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TREATMENT OF Cr^{3+} CONTAMINATED SOIL BY SOLID TEA WASTAGE I. A STUDY OF PHYSIOLOGICAL PROCESSES OF *VIGNA RADIATA*

RAFIA AZMAT[1*], YASMEEN AKHTER[2], TEHSEEN AHMED[3] AND SARA QURESHI[1]

[1,3]Department of Chemistry, [2]Department of Botany
[1,2]Jinnah University for Women 5C Nazimabad 74600 Karachi, Pakistan
[3]University of Karachi, Pakistan
[*]Corresponding author's E-mail: rafiasaeed200@yahoo.com

Abstract

This study describes the option of using domestic tea waste in soil contaminated with the Cr^{3+} trace metal due to industrial and mine activity, continuously discharging in the land and aquatic resources. This disposal of industrial wastage without proper treatment is responsible for the lowering of crop productivity with the accumulation of essential and non essential trace metals in the plants. On the other hand domestic waste management in soil and aquatic resources are also accountable for the reduced field productivity. This research discusses the proper domestic waste management in the agriculture land for the cultivation of crop in the contaminated soil. *Vigna radiata* has been selected as a crop to check the effects of Cr^{3+} and its deletion in the contaminated soil. The highest yield was obtained when soil was mixed with tea wastage instead of spreaded tea wastage. Seed germination, morphology and physiology of 15 days old plant showed remarkable improvement in the plant growth including seed germination with activated tea wastage in the presence of Cr^{3+} as compared to those plants which were grown in Cr^{3+} contaminated soil only. Biochemical analysis of seedling showed an increase in the concentration of chlorophyll, carbohydrates, protein and amino acids, which confirms the remediation of contaminated soil through tea wastage. It was concluded that proper use of domestic waste can be helpful to increase the soil fertility and can concentrate the heavy toxic metals in it through complex formation.

Introduction

Adsorption is a promising alternative method to treat industrial effluents, mainly because of its low cost and high metal binding capacity (Kailas *et al*., 2007; Utomo & Hunter, 2006; Yan-xin, 2001; Cay *et al*., 2004). Tea waste is one of the low cost and easily available adsorbent having strong adsorptivity towards metals like Cd, Zn, Ni, and Pb (Amarasinghe & Williams, 2007; Singh *et al*., 1993; Ahluwalia & Goyal, 2005) because of the soft colloid and chemical components like palmitinic acid of fatty group, terpenes and di-Bu phthalate present in it (Amarasinghe & Williams, 2007; Shyamala *et al*., 2005; Mahvi *et al*., 2005). In under developing countries using sewage sludge in agriculture fields is the risk of soil contamination with heavy metals and their possible transference to humans *via* food chain (Quaff & Ashhar, 2005; Mozumder *et al*.,2008). Heavy metals, however, are regarded as inhibitors of enzymatic and microbiological activity of soil. This is because if added to soil (whether on purpose or by accident) they cause quantitative and qualitative changes in the composition of microflora and in enzymatic activity (Wyszkowska, 2002; Vartika *et al*., 2007)).

Chromium (Cr) concentration in the environment is increasing day by day due to its excessive use in leather processing, refractory steel, and chemical manufacturing industries (Andaleeb *et al*., 2008). Due to its wide industrial use, chromium is considered a serious environmental pollutant. Excess of Cr in the environment causes hazardous effects on all living beings including plants (Arun *et al*., 2005). A gradual decrease was observed for various morphological parameters like root fresh and dry weights, shoot fresh and dry weights and plant height with increase in Cr levels (Andaleeb *et al*., 2008). Contamination of soil and water by chromium (Cr) is of recent concern. Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition (Azmat & Khanum, 2005; Pacha, 1986). In this context present study has been made to detoxification of metal within the soil by using tea wastage.

The objective of this study was to evaluate the use of tea waste in soil contaminated with Cr^{3+} and its effect on some biochemical parameters of mung bean plants as no report of use of tea waste in soil contaminated with Cr^{3+} was available except some physical kinetics parameters for the removal of metal by tea waste were reported in literature. Tea waste was applied in two ways (i) tea waste was blended with the contaminated soil and (ii) tea waste spread on the surface of contaminated soil.

