Effect of ethylene diamine tetra acetic acid chelator in the presence of chromium on growth and some physiological characteristics of sunflower

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Abstract

Chromium is a heavy metal toxic for plants that due extensive industrial applications over the last decade, has become a serious environmental pollution. On the other hand, chelator dissolution and absorption of metal by the plant increase and their use in phytoremediation is important. The aim of this study was to evaluate the effect of different concentrations of chromium and ethylene diamine tetra acetic acid (EDTA) chelator on sunflower (*Heliantus annuus* L.) through the study of growth parameters, chlorophyll, and soluble sugar amounts and Cr accumulation in plants under hydroponic culture. In order to plant sunflower were treated with different concentrations of Cr (1, 2, 4 and 6 ppm) and EDTA (0, 1 and 3 mg/L) in the medium Hoagland. The results showed that the use of chromium reduced the length of shoots and roots, and chlorophyll, while it increased soluble sugars. Addition of EDTA to the metal increased the shoot and root length and chlorophyll content while it reduced soluble sugars. According to the results, the use of chromium heavy metal in medium increased the amount of it in shoot and root. EDTA application, particularly with higher levels of chromium led to its accumulation in shoot and root. This research suggested positive role of application of EDTA in the presence of chromium heavy metal in increasing the power of phytoremediation of sunflower.

Keywords: chrome; ethylene diamine tetra-acetic acid; sunflower; photosynthesis; growth

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Introduction

Heavy metals pollution affects the atmosphere, water, and soil in many places around the world (Meagher, 2000; Raskin and Enley, 2000; Cunningham et al., 1997). Excessive accumulation of heavy metals in agricultural soils leads to more uptake by plants and thus affects the quality and safety of food (Wang et al., 2001). This can be considered as a danger to human health (Liphadzi et al., 2006).

Chromium is an abundant element on the earth that is considered as one of the most important environmental pollutants in recent decades (Panda and Choudhury, 2005). Research has shown that low concentrations of chromium can increase plant growth (Samantaray et al., 1998). While higher concentrations of chromium is highly toxic for humans, animals, and plants and can increase the risks of developing cancer and genetic disorders (Zhang et al., 2007). It is reported that poisoning of plants by chromium

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can reduce plant growth, pigment content, and change the activity of enzymes and damage the ultrastructure of chloroplasts and cell membranes (Hu et al., 2005). Cadmium effects on the different stages of photosynthesis (include the carbon dioxide fixation, transmission electron, and photo phosphorylation)(Arun et al., 2005).

Phytoremediation is a cost-effective technology and safe for the environment which improves soil contaminated with heavy metals (Saifullah et al., 2010). One of the major problems with phytoremediation is the lack of mobility of metals in soil (Fazal et al., 2010). Chelators effectively increase the solubility of heavy metals in soil and subsequent absorption by plants. Adding chelators increase absorption and accumulation of heavy metals by plants and thereby reduces their levels in soil (Chen et al., 2003).

Ethylene diamine tetra-acetic acid (EDTA) is a synthetic chelators which has high capacity for metals and its complex with metals easily is absorbed by plants (Liphadzi et al., 2006). Some species are resistant to a certain amount of heavy metals in soil and have the ability to absorb and stabilize them in their internal organs. However, in some plants the toxicity effects due to absorption of heavy metals are not so visible and this can pose threats for animal or human health (Chatterjee and Chatterjee, 2000).

It is reported that sunflower is resistant to heavy metals. This plant can accumulate high levels of chromium in roots and at the same time produce high biomass (Piroozand Kalantari Manochehri, 2012). In this regard, it has been shown that sunflowers can accumulate significant amounts of Pb when exposed to a chelator such as EDTA (Huang and Cunningham, 1996; Blaylock et al., 1997). According to the above description, the aim of this study was to find the effects of EDTA chelator with different levels of chromium on growth indices and some characteristics of sunflower plant.

Method and Materials

Hybrid Haysvn33 sunflower seeds (*Helianthus annuus L.*) were used in the study. Seeds were first disinfected with sodium hypochlorite 3% for 3 minutes. Then they were put on wet paper to germinate. After 10 days seedlings were planted in plastic pots containing sand and were irrigated with Hoagland solution. In the next step, 30-day-oid seedlings were transferred to dishes containing the Hoagland solution with two different concentrations (1 and 3 mg per liter) EDTA and 1, 2, 4, and 6 ppm Cr6 in the form of sodium dichromate. In each container 20 sunflower plants were planted. Each dish was aerated regularly during the 24 hours by aquarium pumps. Dishes were placed in the lighting condition of 16 hours light and 8 hours darkness, and day and night temperature 22±4 °C. After 14 days, the plants were excised from the dishes and were prepared for the measurements.

