Indian Journal of Experimental Biology
Vol. 52, December 2014, pp. 1206-1210

Chromium accumulation potential of *Zea mays* grown under four different fertilizers

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Received 29 January 2014; revised 03 November 2014

Chromium (Cr) contamination in soil is a growing concern in sustainable agriculture production and food safety. We performed pot experiment with chromium (30 mg/ soil) to assess the accumulation potential of *Zea mays* and study the influence of four fertilizers, viz. Farm Yard Manure (FYM), NPK, Panchakavya (PK) and Vermicompost (VC) with respect to Cr accumulation. The oxidative stress and pigment (chlorophyll) levels were also examined. The results showed increased accumulation of chromium in both shoots and roots of *Zea mays* under FYM and NPK supply, and reduced with PK and VC. While the protein and pigment contents decreased in Cr treated plants, the fertilizers substantiated the loss to overcome the stress. Similarly, accumulation of Cr increased the levels of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) indicating the enhanced damage control activity. However, these levels were relatively low in plants supplemented with fertilizers. Our results confirm that the maize can play an effective role in bioremediation of soils polluted with chromium, particularly in supplementation with fertilizers such as farm yard manure and NPK.

Keywords: Bioremediation, Environment, Farm yard manure, Heavy metal stress, Leather industry, Maize, NPK, Panchakavya, Phytoremediation, Soil pollution, Vermicompost.

Chromium (Cr) is the principal heavy metal leached into the environment by tannery and dye industry which cause significant undesirable biological and ecological effects[1,2]. Cr affects higher vascular plants in their growth, reproduction and development[3]. Usual physicochemical methods for metal remediation such as filtration, acid leaching, electrochemical processes or ion exchange are not only expensive but also spoil the soil quality[4]. Phytoremediation is the most widespread bioremediation method and prospective approach wherein plants are used to remove toxic and heavy metals from polluted soils[1].

Plants such as *Helianthus annuus* L., *Zea mays L.*, *Brassica juncea*, *etc.* are known for their tolerance to heavy metals, and hence play vital role in phytoremediation[5,6]. The microbe associated phytoremediation (MAP) technology is also used in the remediation of various sites contaminated with metals, hydrocarbons and pesticides, *etc.*[7]. Phytoextraction of metals involves two strategies, viz. genetic engineering and chelating agents[8]. Factors such as soil pH, organic matter, Cation Exchange capacity (CEC), *etc.*, influence the solubility and accessibility of metals to plants[9]. Organic acids present in manure like compost, cattle manure, *etc.* chelate metals. Organic chelating materials from farm sources are inexpensive and degradable.

The bioavailability of metals also changes with inorganic amendment by developing binding sites[10]. Present work compares the efficiency of fertilizers in phytoremediation of chromium through maize plants. In this study, we examined the responses and tolerance of *Zea mays* exposed to chromium and analyzed the influence of four different fertilizers on metal accumulating capability, pigment (chlorophyll) and protein content, and antioxidant activities of the plant.

Materials and Methods

*Experimental set up*ð Pot culture experiments were conducted under uniform conditions of light, soil and water in separate pots. Soil was collected from nursery which has normal soil properties and not contaminated. Sterilized soil filled pots were divided into 8 groups of two pots each and the test groups were added with 30 mg/ concentration of chromium

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as $K_2Cr_2O_7$. Equal number of seeds (15) were sown in each pot and after germination, the number of plants reduced to 8 plants per pot. One pot each served as plain control (with water) and another test control (with chromium). The experiment was carried out for 30 days in outdoor and the pots were irrigated with 500 ml of water each on alternate days. The study was carried out during January.

