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Reduction in phytotoxicity of chromium using ACC-deaminase containing bacteria

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Abstract

Extensive use of chromium in tanning and leather industry has created serious concerns for human health and environment. The tannery industry in Pakistan is one of the most developed industry therefore results in discharge of large amount of untreated water in water courses and surrounding soils. The contamination may lead to many phytotoxic effects however certain bacteria may potentially ameliorate the phytotoxic effect of chromium. The present work was designed to check the toxic effects of various concentrations of chromium (ranging from 0 to 100 mg/l of Cr) on growth of Triticum aestivum in multiple sets of experiments. The reduction in phytotoxicity through bacterial inoculation was also assessed in the study. The growth parameters such as shoot length, root length, shoot fresh and dry weights were recorded. It was observed that the increasing concentration of chromium significantly reduced the plant growth. As the minimum growth in terms of root and shoot length and plant biomass was observed at chromium concentration of 100 mg/l. However the study also revealed that inoculation with plant growth promoting bacteria (CC7+ACC-14) reduced the toxic effects of chromium and significantly promoted root and shoot growth under controlled conditions. Thus it is finally concluded that wastewater containing chromium could potentially be utilized for plants when inoculated with certain plant growth promoting bacteria.

Key words: PGPR, Chromium, phytotoxicity, wastewater, wheat

Introduction

Man-made chromium sources lead to chromium pollution, and the use of Cr in the industry has increased significantly during the last couple of decades (Han *et al*., 2004). The world production of Cr is in the order of 107 tons per year, 60–70% is used in alloys, including stainless steel, and 15% is used in chemical industrial processes, mainly leather tanning, pigments, electroplating, wood preservation, textile dyeing, and metal processing industries (Vernay *et al*., 2008). Reportedly up to 630 mg kg^{-1} of chromium has been detected in soil of Pakistan in the vicinity of tannery effluents (Khan, 2001) and around 2700 mg kg^{-1} was observed in India (Raju and Tandon, 1999).

Chromium is recognized s non-essential plant nutrient however trace amounts of chromium is required for animals (Bluskov et al., 2005). But in few studies it has been reported that traces of chromium may increase plant productivity (Prasad et al., 2010). However at higher concentration chromium is toxic to plants due to many interference with other essential nutrients (Scoccianti *et al.*, 2006 Abreu et al., 2002). Chromium is known to affect metabolic processes via oxidative stress causing chloroplast and pigment alterations (Panda and Choudhury, 2005; Scoccianti *et al.,* 2006).

Wastewater with high chromium (VI) concentrations is discharged by different industrial processes into environment without any treatment (Jiang *et al*., 2010). The waste produced by tanneries is included among the main industrial sources of pollution. Such wastewater released from leather industries carries very heavy metals, phenolics, sulphates and phosphates(Chandra *et al*., 2009). At high concentrations, chromium is mutagen, teratogen and carcinogen (Stasinakis *et al*., 2003). When such contaminated water is used for irrigation this impair plant growth and proliferation (Dube *et al*., 2003).

Several authors have reported that diluted industrial effluents have plant growth and yield improving effect on crop plants (Bose and Bhattacharyya, 2008). Therefore treated waste water is a better option for irrigation in present climate change scenarios. Therefore the present study is designed to study the phytotoxic effects of chromium contaminated wastewater on growth of *Triticum aestivum* and reduction of phytotoxicity through microbial inoculation.

Materials and Methods

The phytotoxic effects of chromium were studied using potassium dichromate ($K_2Cr_2O_7$) as chromium source on seeds of wheat (*Triticum aestivum*) variety CHAKWAL 50 (source: NARC).

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Inoculum preparation

Equaling population density of 10^7-10^8 cfu/ml of culture was obtained for the pre isolated strains ACC-14 and CC7 in minimal salt medium (Bangash *et al.*,2013) through incubation at 28-30 °C for 48 h.

Experimental setup

Different experiments were conducted on wheat plant and seeds to test the effect of different concentrations of chromium on seed germination, root and shoot growth of wheat seedlings under axenic conditions. Different concentration of chromium ranging from 0, 20, 40, 60, 80 upto 100 mg L^{-1} of chromium were prepared using $K_2Cr_2O_7$ salt whereas, for control distilled water was used.

