table 3). A maximum (significant) increase in acid phosphatase contents and peroxidase activity was shown by K_2CrO_4- and $K_2Cr_2O_7$- (20 μg ml^{-1}) treated plants in comparison with the control treatment (Table 3).

Discussion

Chromium is the 17th most abundant element in the Earth's mantle, and due to its extensive anthropogenic use its concentration has increased in the last few years [15, 16]. Plants can take up chromium through carriers of important ions such as sulphates. In a plant's chromium uptake, accumulation, translocation, and toxicity depend on its speciation [17].

When *Zea mays* plants were treated with $CrCl_3$, K_2CrO_4, and $K_2Cr_2O_7$, a significant decrease was observed in seed germination compared to control, except in the case of 10 μg ml^{-1} K_2CrO_4. Similar results also were observed by López-Luna et al. [18], where some reductions in seed germination percentage were noticed in plants treated with a high concentration of chromium salt. In the case of hexavalent salts (K_2CrO_4, $K_2Cr_2O_7$), increases in salt concentrations (20 μg ml^{-1}) caused significant reduction in shoot length and fresh weight of seedlings compared to control treatment. Reduction in shoot length also was observed in many plants grown in a chromium-contaminated environment. Mallick et al. [19] also reported a significant decrease in shoot length and reduction in various growth parameters of *Zea mays* when initially supplemented with 9 μg ml^{-1} Cr (VI) after 7 days. Three chromium salts, $CrCl_3$, K_2CrO_4 and $K_2Cr_2O_7$, (10-20 μg ml^{-1}), showed a significant increase in chromium content compared to control (Table 3). In *Pennisetum purpureum, Dyera costulata,* and *Brachiaria decumbens,* accumulation of chromium in plants also is reported by Mant et al. [20] and Gafoori et al. [21]. The chemical form that is transported in plant tissues is not understood yet, but some investigators have reported that Cr (VI) can move from one part of a plant to another. Few scientists

have reported the reduction of more toxic and mobile from Cr (VI) into less toxic Cr (III) inside plant tissues, especially in root cells. In *Zea mays* plants, chromium mainly accumulated in the chloroplast membranes [22]. It has been observed that plant tissues that accumulate iron can also accumulate some quantity of chromium. Plants growing in neutral to alkaline soil contain more chromium contents as compared to those that grow in acidic environments.

A significant increase was observed in acid phosphatase contents and peroxidases activity at all concentrations of $CrCl_3$, K_2CrO_4, and $K_2Cr_2O_7$ compared to control (Table 3). Some other researchers have also reported an increase in guaiacol peroxidase (POX) and SOD activity in shoots of *T. aestivum* exposed to Cr (VI) [23, 24]. Acid phosphatase liberates phosphate in the soil, which helps in the growth of plants in the form of inorganic phosphorous [25]. The conventional methods used for the reclamation of chromium pollution are costly and expensive, which leads to secondary problems in the environment. In recent years, environmental researchers have emphasized using plant biomass that can accumulate heavy metals [11]. In contrast to the conventional methods, phytoremediation can be useful to remediate metal-polluted soil and thus improve its quality and fertility.

Conclusion

From the above results we can conclude that the *Zea mays* plants grown in the chromium-contaminated environment not only accumulate chromium in their tissues (root and shoot), but also convert the most toxic and mobile Cr (VI) into less toxic and immobile Cr (III), thus utilizing the most fast and successful strategy to clean up chromium-rich soil.

Acknowledgements

The University of the Punjab, Lahore, Pakistan is greatly acknowledged for financial support for this research.