Measurement the length, fresh, and dry weight of roots and shoots of sunflower

A ruler was used to determine the length of roots and shoots. To determine the dry weight, the plants were placed in an oven set at 80 °C for 24 hours. Digital scale was used to determine the dry and fresh weight.

Measurement of photosynthetic pigments

At first, 1 g of fresh leaf weight was pulverized with 10 ml Acetone 80%. The solution was purified with Whatman 2 filter paper. Then, the absorbance of the solution at wavelengths of 645 and 663 nm were measured by a spectrophotometer (Jenson, 1987). Chlorophyll content was calculated using the following formulas in milligrams per gram fresh weight:

$$Chla=0.0127A663 - 0.00269A645$$
$$Chlb=0.0229A645 - 0.00468A663$$
$$Chlt=0.0202A645 + 0.00802A663$$

Soluble sugars assay

To determine the soluble sugars, shoots and roots were dried in the oven at 90 °C for 48 h. They were then weighed and 10 ml ethanol (70%) was added. Then the samples were placed in Petri dishes for 7 days at 4 °C. Soluble sugars contents were determined by measuring the absorbance at 485 nm spectrophotometrically with Kochert (1978) method. Glucose standard curve was used to estimate the soluble sugars concentration (mg g-1 DW).

Measuring the amount of chromium

In order to measure the concentration of chromium to 0.05 grams of dried root and shoot tissue 3 ml of concentrated nitric acid was added separately. In order to complete the digestion, solution was slowly heated for 48 hours until it finally became transparent and colorless. The solution volume was increased to 25 ml with distilled water and was used to measure the absorption of chromium by atomic absorption spectrophotometry at 357.9 nm (Sauna, AA-GBC).

Statistical analysis

The statistical significance of the difference between means was evaluated by Duncan-test on SPSS 11.5 for each treatment and control. The results are given at p≤0.01.

Results

Length of shoot and root

The results showed the maximum shoot length in the treatment of 3 mg/l EDTA with 1 ppm of Cr while the minimum length was measured in 4 and 6 ppm Cr treatments. The use of chromium at concentrations of 4 and 6 mg per liter resulted in decreased length shoot while the use of EDTA with the metal led to an increase in shoot length compared to the control and treatment of chrome without chelator (Fig. I). Also largest roots were related to treatment with 1 mg EDTA and 2 and 4 ppm of chromium, and the shortest roots were observed in control. Chrome application at all tested concentrations resulted in increased root length (Fig. II).

Shoot and root dry weight

Chrome application at various concentrations had no significant effect on the dry weight. Application of EDTA at concentration of 1 mg/l with metal led to increased dry weight

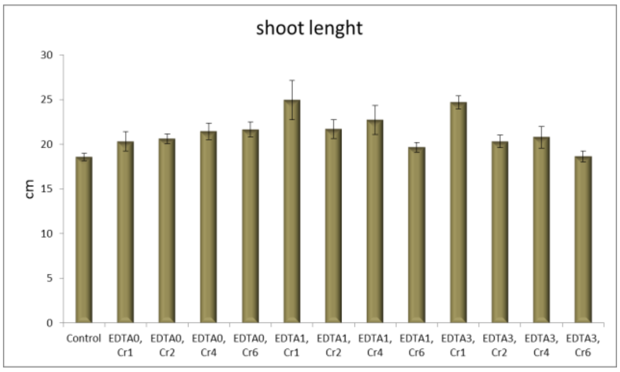

Fig. I. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA (0, 1, and 3 mg /L) and Cr (1, 2 , 4, and 6 ppm) on shoot length of sunflower

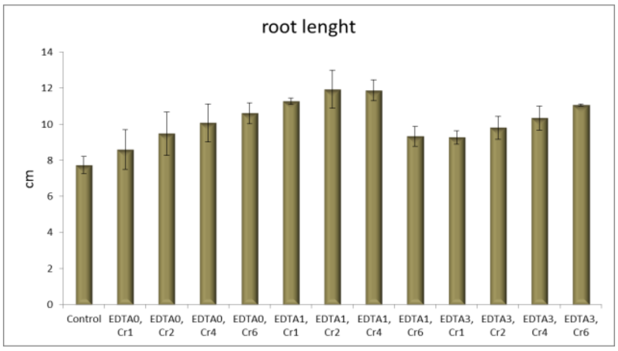

Fig. II. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA(0, 1, and 3 mg /L) and Cr (1,2 , 4, and 6 ppm) on root length of sunflower

