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CHANGES IN SOME BIOPHYSICAL AND BIOCHEMICAL PARAMETERS OF MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] GROWN ON CHROMIUM-CONTAMINATED SOILS TREATED WITH SOLID TEA WASTAGE

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Abstract

The success of solid tea wastage treatment technology in remediating chromium (111) contamination in the soil has been demonstrated on growth of *Vigna radiata*. The present research was designed to study the effect of chromium (Cr^{3+}) on plant growth, potassium (K), phosphorus (P), protease activity and proline profile of *Vigna radiata* as a bioindicator in the presence and absence of the solid tea surface as a biosorbent to control the mobility of Cr^{3+} in the soil. Results showed toxic effects of Cr^{3+} on plant growth and development, which include high protease activity with prominent proline and decreased potassium and phosphorus contents at elevated concentration of metal. Proline content is the only amino acid that accumulates to a greater extent in the leaves of plants under stress. An increase in proline contents in leaves, stem and root with high concentration of Cr^{3+} gets reduced in a solid tea wastage amended plants. Metabolic alteration by Cr^{3+}exposure and their control by solid tea wastage already described in the first report, showed direct effect on enzymes or other metabolites or by its ability to generate reactive oxygen species which may cause oxidative stress. It is suggested that the plant can grow under chromium stress if some suitable adsorbent (like tea wastage) is mixed with the soil which can protect the plants from the phyto-toxicity of Cr^{3+} by altering various metabolic processes.

Introduction

Soil contamination with heavy metal is now a days a worldwide problem leading to agricultural losses and hazardous health problems as metals enter in to food chain (Azmat & Khanum, 2005; Azmat *et al*., 2007). Cr^{3+} is phytotoxic above certain threshold level important for the animal and human being but toxic at certain level for both. Samantaray *et al*., (1998) reported that high concentrations of chromium exhibited severe chlorosis, necrosis and a host of other growth abnormalities and anatomical disorders including the regulation of the mineral metabolism, enzyme activity and other metabolic processes. Cr^{3+} is taken up by plants because of its mobile nature in soil. Since trivalent and hexavalent Cr may interconvert in the soil and soil immobilize both trivalent and hexavalent chromium. It is difficult to asses separately the effects of the two types of Cr on plants. Consequently, it might be appropriate to use the term Cr toxicity in plants (Arun *et al*., 2005) instead of toxicity of trivalent or hexavalent Cr. The effects of chromium on plant growth, crop yield, uptake and distribution in vegetative and reproductive parts are not yet fully understood. Although a number of studies were made to investigate the chemistry of chromium in soil and its uptake by plants and found that it is a stimulant for plant growth (Arun *et al*., 2005). Barcelo *et al*., (1985) described the inhibition of P and K translocation within the plant parts when bean plants were exposed to Cr in nutrient solutions.

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Wani *et al*., (2008) reported the role of mesorhizobium strain RC3 in soil amended with Cr, and found that inoculation of RC3 strain results in plant growth-promoting substances which reduces the Cr by 14, 34 and 29% in root, shoot and grains respectively with the increase in N contents by 40 to 46% in root and shoot. Conventional methods to alleviate the toxicity of chromium include its chemical reduction followed by precipitation, ion exchange and adsorption on activated coal, alum or ash. Most of these methods require high energy or large quantities of chemicals. However, no information is available on the role of solid tea waste in a soil contaminated by Cr on the plant growth and their important nutritive values. Moreover, to the best of our knowledge, there is no report on the effect of chromium on the growth promoting potentials of the solid tea wastage in the potassium, phosphorus, protease and proline activity as a bio-indicators of environmental stress and their function in soil remediation. This research was designed to demonstrate the effect of solid tea wastage on chromium contaminated soil as a new technology to remediate the metal through adsorption on the surface of solid tea wastage, which immobilized the metal in the soil. The results of remediation have been checked on some important parameters of *Vigna radiate*.