Materials and Methods

The pot experiments were carried out under natural conditions in the field of Department of Chemistry, Jinnah University for Women, Karachi, Sindh, in a randomized block design with two treatments and five replicates. The plants were maintained in control garden soil for 7 days and on the 8th day chromium as chromium chloride was applied in three concentrations viz., 50, 100, 200 ppm and referred as set (1) experimental plants, set (2) consists of pots in which 3gm tea wastage was thoroughly mixed with soil and set (3) consists of soil with tea waste spread on surface of soil (3gm) referred as treated plants. The plants were watered daily and Cr^{3+} as $CrCl_3$ in solution were added in soil in alternative days. After 15 d of exposure to the treatments, the plants were harvested, homogenized, and centrifuged, and the supernatants were analyzed for three key enzymes Alkaline phosphatase (ALP) by p-nitrophenol method. The estimation of alkaline phosphate (ALP) based on principal that hydrolyzed the para-nitro phosphate at pH 9 to 10 at 37^oC and produce para-nitro phenol and phosphate with diethylamine buffer, magnesium chloride and p-nitro phenyl phosphate as a substrate. The absorbance was recorded at 405 nm.

$$p-nitro\ \ Phenyl\ \ Phosphate\ +\ H_2O \xrightarrow{AP} Phosphate\ +\ p-nitro\ \ Phenol$$

Aspartate aminotransferase (GOT/AST) is responsible to convert the α-ketogluterate and α-aspartate to α-glutamate and oxalo acetate respectively by transfer of amino group. The formed oxaloacetate was reduced to L-maltate in presence of enzyme maltase dehydrogenase with simultaneous oxidation of reduced NADH in to $NADP^+$. The activity is measured by using Tris buffer, L-aspartate MDH, LD and NAD. The absorbance was measured spectophotometrically at 340 nm.

Alanine amino transferase (GPT/ALT) ALT catalyzes the transfer of amino group from amino to α-ketogluterate to form 2-glutamate pyruvate. The pyruvate is then reduced to lactate in the presence of enzyme lactate dehydrogenase with simultaneous oxidation of reduced NADH into $NADP^+$. The absorbance was measured at 340 nm. The ALT activity was measured by Tris buffer, L-aniline, α-oxogutarate, LD and NADH using Kits (Rao & Deshpande, 2007).

Estimation of carbohydrate and proteins were carried out by methods given by Lowry *et al*., (1951) and Montgomery (1957) respectively. Chlorophyll was estimated using 80% acetone. All determinations were performed in five replicate and data was subjected to statistical analysis.

Results and Discussion

Morphological and physiological processes: The study revealed that Cr causes significant decrease in fresh and dry weight, length of root and shoot, protein, carbohydrate, chlorophyll and amino acids in *Vigna radiata*. The accumulation of chromium by germinating seedlings appears to be significantly affected by Cr concentration and occurred in a linear manner (Azmat *et al*., 2005). Cr-treated plants showed growth depression and decrease in fresh and dry weight too as reported earlier (Rai & Mehrortrs., 2008). It was found that dry matter production was severely affected by Cr (III) concentrations above 100 ppm. The reduction in plant height might be mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant (Table 1). In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in plant height. The effect of Cr on water relations was highly concentration dependent, and primary and first trifoliate leaves were affected differently (Azmat *et al*., 2007). The seedlings which were grown in spreaded tea wastage and soil mixed with the tea wastage showed remarkable effect on the growth as well as physiological parameters. There were significant improvements in growth of the plants, indicating the adsorption of toxic metal on surface applied (Tables 1 & 2). Results reported in Table 2 showed decline in protein contents ($p<0.05$) at elevated concentration of Cr^{3+} with elevated concentration of carbohydrate ($p<0.001$) and amino acid ($p<0.05$) that may be attributed with use of protein under stress and non utilization of carbohydrate whereas plants grown with tea wastage reflects the effect of tea wastage as a manure and biosorbent surface which showed inhibitory effects on the mobility of toxic metal Cr^{3+} and proves that tea wastage could be utilized to increase the soil fertility even in draft condition of toxic metals.