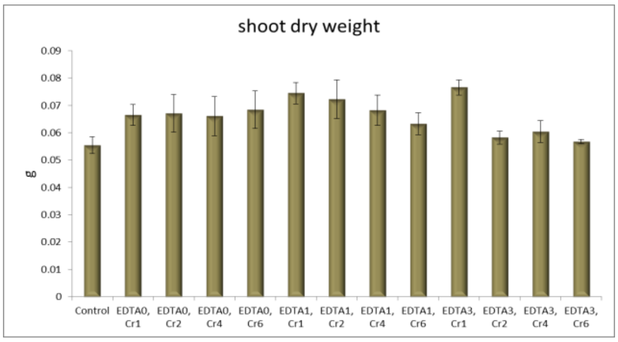

Fig. III. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA (0, 1, and 3 mg /L) and Cr (1, 2, 4, and 6 ppm) on shoot dry weight of sunflower

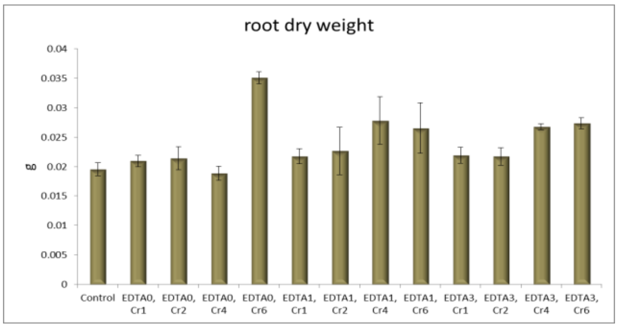

Fig. IV. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA (0, 1, and 3 mg /L) and Cr (1,2 , 4, and 6 ppm) on root dry weight of sunflower of shoots compared with the treatment without EDTA (Fig. III).

As shown in Figure (III), the minimum dry root weight was observed in the control. Application of chromium at concentration of 4 ppm resulted in a significant decrease in the dry root weight. On the other hand, application of EDTA at concentrations of 1 and 3 mg/l in combination with chromium led to increase in the dry weight compared to control and no-metal treatment (Fig. IV).

Chlorophyll pigments

Mean comparison showed the highest chlorophyll a in EDTA3, Cr 4 (EDTA 3 mg/l and 4 ppm chromium) treatment and the lowest in the treatment of 4 ppm Cr. Use of EDTA at both 1 and 3 mg/l levels led to an increase in chlorophyll a compared to the treatment without EDTA (Fig. V).

The results of this study showed that the use of chromium at all concentrations tested, led to a significant decrease in the amount of chlorophyll b compared to control, so the minimum chlorophyll b was observed at 4 ppm. The use of EDTA at concentrations of 1 and 3 mg/l with metal increased chlorophyll b in comparison with the treatment without EDTA so that the maximum amount of chlorophyll b was seen in the treatments (EDTA3, Cr4), (EDTA3, Cr2), (EDTA3, Cr1), and (EDTA1, Cr4) (Fig. VI).

Chrome application at all levels led to a decline in total chlorophyll content, so the least amount of chlorophyll was found at the highest concentration of chromium. Application of EDTA at both levels (1 and 3 mg/l) with Cr raised total chlorophyll so that the maximum amount of total chlorophyll was observed in EDTA3, Cr4 treatment (Fig. VII).

Soluble sugars

As Fig. (VII) shows, treatment of chromium at concentrations of 1 and 2 ppm reduced soluble sugar content in leaf compared to the control. Application of EDTA, especially at concentration of 3 mg with increasing chromium raised organic sugars content in the leaves of sunflower. On the other hand, soluble sugar content in root was high in all Cr treatments compared to the control. The use of EDTA in