Germination parameters

Plants sample were studied for root length, shoot length, root and shoot biomass.

Jar experiment (wastewater)

Seeds of wheat were placed in wastewater for 112 hours . Glass jar experiment was performed in each jar, 50 ml solution was added. Pre-germinated seedlings (afterone week of germination) were transferred into jars containing chromium solution of different concentrations (0, 50 and 100 mg/l) with and without inoculum. To compare the effect of inoculation 5 ml inoculum of each strain (CC7 and ACC-14) was added in the jars (with inoculum). Wastewater was continuously monitored after introducing the inoculated seeds for pH and EC and compared to the control using portable multi-meter probe (MV 400+).

Table 1: Chromium toxicity to the wheat seedlings in petri plate experiment

Cr concentration (mgL^{-1})	Shoot length(cm)	Root length(cm)	Shoot fresh weight(g/plant)	Root fresh weight(g/plant)	Shoot dry weight(g/plant)	Root dry weight(g/plant)
0	5.23 ± 0.52^a	1.53 ± 0.16^a	0.18 ± 0.016^a	0.004 ± 0.0006^a	0.004 ± 0.0006^a	0.003 ± 0.0007^a
20	4.56 ± 0.50^b	1.44 ± 0.13^b	0.04 ± 0.01^b	0.003 ± 0.0005^b	0.0039 ± 0.0006^b	0.002 ± 0.0005^b
40	4.48 ± 0.32^b	1.19 ± 0.17^c	0.03 ± 0.003^c	0.0019 ± 0.0002^c	0.0026 ± 0.0004^c	0.001 ± 0.0002^c
60	3.85 ± 0.21^c	0.75 ± 0.14^d	0.01 ± 0.004^d	0.0013 ± 0.0002^d	0.0025 ± 0.0005^c	0.0008 ± 0.0001^d
80	3.29 ± 0.12^d	0.57 ± 0.10^d	0.01 ± 0.003^d	0.0005 ± 0.0001^e	0.0020 ± 0.0007^d	0.0004 ± 0.0001^e
100	2.66 ± 0.15^e	0.34 ± 0.04^e	0.006 ± 0.003^d	0.0004 ± 0.0001^e	0.0012 ± 0.0002^e	0.0003 ± 0.0001^f

Data of columns indexed by same letter are not significantly different according to Fisher's protected LSD test ($p < 0.05$)

Chromium toxicity to seed (petri plate assay)

Petri plate experiment was conducted to check the seed germination under varying chromium concentration. Five seeds per plate were incubated for each concentration the seeds were placed on filter paper moistened with the contaminated water (0 mg L^{-1} was considered as control). The petri-plates were incubated at 25ºC temperature for 7 days and plant growth parameters were assessed.

Chromium toxicity to plants (Wastewater)

Similar to the petri plate experiment toxicity of chromium was assessed at different chromium concentration in liquid. The seeds were soaked in liquid chromium solution ranging from 0-100 mg L^{-1}. Toxicity was assessed by monitoring root and shoot length and biomass.

Jar experiment (Sand)

Seeds of wheat variety CHAKWAL 50 were grown in sand and placed in 12 h light for 2 weeks. Five ml inoculum of each strain (CC7 and ACC-14) was added in jars (with inoculum). The effect of inoculation on growth of wheat seedlings was assessed at three varying concentrations that is 0 mg L^{-1}, 50mgL^{-1} and 100 mg L^{-1}.

Results

Evaluation of chromium toxicity to wheat seedlings in petri plate experiment

Petri-plate experiment was conducted to assess the effect of different concentrations of chromium on growth parameters of wheat seedlings. Table 1 represents the detailed effect of chromium concentration on growth of wheat seeds. Overall there is a significant reduction growth parameters with increasing concentration of chromium.