Enzymes activity: An increase and decrease in the enzymes activity could reflect the current situation of environments as biomarkers (Scoccianti *et al*., 2008; Marc *et al*., 2005). Results showed that AST enzymes activity initially increases at 50ppm of Cr (589.56) as compared with the control plants ($p<0.05$) and gradually decreases with the increase in concentration of Cr. This showed that elevated concentration of Cr (III) alters the enzyme activity. Set 2 (Mixed tea plants) showed significant effect of tea wastage as an adsorbent surface thoroughly mixed with the soil contaminated with the Cr to use the solid domestic wastes to control the pollution cause by heavy metals. Results of tea wastage treated plant showed that at 50 ppm, the activity of AST enzyme (Fig. 1) reaches approximately to the normal value (556.97) when compared with control (556.97).This reflects that Cr remediation which may occur through adsorption on the solid tea surface (Wasewar *et al*., 2007). But increase in the concentration of Cr showed negative effects on the AST enzymes activity. Set 3 (Spreaded tea waste) plants showed no remarkable effects on the AST enzyme activity where continuous decrease in enzyme activity were observed which showed that for proper adsorption of heavy metal it is essential to mixed the solid tea wastage with soil thoroughly.

Table 1.Effect of Cr and solid tea wastage as a biosorbent manure on fresh and dry weight of *Vigna radiata*.

$CrCl_3$ (ppm)	Fresh weight	Dry weight
	Root	
0	0.544	0.198
50	0.355	0.075
50+ mixed tea	0.399	0.00851
50+spray tea	0.3634	0.00812
100	0.2115	0.0067
100+ mixed tea	0.229	0.0681
100+spray tea	0.218	0.00541
200	0.114	0.0050
200+ mixed tea	0.118	0.037
200+spray tea	0.116	0.0367
	Stem	
0	0.3853	0.2133
50	0.198	0.00158
50+ mixed tea	0.1831	0.00232
50+spray tea	0.175	0.00189
100	0.169	0.0010
100+ mixed tea	0.1732	0.00143
100+spray tea	0.1710	0.00133
200	0.0109	0.009
200+ mixed tea	0.1534	0.00163
200+spray tea	0.1421	0.00141
	Leaves	
0	0.2	0.0544
50	0.09	0.0051
50 + mixed tea	0.1258	0.0134
50 + spray tea	0.1141	0.0011
100	0.0949	0.0039
100 + mixed tea	0.0643	0.0006
100 + spray tea	0.0541	0.0005
200	0.015	0.0020
200 + mixed tea	0.1048	0.0167
200+ spray tea	0.0101	0.009

The ALT showed great variation in its activity (Fig. 2). Initially it decreases ($p<0.001$) and then slightly increases with the elevated concentration of the Cr^{3+} i.e., 100 ppm and 200 ppm. Set 2 plants again showed slight improvement in the enzymes activity but not to that of control one. This showed that the toxicity of metal to the biomarker, ALP ($p<0.05$) showed completely different response to the heavy metal Cr which showed an increase in enzyme activity to overcome the heavy metal stress. This may be attributed with decrease or increase in the enzyme activity due to the denature of site of interaction of enzymes under metal stress.

The results of the analyses, reported hereby prove that activity of individual enzymes is quite a sufficient indicator of soil fertility. Enzymatic activity of soil is a reliable measure of current biological status. Naturally occurring amounts of heavy metals do not disturb the biochemical balance of soil. Slightly higher quantities of heavy metals might stimulate the activity of soil enzymes, whereas in larger they will have an inhibitory effect (Wyszkowska, 2002). Heavy metals, however, are regarded as inhibitors of enzymatic activity. It was observed that the potential soil fertility index decreased at higher concentration of chromium contamination and negatively affect the germination processes and altered the activity of enzymes correlated with chromium. On the other hand it is interesting to note that the activity of two enzymes decreased up to 100 ppm level of Cr while ALP (3) was found to be increased at all applied doses. It may be due to stress caused by the accumulation of Cr. The experiments revealed that chlorophyll a and b decrease with the concentration of Cr (Azmat *et al*., 2005) but improved in tea waste amended soil. These results showed that chlorophyll as a vital pigment for life of plants is not tolerant to chromium and its toxicity was correlated with the degree of soil contamination. Soil amended with the solid tea wastage showed increase in concentration of chlorophyll contents (Table 2). Carbohydrate contents of *Vigna radiata* showed an increase in concentration which may be related with the metal toxicity and alteration in key enzymes activity of protein and carbohydrate metabolism. Set 2 and set 3 plants showed decrease in carbohydrate contents due to the adsorption of Cr metal on tea wastage. The effect of Cr (III) on the activity of AST, ALT and alkaline phosphatase depended on the rate of soil contamination with chromium. Our results are similar to those reported by Wyszkowska (2002) in which he reported that in the objects polluted with 100 mg Cr kg^{-1} of soil, plants became necrotic at the stage of seedlings and in the soil treated with 150 mg Cr kg^{-1} of soil, the emergence of plants was inhibited.