Materials and Methods

The pot experiments were carried out in natural conditions in the field of the Department of Chemistry, Jinnah University for Women, Karachi, Sindh, in a randomized block design with two treatments and five replicates as described by Azmat *et al*., (2010). The plants were maintained in control garden soil for 7 days and on 8th day the chromium as chromium chloride was given in three treatments viz., 50,100, 200 ppm and referred as set (1) experimental plants whereas treated plants were referred as set (2) consist of pots in which 3gm tea wastage was thoroughly mixed with soil and set (3) consist of soil with tea waste spread on surface of soil (3gm). The plants were watered daily and Cr^{3+} as $CrCl_3$ in solution was added in soil in alternative days (Azmat *et al*., 2010).

After 15 days of exposure to the treatments, the plants were harvested, homogenized, centrifuged and the supernatants were analyzed for proline and protease activity. Proline contents was extracted from 0.5 g of fresh shoot and root into 10ml of 3% Sulfosalicylic acid and filtered through Whatman No.2 filter paper. Proline was determined by ninhydrine method as described by Btaes *et al*., (1973) on Schimadzo UV/Visible Spectrophotometer 180 A using pure proline as a standard. Absorbance was measured at 570nm.

Protease activity was determined by the method described by Ainous (1970) using 1% NaCl in phosphate buffer (pH 7.5) in which 1gm fresh weight of seedlings was placed in the mortar and casin solution was added in supernatant and incubated for 1hr at 50 C. After incubation 1 ml TCA was added then centrifuged to 10 min. 0.5 ml folin phenol reagent was added in the supernatant and absorbance was recorded on Spectrophotometer at 570nm.

P metabolites was determined by mixing with hydrolyzing extract with 5ml 0.1M acetate buffer pH 4.0, 0.5ml 1% Ammonium molybdate in 0.05N H_2SO_4, 0.5ml 1% Na-ascorbate. To avoid the delay in the conversion of the blue color of molybdate-phosphoric complex, 1mM $CuSO_4.5H_2O$ was added into the ascorbate solution. The blue color of the complex was obtained after 10 min and the absorption was determined using spectrophotometer 180 A” at 620 nm. Potassium was determined by flame photometer after preparing ash and then dissolved in Nitric acid and Hydrochloric acid as described by Azmat & Khanum (2005).

Statistics: Values are mean values ± s.e. Differences among treatments were analyzed by 1-way ANOVA, taking $p<0.05$ as significant according to Tukey's multiple range test.

Results and Discussion

Initially an increase in the root length at 50ppm was observed which may be due to increased relative proportion of pith and cortical tissue layers that later on reduced at further increase in concentration of Cr. The reduction in root growth could be due to the direct contact of seedlings roots with Cr in the soil causing a collapse and subsequent inability of the roots to absorb water from the medium (Barcelo *et al*., 1986) or may be related with the inhibition of the root cell division/ root elongation or to the extension of a cell cycle in the roots. The seedlings with tea waste showed approximate normal root length especially in set 3 plants showed normal growth rate (Fig. 1) reflects the remediation of Cr or immobility or complex formation of metal with applied tea surface. Adverse effects of Cr on plant height and shoot growth were observed (Fig. 2) which is successfully controlled by solid tea surface. 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.