*Fertilizers*ð Four different fertilizers (Farm Yard manure (FYM), NPK, Vermicompost (VC) and Panchkavya (PK) were selected to analyze the enhancement capability of phytoextraction and tolerance potential of *Zea mays* under chromium toxicity. FYM, NPK, VC and PK were purchased from Saradha Nursery, Kumbakonam, and NPK from commercial fertilizer shop. Eight pots were filled with soil mixed with different fertilizers as follows: 1/pot FYM, 20:20:10; NPK, 200g/pot; VC and PK 1g/pot as per usual concentration used by researchers. Plants were irrigated regularly with water and removed carefully on 30th day and physical and biochemical parameters were analyzed.

*Experimental design*ð The experimental design had four groups based on the fertilizers studied, and one test control as given below:

[Control] Soil without fertilizer Test control - 30 ppm Cr polluted soil

Group I Control - Soil + FYM Group I Test - 30 ppm Cr polluted soil + FYM

Group II Control - Soil + NPK Group II Test ï 30 ppm Cr polluted soil + NPK

Group III Control - Soil + PK Group III Test ï 30 ppm Cr polluted soil + PK

Group IV Control - Soil + VC Group IV Test ï 30 ppm Cr polluted soil + VC

One with plain water served as control.

*Pigment analysis*ð Fresh leaf samples (0.5 g) of 30 days old plants were centrifuged for 10 min at 3000 rpm after addition of 5 ml of 80% acetone and the supernatant was collected. The precipitate was re-extracted with 80% acetone until the extract lost its green color. All the supernatants were combined and made up to a known volume with 80% acetone. Absorbance was measured at 645 nm and 663 nm for Chlorophyll (*Chl*) a and *Chl* b, respectively, and were calculated as reported earlier including total chlorophyll[11].

*Protein and antioxidant enzymes*ð Protein content of experimental plants was analyzed using Lowry method[12]. The whole plant was taken for analysis. Cr induced toxicity and the influence of fertilizers was measured by estimating the antioxidant enzyme levels. Super Oxide Dismutase (SOD) was assayed following Kakkar *et al*[13], catalase (CAT) activity, by Luck *et al*[14], and peroxidase (PER) estimation by Reddyõs method[15].

*Chromium in root and shoot*ð The harvested plants were separated out as roots and shoots, crushed into powder and incinerated at high temperature. About 0.1ï1.0 g of powdered sample or 1-20 ml of liquid samples were taken in a known weight silica crucible and kept in a muffle furnace at 450-500 °C for 4-5 h till it turned into ash. For liquid samples, the excess volume was reduced to 1-2 ml before made into ash. The ash was dissolved in suitable acid. Depending on the need, the ash was subjected to digestion as described below.

Samples were digested with 1:1 concentrated nitric acid and distilled water on hot plate for 4-5 h. They were kept in open condition. The loss of acid by vaporizations was adjusted by adding the same acid mixture. After cooling they were made up to 100 ml by adding distilled water. They were filtered through Whatman No. 1 filter paper and analyzed using flame Atomic Absorption Spectrometer[16].

Variation in the level of pigments, protein, antioxidant enzymes and chromium in shoot and root were analyzed. The ótô test analysis was performed for the data obtained and the results were compared.

Results and Discussion

*Pigments*ð The ótô test results showed significant differences in *Chl* a, b and total Chlorophyll levels of *Zea mays* (*P*' 0.5). Maximum reduction in *Chl* was observed in test control group which received Cr alone with values 0.65, 0.4 and 1.05 mg/g, respectively (Fig. 1). The levels of pigments have shown significant increase in fertilizer treated plants compared to test control. Highest total *Chl* was 2.82 mg/g in NPK+water treated plants. Among the test groups treated with Cr and fertilizers, the FYM had 2.27 mg/g followed by NPK and VC with 1.98 mg/g each, and PK 1.75 mg/g. The increased pigment content could be attributed to the enhanced nutrient supply by the fertilizer.

Chromium degrades ŭ-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis[17]. Significantly reduction of photosynthesis by Cr exposure was evident by the reduced chlorophyll content. The increased *Chl* content in the Cr+fertilizer treated groups compared to the test control suggests that fertilizers attenuate the damage caused by Cr to some extent and stimulates growth.