Table 2. Effect of Cr and solid tea wastage as a biosorbent manure on nutritions of *Vigna radiate*.

Concentration (ppm)	Control	Set 1 $CrCl_3$	Set 2 $CrCl_3$ + Mix. Tea	Set 3 Tea + $CrCl_3$
		Chlorophyll "a" (mg/gm)		
50		0.761	0.862	0.787
100	1.176	0.517	0.641	0.539
200		0.321	0.421	0.0.392
		Chlorophyll "b" (mg/gm)		
50		0.0875	0.1915	0.181
100	0.243	0.0524	0.0728	0.0633
200		0.042	0.04996	0.051
		Protein test (g/dL)		
50		3.177	3.134	3.027
100	4.102	2.855	2.941	2.877
200		2.726	2.920	2.598
		Carbohydrate test (mg/dL)		
50		48.093	32.350	25.708
100	14.173	43.108	19.550	26.78
200		47.311	39.100	28.15
		Amino acid test (%)		
50		1.00	1.142	1.04
100	0.718	0.956	0.82	0.878
200		0.63	0.788	0.786

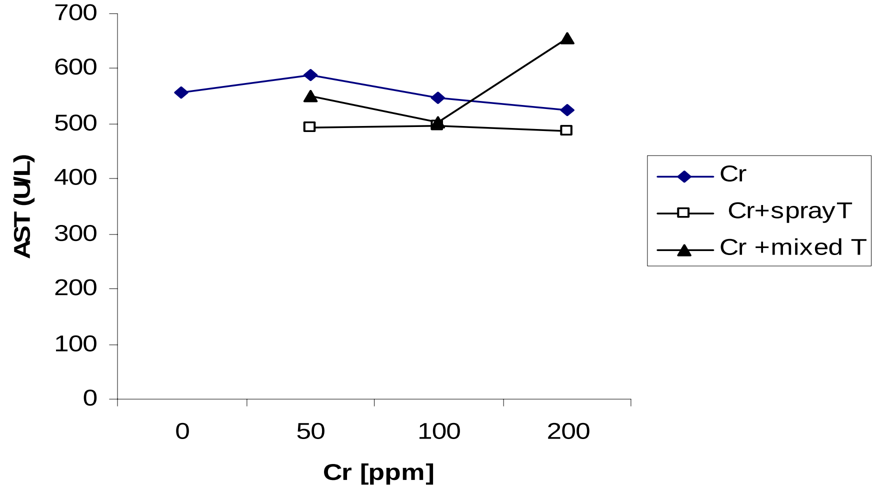

Fig. 1. Effect of Cr and solid tea wastage as a biosorbent manure on enzyme AST activity of seedlings of *Vigna radiata*.

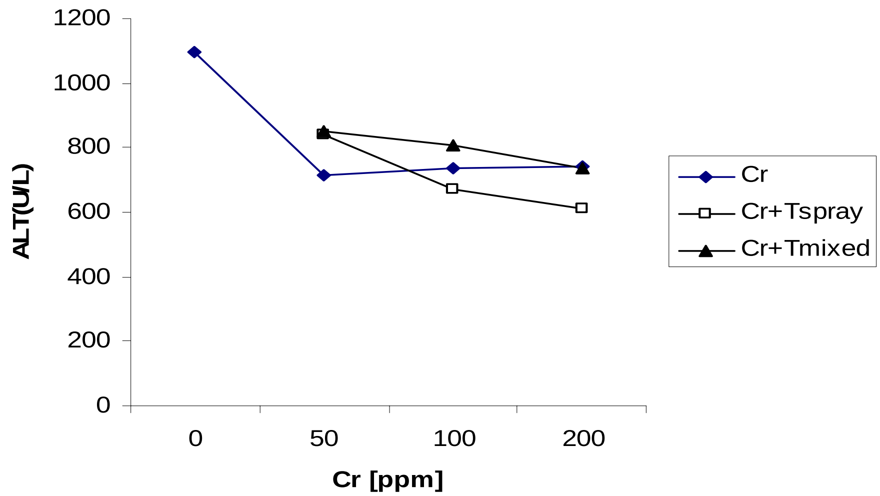

Fig. 2. Effect of Cr and solid tea wastage as a biosorbent manure on enzyme ALT activity of seedlings of *Vigna radiata*.