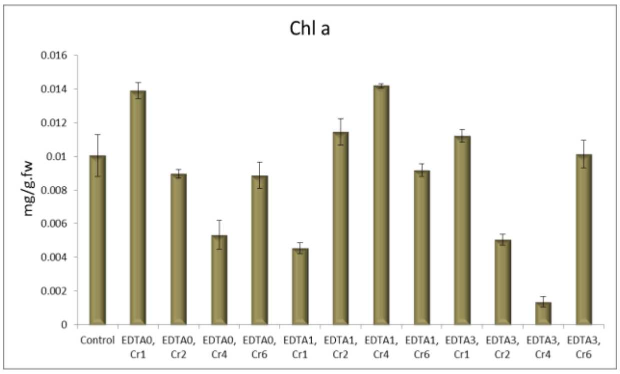

Fig. V. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA (0, 1, and 3 mg /L) and Cr (1, 2, 4, and 6 mg /L) on Chl a content

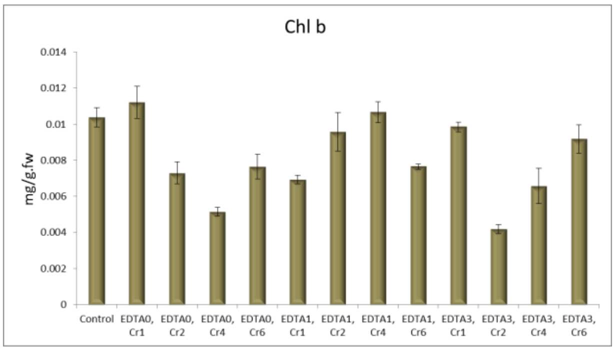

Fig. VI. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA(0, 1, and 3 mg /L) and Cr (1, 2, 4, and 6 ppm) on Chl b content

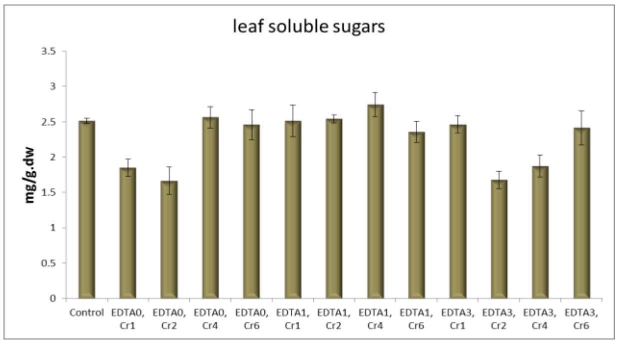

Fig. VII. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA(0, 1, and 3 mg /L) and Cr (1, 2, 4, and 6 ppm) on soluble sugars content in leaves of sunflower

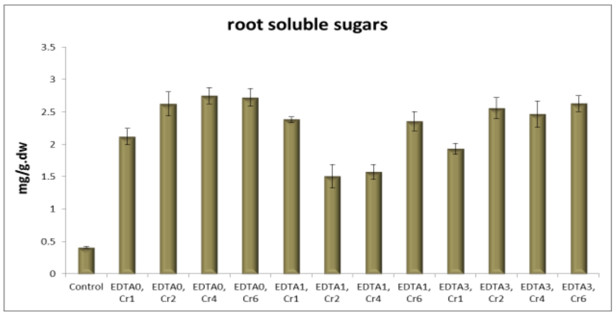

Fig. VIII. The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA(0, 1, and 3 mg /L) and Cr (1, 2 , 4, and 6 ppm) on soluble sugars content in root of sunflower

Table 1
The effect of different concentrations of ethylene diamine tetra-acetic acid, EDTA (0, 1, and 3 mg /L) and Cr (2 and 6 ppm) on Cr content in shoot and root of sunflower

Treatment	Control	EDTA 0 Cr2	EDTA 0 Cr6	EDTA 1 Cr2	EDTA 1 Cr6	EDTA3 Cr2	EDTA3 Cr6
shoot Cr (ppm)	0 g	17.3 f	54.7 c	43.6 e	69.5 b	50.3 d	93.6 a
Root Cr (ppm)	0 g	574.5 f	1567.6 b	738.6 d	1127.5 c	580.4 e	1600.4 a

treatments containing chromium caused increase in root soluble sugar content and this increase was particularly significant at high concentrations of chromium (Fig. VIII).

Chrome accumulation

The use of chromium heavy metal at concentrations of 2 and 6 ppm increased the amount of chromium in shoot .The use of EDTA in combination with chromium especially at a concentration of 6 ppm led to increased leaf Cr. Also, with an increase in concentration of chromium, so did the amount of this metal in roots. In fact, the amount of this metal increased in roots. As shown in Table 1 using EDTA 3 mg/l with 6 ppm chromium resulted in highest chromium (Table1).