Effect of different concentrations of chromium on growth of wheat in wastewater

Table 2 summarizes the effect of different concentrations of chromium on wheat growth in wastewater. Similar to the petri plate experiment there is a significant decline in growth parameters. The highest phytotoxicity is observed for 100ppm concentration (dry root weight = 0.0001g/plant) compared to the 0ppm (0.0032 g/plant). However, the ameliorative effect of inoculation was also evident in the Table 2. The effect of inoculation was more prominent at low concentration of chromium that is at 0 ppm and 50 ppm. The effect is evident for all the observed growth parameters that is root length, shoot length, root and shoot biomass. Similar to the petri plate experiment growth retardation was observed for all the growth parameters. Figure 1 depicts that there is significant retardation in shoot and root growth with every 20 ppm rise in concentration. This decline is consistent for the biomass reduction with wastewater.

Effect of inoculation on growth of wheat seedlings

The effect of inoculation on the growth of wheat seedling under chromium stress indicated a significant ($P<0.05$) improvement in growth parameters as compared to the uninoculated plants (Table 3). The growth in terms of shoot length, root length and root and shoot biomass was significantly higher as compared to their respective uninoculated one at 0 mg L^{-1} and 50 mg L^{-1}. However the effect of inoculation weakens as the concentration approaches to 100 mg L^{-1} of chromium.

chromium in synthetic wastewater. There was a continuous rise in EC in all the concentrations. Although the EC of wastewater with inoculated seeds was lower but the difference to the uninoculated ones was very minimal. A noticeable trend was observed for 50 ppm concentration where EC showed a steady increase till the end of experiment in wastewater with inoculated seeds where in uninoculated seeds there was a steep decline in EC.

Figure 2 represents the change in pH of wastewater. Initially the pH of the wastewater was slightly acidic but with the passage of time the pH began to rise in both treatments. The change was more obvious in wastewater with inoculated seeds the change in pH was more evident and approaches to neutral. Where in wastewater with uninoculated seeds the pH approaches to basic (7.8 at 50 ppm) compared to uninoculated one (7.2 at 50 ppm).

Discussion

In the present study growth retarding effect of different concentrations of chromium was observed on

Table 2: Effect of inoculation on chromium toxicity to wheat seedlings in wastewater

Cr concentration (mg L^{-1})	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
0	12.01^{a}	11.6^{a}	0.0814^{a}	0.002^{a}	0.014^{a}	0.0024^{a}
20	6.4^{b}	3.68^{b}	0.074^{b}	0.0018^{b}	0.009^{b}	0.0007^{b}
40	5.3^{b}	2.63^{c}	0.055^{c}	0.0015^{c}	0.007^{c}	0.0003^{c}
60	4.75^{c}	2.24^{d}	0.017^{d}	0.0005^{d}	0.005^{d}	0.0002^{d}
80	3.633^{d}	1.2^{e}	0.004^{e}	0.00013^{e}	0.00703^{d}	0.00016^{d}
100	2.803^{e}	0.803^{e}	0.002^{e}	0.0016^{e}	0.00406^{e}	0.0016^{d}

Data of columns indexed by same letter are not significantly different according to Fisher's protected LSD test ($p < 0.05$)

Table 3: Reduction in phytotoxicity of chromium through inoculation

Cr concentration (mg L^{-1})	Shoot length(cm)	Root length(cm)	Shoot fresh weight(g/plant)	Root fresh weight(g/plant)	Shoot dry weight(g/plant)	Root dry weight(g/plant)
0 (I)*	11.65 ± 0.57^{a}	2.84 ± 0.14^{a}	0.11 ± 0.01^{a}	0.24 ± 0.001^{a}	0.008 ± 0.001^{a}	0.0032 ± 0.0026^{a}
0 (UI)**	10.03 ± 0.68^{b}	3.66 ± 0.14^{b}	0.04 ± 0.01^{b}	0.15 ± 0.003^{b}	0.0065 ± 0.001^{b}	0.0022 ± 0.0002^{b}
50 (I)	4.13 ± 0.15^{c}	1.56 ± 0.57^{c}	0.30 ± 0.10^{a}	0.25 ± 0.001^{a}	0.0034 ± 0.0004^{c}	0.0007 ± 0.0001^{c}
50 (UI)	3.57 ± 0.25^{d}	1.26 ± 0.52^{d}	0.13 ± 0.003^{b}	0.010 ± 0.001^{b}	0.0023 ± 0.0004^{d}	0.0004 ± 0.00006^{d}
100 (I)	2.06 ± 0.15^{e}	1.15 ± 0.05^{e}	0.0096 ± 0.001^{a}	0.0018 ± 0.001^{c}	0.0025 ± 0.0003^{d}	0.0008 ± 0.00009^{c}
100 (UI)	2.03 ± 0.20^{e}	1.10 ± 0.05^{e}	0.0074 ± 0.001^{a}	0.001 ± 0.0006^{c}	0.0016 ± 0.0002^{e}	0.0001 ± 0.00001^{e}