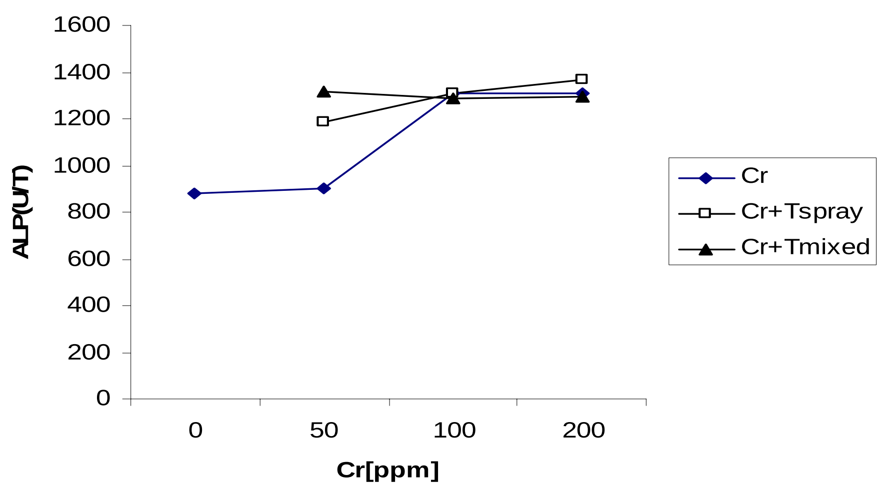

Fig. 3. Effect of Cr and solid tea wastage as a biosorbent manure on enzyme ALP activity of seedlings of *Vigna radiata*.

Mechanism of remediation of Cr^{3+}: The mechanism of remediation of Cr^{3+} based on adsorption of Cr^{3+} on solid tea wastage within the soil where it was found that in the pot which contained thoroughly mixed tea waste with the garden soil shows soil stony structure and the plants of this pot were quite erect and more healthy as compared to plants with Cr^{3+}and with no Cr^{3+}. The available biochemical experimental data presented here showed that plants with mixed tea showed more tolerant morphological as well as physiological parameters. The remediation mechanism for the adsorption of heavy metal Cr^{3+} using tea waste presented here showed that soft colloid and chemical components like palmitinic acid of fatty group, terpenes and di-Bu phthalate play a key role for complex forming with the metals, reduced the mobility of metal in the contaminated soil and reduced the accumulation of Cr^{3+} in plant tissues in the early stage of development of seedlings

Conclusion

These results suggest that toxic metal mobility in the plant can be controlled by application of some suitable adsorbing surface like solid tea waste, in which metal bind with the surface and that surface can also act as manure of the soil to increase the soil fertility as a remediater. However more researches on other metals with other crops with tea wastage should encourage to resolve the matter of metal toxicity to protect the food chain from adverse effect of metals on human health.

References

Ahluwalia, S.S. and D. Goyal. 2005. Removal of heavy metals by waste tea leaves from aqueous solution. *Eng. in Life Sci*., 5(2): 158-162.

Amarasinghe, B.M. and W.P.K. Williams. 2007. Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater. *Chem. Eng. J*., 132(1-3): 299-309.

Andaleeb, F., Z.M. Anjum, M. Ashraf and K.Z. Mahmood. 2008. Effect of chromium on growth attributes in sunflower (Helianthus annuus L.). *J. Environ. Sci*., 20(12): 1475-1480.

Arun, K., S.C. Carlos, H. Loza-Taverac and S. Avudainayagam. 2005. Chromium toxicity in plants. *Chemosphere*, 31(5): 739-753.

Azmat, R. and R. Khanum. 2005. Effect of Chromium on uptakes of minerals in Bean plant *Pak. J. Biol. Sci*., 8(2): 281-283.

Azmat, R., R. Parveen and I.I. Naqvi. 2007. Effect of chromium combined with atrazine on potassium, sodium, manganese, iron and phosphate in roots and shoots in bean *Vigna radiata* (L.) Wilczek. *Saudi J. Chem. Soc*., 11(1): 111-120.