Protein level— Mean protein level of Cr treated plants have shown significant decrease (22%) compared to control (Fig. 2a). The test control with Cr had minimal protein content (17.13 mg/g) while the control group with water alone had 22.25 mg/g. However, application of fertilizers increased protein content of the plants; marginally in water treated groups and the test group II and III (NPK and PK); and significantly in group I and IV (FYM and VC). The efficiency of fertilizers in terms of protein content of Cr treated plants was in following order FYM>VC>NPK>PK.

The present study indicates consistent decrease in protein content in groups under Cr treatments that explain antioxidant defense activity. Decreased protein content under heavy metal stress is attributed to enhanced protease activity, structural and competent functional modifications by the disintegration of proteins[18], DNA-protein cross-links[19], interaction with SH residues of proteins and substitute them with heavy metals in metalloproteins, etc.[20]. Earlier workers have shown that cadmium by preventing the uptake of Mg and K decreases protein content and encourage posttranslational modification[21], reduction in synthesis or enhancement in protein catabolism[22].

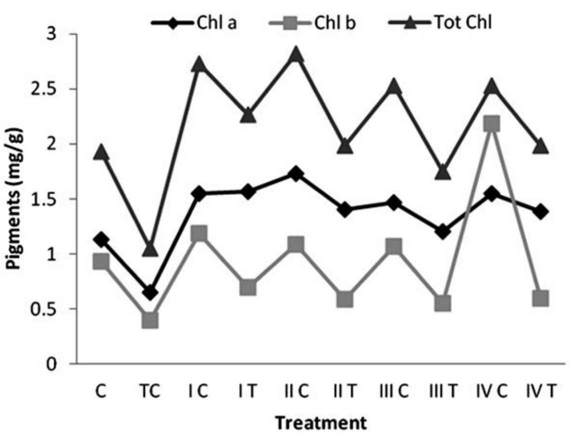

Fig. 1— Effect of different fertilizers under chromium stress on pigment level in *Zea mays*. [C, Control; TC, Test Control Cr; I C, Group I Control (water+FYM); I T, Group I Test (Cr+FYM); II C, Group II Control (water+NPK); II T, Group II Test (Cr+NPK); III C, Group III Control (water+PK); III T, Group III Test (Cr+PK); IV C, Group IV Control (water+VC); IV T, Group IV Test (Cr+VC)]

Antioxidant enzymes— The role of antioxidant enzymes in stress condition is vital for both plants and animals. They fight free radicals formed in such situations which would otherwise damage the cells. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) convert such toxic free radicals to nontoxic moity. SOD is a biomarker of environmental stress and crucial component of plant antioxidation system[23]. It converts superoxide anion O^{2-} to H_2O_2 in cytosol, mitochondria and chloroplast and helps in cellular defense mechanisms against the risk of OH formation[24]. High accumulation of ROS increases SOD for activating the antioxidative defense enzymes to slow down the oxygen radical accumulation and de-novo synthesis of the enzymatic proteins under heavy metal stress[25]. Increased CAT activity was predictable as the increased SOD leads to H_2O_2 formation which will be further detoxified by CAT or POD to keep the cellular redox. CAT plays an important role in plant defensive mechanisms in mitochondria and peroxisomes[26] and has major role in scavenging free radicals, especially H_2O_2 produced by photorespiration[27] and stress conditions[28]. By catalyzing H_2O_2 to H_2O and O_2 via two-electron transfer it prevents OH^{-} ions and protects nucleic acids, proteins, and lipids against ROS[29].