References

1. LI L., LI F. S., XIONG D. Q., SONG, C. Y., Heavy metal contamination and their distribution in different size fractions of the surficial sediment of Haihe River, China. Environ. Geol. **50**, 431-438, **2006**.
2. ZHANG M. Y., CUI L. J., SHENG L. X., WANG Y. F., Distribution and enrichment of heavy metals among sediments, water body and plants in Hengshuihu wetland of Northern China. Ecol. Eng. **35**, 563-569, **2009**.
3. WANI P. A., KHAN M. S., ZAIDI A., Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (*Vigna*) on growth symbiosis seed yield and metal uptake by greengram plants. Chemosphere. **70**, 36-45, **2007**.
4. NOURI J., MAHVI A. H., JAHED G. R., BABAEI A. A., Regional distribution pattern of groundwater heavy metals resulting from agricultural activities. Environ. Geol. **55**, 1337-1343, **2008**.
5. ATAFAR Z., MESDAGHINIA A., NOURI J., HOMAEE M., YUNESIAN M., AHMADIMOGHADDAM M., MAHVI A. H., Effect of fertilizer application on soil heavy metal concentration. Environ. Monitor. Assess. 1-7, **2008**.
6. MUSARRAT J., ZAIDI A., KHAN M. S., SIDDIQUI M. A., AL-KHEDHAIRY A. A., Genotoxicity assessment of heavy metal-contaminated soils. M.S. Khan, A. Zaidi, R. Goel, J. Musarrat (Eds.), Biomanagement of metal-contaminated soils, Springer Wien, New York, pp. 323-342, **2011**.
7. BRUNETTI G., FARRAG K., ROVIRA P. S., NIGRO F., SENESI N., Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. Geoderma. **160**, 517-523, **2011**.
8. DONG J., WU F., HUANG R. and ZANG G., A Chromium-tolerant plant growing in Cr-contaminated land. Int. J. Phytoremdiat. **9**, 167-179, **2007.**
9. AKHIONBARE S. M. O., EBE T. E., AKHIONBARE W. N., AC-CHUKWUOCHA N., Heavy metal uptake by corn *(Zea mays*) grown on contaminated soil. Res. J. Agri. Biol. Sci. **6**, 993-997, **2010**.
10. SHAHANDEH, H. AND HOSSNER, L. R. Plant screening for chromium phytoremediation, Int. J. Phytoremediat**, 2,** 31-51, **2000**.
11. SHAMS K. M., TICHY G., FISCHER A., SAGER M., PEER T., ASHTAR B., FILIP K., Aspects of phytoremediation for chromium contaminated sites using common plants *Urtica dioica*, *Brassica napus* and *Zea mays. Plant Soil,* **328***,* 175-189, **2010**.
12. RAND M. C., ARNOLD E., MICHEL J., Standard methods for the examination of water and wastewater. New York: American Public Health Association, **1979**.
13. DAVID R., MURRAY E., Protein synthesis in dark brown leaves. Can. J. Bot. **43**, 817-824, **1965**.
14. IQBAL J., RAFIQUE N., Toxic effect of barium chloride on germination, early seedling growth, soluble proteins and acid phosphatase in *Zea mays* L. Pak. J. Bot. **19**, 1-8, **1986**.
15. AVUDAINAYAGAM S., MEGHARAJ M., OWENS G., KOOKANA R. S., CHITTLEBOROUGH D., NAIDU R., Chemistry of chromium in soils with emphasis on tannery waste sites. Rev. Environ. Contam. Toxicol. **178**, 53-91, **2003**.
16. ZAYED A. M., TERRY N., Chromium in the environment: factors affecting biological remediation. Plant Soil. **249**, 139-156, **2003**.
17. OLIVEIRA H., Chromium as an environmental pollutant: insights on induced plant toxicity. J. Bot. 12, 1-8, **2012**: doi:10.1155/2012/375843.
18. LÓPEZ-LUNA J., GONZÁLEZ-CHÁVEZ M. C., ESPARZA-GARCÍA F. J. AND RODRÍGUEZ-VÁZQUEZ R., Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants. J. Hazard. Mater. **163**: 829-834, **2009**.
19. MALLICK S., SINAM G., KUMAR-MISHRA R., SINHA S., Interactive effects of Cr and Fe treatments on plants growth, nutrition and oxidative status in *Zea mays* L. Ecotoxicol. Environ. Safe. **73,** 987-995, **2010**.
20. MANT C., COSTA S., WILLIAMS J., TAMBOURGI E., Phytoremediation of chromium by model constructed wetland. Bioresource Technol. **97**, 1767-1772, **2006**.

21. GAFOORI M., MAJID N. M., ISLAM M. M., LUHAT S., Bioaccumulation of heavy metals by *Dyera costulata* cultivated in sewage sludge contaminated soil. Afr J Biotechnol. **10**, 10674-10682, **2011.**
22. TOPPI L. SANIT DI., FOSSATI F., MUSETTI R., MIKEREZI, I., FAVALI M. A., Effects of hexavalent chromium on maize, tomato, and cauliflower plants. J. Plant Nutrit. **25**, 701-717, **2002**.
23. LIU D., ZOU J., WANG M., AND JIANG W., Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L. Bioresource Technol. **99**, 2628-2636, **2008**.
24. DEY S. K., JENA P. P., KUNDU S., Antioxidative efficiency of *Triticum aestivum* L. exposed to chromium stress. J. Environ. Biol. 30, 539-544, 2009.
25. WYSS M., PASAMONTES L., RÉMY R., KOHLER J., KUSZNIR E., GADIENT M., MÜLLER F., VAN LOON APGM., Comparison of the thermostability properties of three acid phosphatases from molds: Aspergillus fumigatus phytase, A. niger phytase, and A. niger PH 2.5 acid phosphatase. Appl Environ. Microbiol. **64**, 4446-4451, **1998**.