Azmat, R., R. Parveen, I.I. Naqvi and S. Shoukat. 2005. Effect of Cr (III) combined with atrazine on protein, carbohydrate, amino acid and chlorophyll content in *Vigna Radiata* (L.) Wilczek. *International J. Biol. & Biotechnol*., 2(2): 433-439.

Cay, S., A. Uyanik and A. Ozasik. 2004. Single and binary component adsorption of copper (II) and cadmium (II) from aqueous solutions using tea-industry waste. *Separation and Purification Technol*., 38(3): 273-280.

Kailas, L., Y. Ravichandra, K.M. Anil and V. Godbole. 2007. Adsorption mechanism for the adsorption of heavy metals using tea waste as an adsorbent. *Manager's J. Eng. & Technol*., 3(1): 41-46.

Lowry, O.H., N.J. Rosenberg, A.L. Farr and R.J. Randall. 1951. Protein measurements with folin-phenol reagents. *J. Biol. Chem*., 193: 265-275.

Mahvi, A.H., D. Naghipour, F. Vaezi and S. Nazmara. 2005. Teawaste as an adsorbent for heavy metal removal from industrial wastewaters. *American J. Appl. Sci*., 2(1): 372-375.

Marc, D., B. Eric, C. Sylvie, M. Eric, M. Jean-François and F. Jean-François. 2008. Antioxidant enzyme activities as affected by trivalent and hexavalent chromium species in Fontinalis antipyretica Hedw. *Chemosphere*, 73(3): 281-290.

Montgomery, R. 1957. Determination of glycogen. Arch. *Biochem. Biophys*., 67: 378-386.

Mozumder, S.I., M.R. Khan and A. Islam. 2008. Kinetics and mechanism of Cr (VI) adsorption onto tea-leaves waste. *Asia-Pacific J. Chem. Eng.,* 3(4): 452-458.

Pacha, J. 1986. Wpµyw trĐjwarto_ciowego chromu na aktywno_wybranych enzymĐw w glebie. *Acta Biol. Siles.,* 3(20): 95.

Quaff, A.R. and M.M. Ashhar. 2005. Removal of arsenic (III) by activated tea waste and activated charcoal: a comparative study. *India. J. Ind. Water Works Assoc.,* 37(1): 63-69.

Rai, V. and S. Mehrotra. 2008. National Botanical Research Institute, Lucknow, India. *Environ. Monitoring & Assessment*, 147(1-3): 307-315.

Rao, B.S. and Deshpande. 2007. *Experimental Biochemistry*. I,k International Pvt. Ltd. New Delhi. 1100116 India

Scoccianti, V., M. Iacobucci, M.F. Paoletti, A. Fraternale and A. Speranza. 2008. Species-dependent chromium accumulation, lipid peroxidation and glutathione levels in germinating kiwifruit pollen under Cr (III) and Cr (VI) stress. *Chemosphere*, 73(7): 1042-1048.

Shyamala, R., S. Sivakamasundari and P. Lalitha. 2005. Comparison of the adsorption potential of biosorbents-waste tea leaves and rice husk, in the removal of chromium (VI) from waste water. *J. Ind. Pollution Control*, 21(1): 31-36.

Singh, D.K., D.P. Tiwari and D.N. Saksena. 1993. Removal of lead from aqueous solutions by chemically treated used tea leaves. *Ind. J. Environ. Health*., 35(3): 169-77.

Utomo, H.D. and K.A. Hunter. 2006. Adsorption of divalent copper, zinc, cadmium and lead ions from aqueous solution by waste tea and coffee adsorbents. *Environ. Technol.,* 27(1): 25-32.

Vartika, R., K.A.K.S. Sayyada Rawat and S. Mehrotra. 2007. Disruption of elements uptake due to excess chromium in Indian medicinal plants. *Biological Trace Element Research*, 120(1-3): 127-132.

Wang, Yan-xin. 2001. Removal of heavy metals from wastewaters using low cost sorbents: applications of biomass and geomaterial to environmental protection. *Dixue Qianyuan,* 8(2): 301-307.

Wasewar, K.L., Y. Ravichandra, K.M. Anil and V. Godbole. 2007. Adsorption mechanism for the adsorption of heavy metals using tea waste as an adsorbent. *India i-Manager's J. Eng. & Technol.,* 3(1): 41-46.

Wyszkowska, J. 2002. Soil contamination by chromium and its enzymatic activity and yielding, *Polish J. Environ. Stud.,* 11(1): 79-84.

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