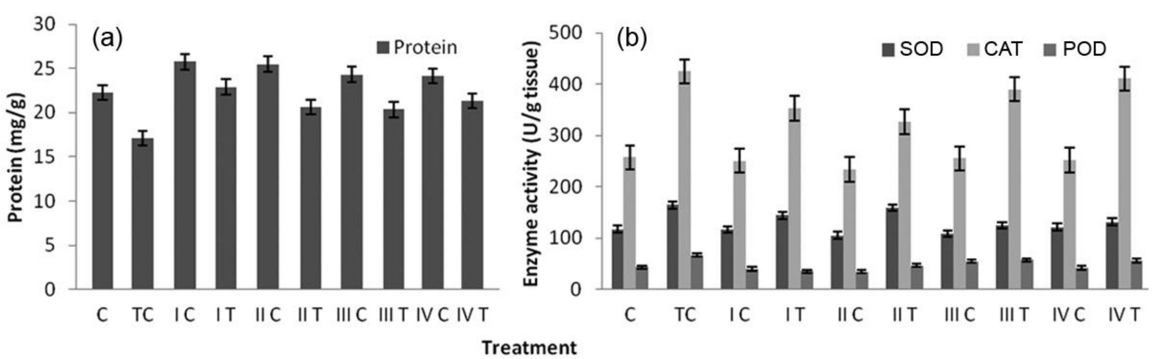

Fig. 2— Effect of different fertilizers under Cr stress on (a) protein level; and (b) antioxidant enzymes in *Zea mays*. Other details are same as in Fig. 1.

Here, we observed increased SOD, CAT and POD activity to the extent of 40, 65 and 54%, respectively in the test control (Cr) plants compared to control (Fig. 2b). However, the SOD showed 24% decrease in group III test (Cr+PK); CAT, 23% in group II test (Cr+NPK); and POD, 47% in group I test (Cr+FYM) compared to test control. The mild decrease in the antioxidant levels in water+fertilizer treated plants possibly indicate the attenuation role played by the fertilizers.

Chromium uptake

The Cr content in the roots was higher than that of the shoots (Fig. 3). The plants grown in FYM-applied soil had the highest concentration of Cr (498 µg/g and 290 µg/g) followed by NPK test group (472 µg/g and 257 µg/g), higher than even the control (413 µg/g and 179 µg/g), respectively in root and shoot. Compared to control, shoot and root concentrations of group III and IV (Cr+PK and Cr+VC) showed 6, 35% reduction in shoot Cr level; and 12, 24% in root Cr level, respectively. The order of chromium accumulation in the presence of tested fertilizers by *Zea mays* was FYM>NPK>PK>VC in both root and shoot.

The metal uptake in plants is fixed to a chemiosmotic route diagonal to the membrane of intact root cells[30]. *Brassica juncea* accumulates more Cd in their roots as compared to aerial parts[31]. Chromium accumulation varies depending on the plant species, and within the species, different parts[32]. It is reported to be greater in roots followed by leaves[33]. In our earlier study, we have demonstrated that Cr bioaccumulation of macrophytes in the shoot is less than that of the root[34]. It has also been shown that Cr is not uniformly dispersed in roots that act as barriers to symplastic and apoplastic Cr translocation. Hence, Cr transport to shoot gets controlled as the plants have no specific system for Cr transport[30]. Further, the translocation of Cr to shoot from root is a vital factor disturbing accumulation of this metal in above ground tissues[32]. These earlier works explain our observation of higher accumulation of Cr in roots compared to shoots.

The ease of access of heavy metals present in soil is based on soil pH, chemical speciation of the metal CEC, total dissolved solids *etc.*[35]. Phyto availability of metals was highest in fertilizer treated soil because of the reduced soil pH. Phosphorous source in NPK is superphosphate. The monocalcium phosphate (MCP) gets dissolved leading to formation of soluble dicalcium phosphate (DCP) slowly with the discharge of phosphoric acid close to the fertilizer granules. The phosphoric acid changes into phosphate and hydrogen ions. The protons lower the pH around the fertilizer to and thereby ease higher accumulation of heavy metals[36].