*I: inoculated, **UI: Uninoculated; Data of columns indexed by same letter are not significantly different according to Univariate analysis of variance ($p < 0.05$).

Effect of inoculated wheat seedlings on wastewater characteristics

Figure 1 demonstrates the variation in electrical conductivity of the wastewater at three different levels of

growth of wheat seedlings. The toxicity of chromium was more profound in roots that may be due to the fact that plant roots were the first point of contact for those toxic chromium species in the growth media. It was also reported previously that root growth was more adversely affected than shoot growth (Vernay *et al*., 2008). Growth inhibition by chromium and higher sensitivity of root biomass compared to shoot biomass was found for seedlings of *Miscanthus sinensis*, *Sorghum bicolor*, *Triticum aestivum* and *Vigna radiate* (Shanker and Pathmanabhan, 2004; Shanker *et al*., 2004; Arduini *et al*., 2006). The reduction in roots length limits the plants reduction of biomass production (Terry, 1981) by the plant as observed in present study (Table 1).

The effect of inoculation was in the preset study was more profound at lower concentration however the effect of inoculation weakens with raising concentration (Table 3). The increased plant growth and root and shoot biomass compared to uninoculated plants could be attributed to

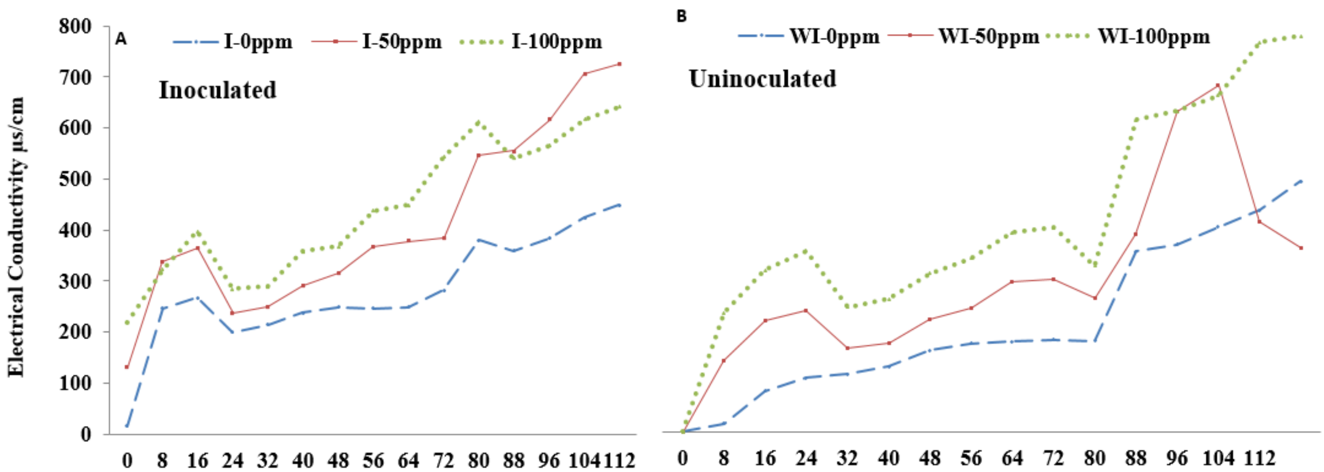

Figure1: Variation in EC (electrical conductivity of synthetic wastewater at different chromium levels. a; effect of inoculation, b; effect of uninoculated wheat seeds