There are some reports that suggest heavy metals such as chromium have inhibitory effects on the plant growth. Research has shown that chromium as an unnecessary element, generally inhibits the growth of plants (Pandey et al., 2005; Samantaray et al., 1998). It is believed that a reduction in aerial organs' length is due to decrease in root growth and thus reduces the transfer of water and nutrients to the aerial parts of the plant. In addition, chrome transferred to shoot causing the disturbance on cellular metabolism and thereby reduces the growth (Shanker et al., 2005).

In this study, using EDTA with heavy metal stimulated the growth. In a study by Zeid and Ghate (2007), growth of bean seedlings irrigated the with effluent containing copper, zinc, and cadmium declined and EDAT could reduce the inhibitory effect of heavy metals on the seedlings growth. Shuang (2006) showed that EDTA reduced the inhibitory effect of Pb on the roots elongation of *Zinnia elegans* and increased number of lateral roots. Also Singh (2011) reported higher levels of 60 milligrams per kg of chromium reduced size and and leaf growth rate in spinach.

Based on the results of this study, the amount of chlorophyll a, b, and total chlorophyll decreased significantly with increasing chromium and the use of EDTA in both concentrations led to improved stress effect on this pigment. Studies have shown that cadmium reduces total chlorophyll, chlorophyll a, b, and carotenoids in higher plants (Sheoran and Singh, 1990).

In this regard, it has been reported that decreasing the amount of chlorophyll due to inhibition of its the biosynthesis. Chlorophyll biosynthesis inhibition is probably due to inhibition of the synthesis of amino acids and the formation of aminolevulinic acid and protochlorophyllide reductase (Vassilev and Yordanov, 1997). Also in barley leaves under cadmium stress, the formation of HPLC is disrupted due to protein synthesis inhibition in the transcription level and this causes photo-oxidation of chlorophyll (Hegedus et al., 2001).

A significant increase was observed in the amount of soluble sugars in plants treated with chromium in comparison with control. Research has shown that plants to increasing of soluble sugars under stress conditions, can keep carbohydrate reserve for cell basal metabolism at an optimal level (Dubey and Singh, 1999). In this study, the use of EDTA at concentration of 3 mg/l decreased root and shoots soluble sugars in comparison with control and the treatment without EDTA.

Our results showed that to increasing the amount of chromium in medium, the this heavy metals content in plant tissues (shoot and root) enhanced. Also the amount of chromium was at root more than shoot. In this regard it seems cadmium accumulation in roots is a resistance mechanism against the damage caused by heavy metals.

It is reported that chromium accumulates in the vacuoles of root cells (Arun et al., 2005). Studies have shown that EDTA- metal complex transported through the apoplastic pathway to inactive form (Javier et al., 2007). Research has shown that chelators affect solubility, mobility, and absorption of metals in the soil or medium (Deepak et al., 2008). In the present study, metal uptake by plants increased in the presence of EDTA- chelator and the amount of chromium accumulated in the plant was more. Generally, the results of this study showed EDTA- chelator reduced the toxic effects of increasing concentrations of chromium in sunflower and this issue can be considered in phytoremediation.

References

Arun, K., C. Shanker.,C. Herminia.,S. Loza-Tavera and **S. Avudainayagamd** .2005. 'Chromium toxicity in plants'. *Environ. Int*. 31: 739-753.

Chatterjee, J. and **C. Chatterjee.** 2000. 'Phtotoxicity of cobalt, chromium and copper in cauliflower'. *Environ Pollut*. 109: 69-74.

Chen, Y.X., Q. Lin.,Y.M. Luo., Y.F. He., S.J. Zhen., Y.L. Yu., G.M. Tian and **M.H. Wong** .2003. 'The role of citric acid on the phytoremediation of heavy metal contaminated soil'. *Chemosphere.* 50:807–811.

Cunningham, S.D., J.R. Shann, D.F. Crowley and **Anderson, T.A**. 1997. 'Phytoremediation of contaminated water and soil. Phytoremediation of soil and water contaminants'. American Chemical Society, Washington, DC., pp. 2-17.

Deepak, K. G.,S. Alok and **V.P. Singh. 2008** .'EDTA enhances lead uptake and facilitates phytoremediation by vetiver grass'. *J Inviron Biol*. 26(6):903-906.