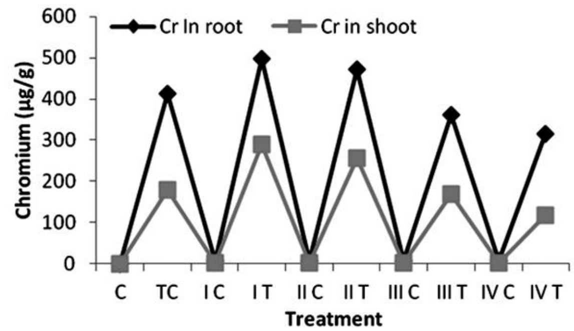

Fig. 3ð Effect of different fertilizers on the uptake of chromium by *Zea mays*. Other details are same as in Fig. 1.

The present study confirms that *Zea mays* are capable of up taking Cr. Roots accumulate more Cr than the shoots. Exposure of *Zea mays* to Cr results in decreased overall growth, and pigment and protein content. However, application of fertilizers restores the damage caused by the heavy metal Cr and shows increased protein and pigment content, and antioxidant activity. Farm yard manure (FYM) and NPK enhances plant growth and development and accumulation of metal. It can be concluded that *Zea mays* can be used as an effective agent in phytoremediation of soils polluted with chromium with the help of fertilizers such as FYM and NPK.

References

1 Lasat M M, Phytoxtraction of toxic metals: a review of biological mechanisms, *J Environ Qual,* 31 (2002) 109.

2 Chandra P, Sinha S & Rai U N, Bioremediation of Cr from water and soil by vascular aquatic plants, In Phytoremediation of Soil and Water Contaminants, ACS Symposium Series #664, edited by Kruger E L, Anderson T A & Coats J R , (American Chemical Society, Washington, DC), 1997, 274.

3 Cervantes C, Garcia J C, Devars S, Corona F G, Tavera H L, Carlos Torres-guzman J & Sanchez R M, Interactions of chromium with micro-organisms and plants FEMS, *Microbiol Rev,* 25 (2001) 335.

4 Dixon B, Bioremediation is here to stay, *ASM News,* 62 (1996) 527.

5 Pilon-Smits E, Phytoremediation, On line at: arjournals.annualreviews.org, *Annu Rev Plant Biol,* 56 (2005) 15.

6 Milner M J & Kochian L V, Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system, *Ann Bot,* 102 (2008) 3.

7 Juwarkar A A, Microbe-assisted phytoremediation for restoration of biodiversity of degraded lands: a sustainable solution, *Proc Natl Acad Sci, India Sec B,* 82 (2) (2012) 313.

8 Lai H Y & Chen Z S, Effects of EDTA on solubility of cadmium. Zinc and lead and their uptake by rainbow pink and vetiver grass, *Chemosphere*, 55 (2004) 421.

9 Singh R P & Agrawal M, Effects of sewage sludge amendment on heavy metal accumulation and consequent responses of *Beta vulgaris* plants, *Chemosphsere,* 67 (11) (2007) 2229.

10 Puschenreiter M, Horak O, Friesl W & Hartl W, Low cost agricultural measures to reduce heavy metal transfer into the food chain ï a review, *Plant Soil Environ*, 51 (1) (2005) 1.

11 Arnon D T, Copper enzymes in isolated Chloroplast polyphenol oxidase in *Beta vulgaris, Plant Physiol*, 24 (1949) 1.

12 Lowry O H, Rosebrough N J, Farr A L& Randall R J, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 193 (1951) 265.

13 Kakkar P, Das B & Viswanathan P N, A modified spectrophotometric assay of SOD, *Indian J Biochem Biophys*, 21 (1984) 130.

14 Luck H, Methods of Enzymatic Analysis (Bergmeyer, H U., ed.), Academic Press, New York, (1963) 886.

15 Reddy K P, Subhani S M, Khan P A & Kumar K B, Effect of light and benzyl adenine on dark-treated growing rice leaves, changes in peroxidase activity, *Plant Cell Physiol*, 24 (1995) 987.