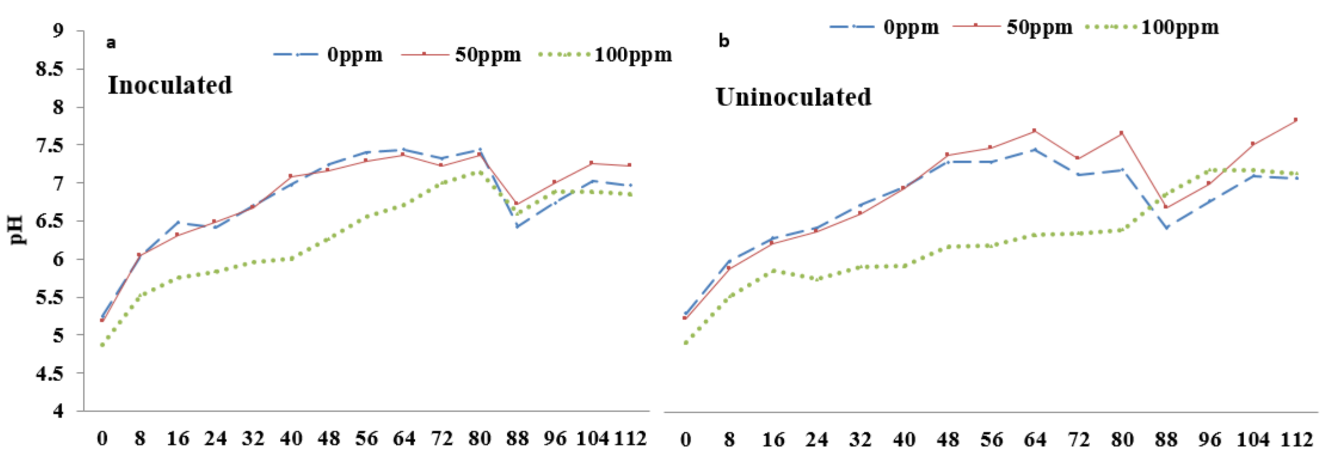

Figure2: Variation in pH of synthetic wastewater at different chromium levels. a; effect of inoculation, b; effect of Uninoculated wheat seeds

access to nutrients therefore less nutrients and water is transported to ariel parts of plants (Banerjee *et al.*, 2008). This can be related to the presently observed decreased shoot length. Previously the similar trend was observed by Rajkumar *et al.* (2006) for wheat plant. The decrease in length of root and shoot of wheat results in promotional activity of PGPR and reduction in growth retarding biochemical like ethylene. Previously increased growth by PGPR (Plant growth promoting rhizobacteria) has been reported due the reduction in ethylene concentration in plant cells (Ashraf *et al*., 2004; Abedin and Meharg, 2002 Bangash *et al.*, 2013). Thus, this could be reason of better performance of inoculated plants in terms of growth as compared to uninoculated plants (Table 2). The uninoculated plants exposed to different concentrations of chromium, showed a marked inhibition in the growth. Plants inoculated with bacterial strains CC7 and ACC-14 exhibited an increase in root length, shoot length, plant fresh and dry weights in presence of chromium. Yadav *et al*. (2010) concluded in his study that inhibitory effects of Cr on seed germination could be due to its depressive effects on amylases activity and ceased the transport of sugars to the embryo axes.

Conclusion

The present study highlighted the growth reduction of wheat when irrigated with chromium contaminated wastewater. However the phytotoxicity could be reduced by using potent PGPR like CC7 and ACC-14. The strains were capable of promoting plant growth even under chromium stress. Therefore these could be opted to ameliorate the toxic effects of chromium at contaminated sites.

References

Abedin, M. J. and A. A. Meharg. 2002. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant soil*, 243: 57–66.

Abreu, M.M., F. Calouro, and M.L.V. Fernandes. 2002. Application of chromium to soils at different rates and oxidation states. II. Influence on uptake of selected nu-trients by radish. *Commun. Soil Sci. Plan*. 13:2269-2277.

Arduini, I., A. Masoni and L. Ercoli. 2006. Effects of high chromium applications on miscanthus during the period of maximum growth. *Environ. Exp. Bot*., 58: 234-243.

Ashraf, M., S. Hasnain, O. Berge and T. Mahmood. 2004. Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. *Biol. Fert. Soils*, 40: 157–162.