Fazal, H., B. Asghari and **M.P. Fuller**. 2010. 'The improved phytoextraction of lead (Pb) and the growth of maize(*Zea mays* L.): the role of plant growth regulators (GA3 and IAA) and EDTA alone and in combinations'. *Chemosphere*. 80: 457-462.

Hegedus, A., S. Erdi and **G. Horvath.** 2001. 'Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedling under cadmium stress'. *Plant Sci.* 160:1085-1093.

Hu, J., G. Chen and **M. Irene**. 2005. 'Removal and recovery of Cr (VI) from wastewater by magnetite nanoparticles'. *Water Res*. 39: 4528-4536.

Javier, H.A., G. Carlos., B. Oihana and **B. José María**. 2007. 'EDTA-induced heavy metal accumulation and phytotoxicity in cardoon plants'. *Environ Exper Bot*. 60 (1): 26-32.

Jenson, A. 1987. 'Chlorophyll and carotenoid: Hand Book of physiology and biochemical method'. Cambridge University Press.PP:294.

Kochert, G. 1978. Carbohydrate determination by phenol sulfuric acid method in: Helebust, J.A. CRAIG, J.S. (ed): Hard book of Ohicologia Method.PP: 56-97.

Liphadzi, M.S. and **M.B. Kirkham.** 2006. 'Availability and plant uptake of heavy metals in EDTA-assisted phytoremediation of soil and composted biosolids'. *S Afr. J Bot*. 72: 391–397.

Meagher, RB. 2000. 'Phytoremediation of toxic elemental and organic pollutants'. *Curr Opin Plant Biol.* 3: 153-162.

Panda, S.K. and **S. Choudhury.** 2005.' Chromium stress in plants'. *Braz J of Plant Physiology*. 17: 95-192.

Pirooz, P.S. and **KH. Kalantari Manochehri**. 2012. 'Study of phytoremediation of chromium by sunflower (*Heliathus annuus* L.)'. First National Conference on phytoremediation, Kerman.

Pandey, V., V. Dixit and **R. Shyam**.2005. 'Antioxidative responses in relation to growth of mustard (*Brassica juncea* cv. Pusa Jaikisan) plants exposed to hexavalent chromium'. *Chemosphere.*61: 40-47.

Raskin, I. and **B.D. Ensley**. 2000. 'Phytoremediation of toxic metals: using plants to clean up the environment'. John Wiley and Sons, N. York, pp: 303.

Saifullah, Z., M.H., E. Meers., G. Abdul Ghafoor., G. Murtaza., M. Sabir, M. Zia-ur-Rehman and **F.M.G. Tack**. 2010. 'Chemically enhanced phytoextraction of Pb by wheat in texturally different soils'. *Chemosphere*. 79: 652–658.

Samantaray, S., G.R. Rout and **P. Das.** 1998.' Role of chromium on plant growth and metabolism'. *Acta Physiol Plant* 20: 201-212.

Shanker, A. K., C. Cervantes., L. Herminia and **S.A. Tavera.** 2005. 'Chromium toxicity in plants'. *Environ Int* 31: 739-753.

Sheoran, I. H. and **R. Singh. R**. 1990. 'Effects of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeon pea (*Cajanus cajan* L.)'. *Photosynth Res* .23: 345-351.

Shuang, C., Z. Qi-xing, W. Shu-he, Z. Wei, C. Lei and **R. Li-ping**. 2006. 'Effects of exogenous chelators on phytoavailability and toxicity of Pb in *Zinnia elegans* Jacq'. *J Hazard Mater*. 146(1-2): 341-6.

Singh, A.K. 2011. 'Effect of trivalent and hexavalent chromium on spinach (*Spinacea oleracea* L)'. *Environ Ecol*. 19:807–810.

Vassilev, A. and **I. Yordanov**.1997. 'Reductive analysis of factors limiting growth of cadmium treated plants –review'. *Plant Physiol*. 23:114-133.

Wang, Q., Y. Dong, Y. Cui and **X. Liu** .2001. 'Instances of soil and crop heavy metal contamination in China'. *Soil Sediment Contam*. 10:497–510.

Zeid, I.M. and **H.M, Ghate**. 2007. 'Response of bean heavy metals in sewage water'. *Pak J Biol Sci* 10(6): 874-879.

Zhang, X. H., J. Liu, H.T. Huang, J. Chen, Y.N. Zhu and **D.Q. Wnag** .2007. 'Chromium accumulation by the hyperaccumulator plant *Leersia hexandra* Swartz'. *Chemosphere.* 67: 1138-1143.