16 APHA, Standard methods for the examination of waste and waste water (14^{th} Edition) American Public Health Association, New York. 1990.

17 Vajpayee P, Tripathi R D, Rai U N, Al M B & Singh S N, Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L, *Chemosphere*, 41(2000) 1075.

18 John P, Ahmad P, Gadgil K & Sharma S, Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *Int J Plant Prod*, 3 (2009) 65.

19 Atesi I, Suzen H S, Aydin A & Karakaya A, The oxidative DNA base damage in tests of rats after intraperitoneal cadmium injection, *Biometals,* 17 (2004) 371.

20 Pál M, Horváth E, Janda T, Páldi E & Szalai G, Physiological changes and defense mechanisms induced by cadmium stress in maize, *Plant Nutr Soil Sci*, 169 (2006) 239.

21 Romero-Puertas M C, Corpas F J, Rodríguez-Serrano M, Gђmez M, del Río L A & Sandalio L M, Differential expression and regulation of antioxidative enzymes by cadmium in pea plants, *Plant Physiol,* 164 (2007) 1346.

22 Monteiro M S, Santos C, Soares A & Mann R M, Assessment of biomarkers of cadmium stress in lettuce, *Ecotoxicol Environ Saf*, 72 (2009) 811.

23 Dazy M, Masfaraud J F & Férard J F, Induction of oxidative stress biomarkers associated with heavy metal stress in *Fontinalis antipyretica* Hedw, *Chemosphere,* 75(2009) 297.

24 Gratão P L, Polle A, Lea P J & Azevedo R A, Making the life of heavy metal-stressed plants a little easier, *Funct Plant Biol*, 32 (2005) 481.

25 Verma S & Dubey R S, Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants, *Plant Sci* 164 (2003) 645.

26 Gupta M, Sharma P, Sarin N B & Sinha A K, Differential response of arsenic stress in two varieties of *Brassica juncea* L., *Chemosphere*, 74 (2009) 1201.

27 Bowler C, Montagu M V & Inzé D, Superoxide dismutase and stress tolerance, Annu *Rev Plant Physiol* 43 (1992) 83.

28 Foyer C H & Noctor, Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context, *Plant Cell Environ* 28 (2005) 1056.

29 Wang Z, Zhang Y, Huang Z & Huang L, Antioxidative response of metal-accumulator and non-accumulator plants under cadmium stress, *Plant Soil* 310 (2008) 137.

30 Zayed A M & Terry N, Chromium in the environment: factors affecting biological remediation, *Plant Soil*, 249 (2003) 139.

31 Mohd Irfan, Aqil Ahmed & Shamsul Hayat, Effect of Cadmium on growth and antioxidant enzymes in two varieties of *Brassica juncea*, *Saudi J. Biol Sci*, 21 (2014) 125.

32 Lahouti M & Peterson P J, Chromium accumulation and distribution in crop plants, *J Sci. Food Agri*, 30 (1979) 136.

33 Remon E, Bouchardon JL, Cornier B, Guy B, Leclerc JC, Faure O, Soil characteristics, heavy metal availability and vegetation recovery at a former metallurgical landfill: implications in risk assessment and site restoration, *Environ Pollut*, 137 (2005) 316.

34 Dheeba B, Sampathkumar P, Kannan K, Growth, Chromium Accumulation Potential and Biochemical Changes of *Vigna radiata* and *Zea mays* Grown with Effective Microbes. *Proc. Natl Acad Sci India Sect B Biol Sci*, 84(2)(2014) 381.

35 Smith S R, A critical review of the bioavailability and impact of heavy metals in municipal solid waste composts compared to sewage sludge, *Environ Int*, 35 (2009) 142.

36 Bolan N S, Naidu R, Syers J K & Tillman R W, Surface charges and solute interactions in soils, *Adv Agron*, 67 (1999) 88.