Banerjee, A., D. Nayak, D. Chakrabortty and S. Lahiri. 2008. Uptake studies of environmentally hazardous $_{51}$Cr in Mung beans. *Environ. Pollut.*, 151: 423- 427.

Bangash, N., A. Khalid,T. M and M. T. Siddique. 2013. Screening rhizobacteria containing Acc-deaminase for Growth promotion of wheat under water stress. *Pak. J. Bot.*, 45: 91-96.

Bluskov, S., J.M. Arocena, O. O. Omotoso, and J. P. Young. 2005. Uptake, distribution, and speciation of chromium in *Brassica Juncea*. *Int. J. Phytoremediat.* 7:153–165

Bose, S. and A. K. Bhattacharyya. 2008. Heavy metal accumulation in wheat plant grown in soil emended with industrial sludge. *Chemosphere*, 70: 1264-1272.

Chandra, R., R. N. Bharagava, S. Yadav and D. Mohan. 2009. Accumulation and distribution of toxic metals in Wheat and Indian mustard irrigated with distillery and tannery effluents. *J. Hazard. Mater*., 162: 1514-1521.

Dube, B. K., T. Kamlesh, J. Chatterjee and C. Chatterjee. 2003. Excess chromium alters uptake and translocation of certain nutrients in citrullus. *Chemosphere*, 53: 1147–1153.

Han, X. F., S. B. Maruthi, L. D. Monts and Y. Su. 2004. Phytoavailability and toxicity of trivalent and hexavalent to *Brassica juncea*. *New phytol.*, 162: 489-499.

Jiang, J., L. Wu, N. Li, Y. Luo and Q. Zhao. 2010. Effects of multiple heavy metal contamination and repeated phytoextraction by *Sedum Plumbizinicicola* on soil microbial properties. *Eur. J. Soil Biol.*, 46: 18-26.

Khan, A.G. 2001. Relationships between chromium biomagnification ratio, accu-mulation factor, and mycorrhizae in plants growing on tannery effluent-polluted soil. *Environ. Int.* 26:417-423.

Prasad, A., A. K. Singh, S. Chand, C. S. Chanotiya, D.D. Patra. 2010. Effect of chro-mium and lead on yield, chemical composition of essential oil, and accumulation of heavy metals of mint species. *Commun. Soil Sci.* Plant 41: 2170-2186.

Rajkumar, M., R. Nagendran, K. J. Lee, W. H. Lee and S. Z. Kim. 2006. Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. *Chemosphere*., 62: 741-748.

Raju, M. and S. N. Tanson. 1999. Operationally determined speciation of chromium in tannery sludges. *Chem. Speciat. Bioavailab*. 11: 67-70.

Shanker, A. K. and G. Pathmanabhan. 2004. Speciation dependant antioxidative response in roots and leaves of Sorghum *bicolor* (L. Moench). V CO_2 under Cr (III) and Cr (VI) stress. *Plant Soil*., 265: 141–151.

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 green gram (*Vignaradiata* (L.) R. Wilczek. cv CO4) roots. *Plant Sci*., 166: 1035–1043.

Stasinakis, A. S., N. S. Thomaidis, D. Mamais, E. C. Papanikolaou, A. Tsakon and T. D. Lekkas. 2003. Effect of chromium (VI) addition on the activated sludge process. *Water Research.*, 37: 2140–2148.

Terry, N. 1981. An analysis of the growth responses of *Beta vulgaris* L. to phytotoxic trace elements. II. Chromium. *Final report to the Kearney Foundation of Soil Science*, July 1975–June 1980.

Vernay, P., C. G. Moussard, L. Jean, F. Bordas, O. Faure, G. Ledoigt and A. Hitmi. 2008. Effect of chromium species on phytochemical and physiological parameters in *Datura innoxia. Chemosphere*, 72: 763–77.

Yadav, S. K., M. Dhote, P. Kumar, J. Sharma, T. Chakrabarti, and A. A. Juwarkar. 2010. Differential antioxidative enzyme responses of *Jatrophacurcas* L. to chromium stress. *J. Hazard. Mater*., 180: 609–615.