Results reported in Table 1 showed the decrease in the concentration of K contents both in root and shoot at increasing concentration of Cr compared with the control plants (p<. 005) which showed a dramatic affect on the plants ability to survive and functioning during metal stress periods. Initial potassium deficiency shows up as yellowing of older leaf blades, lower leaf blades, which is then followed by dieback of the leaf tip and scorching of the leaf margins as the deficiency problem becomes worse (Biddappa & Bopaiah, 1989). Once these conditions occur, wear injury for the turf plants will increase significantly. Factors which can lead to potassium deficiency include leaching in sandy soils or soils irrigated with the contaminated water. Many plant physiologists consider potassium second only to nitrogen in importance for plant growth. Potassium is second to nitrogen in plant tissue levels with ranges of 1 to 3% by weight. Potassium is the only essential plant nutrient that is not a constituent of any plant part. Potassium is a key nutrient in the plants tolerance to stresses (Arun *et al*., 2005). Increase in potassium contents in tea waste amended plants showed more tolerance surviving capability under various stresses (Table 1), such as metals, cold/hot temperatures, drought wear and pest problems (Dahiya *et al*., 2005). And both role of potassium as biophysical and biochemical were visible in this investigation. Potassium acts as catalysts for many of the enzymatic processes in the plant (Azmat *et al*., 2010) that are necessary for plant growth to take place. Another key role of potassium is the regulation of water use in the plant (osmoregulation). This osmoregulation process affects water transport in the xylem, maintains high daily cell turgor pressure which affects tolerance, cell elongation for growth and most importantly it regulates the opening and closing of the stomata which affect transpirational cooling and carbon dioxide uptake for photosynthesis (Azmat *et al*.,2010). Results of tea waste amended soil showed marked effect on the plant physical and biological processes (Table 1) where K contents reaches to the normal value and showed more rapid growth in soil contaminated with the Cr. Metabolic alterations by Cr^{3+} exposure and their control by solid tea wastage was already described by Azmat *et al*., (2010) which showed direct effect on enzymes or other metabolites or by its ability to generate reactive oxygen species which may cause oxidative stress.

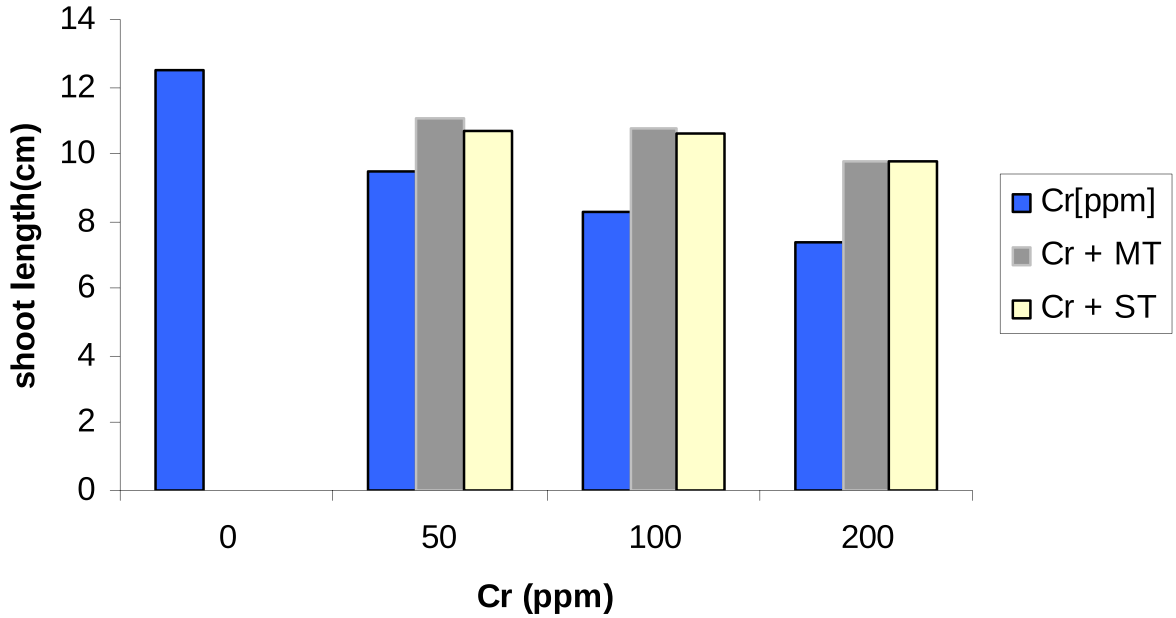

Fig. 1. A plot of effect of tea waste on shoot length of *Vigna radiata* in Cr contaminated soil.

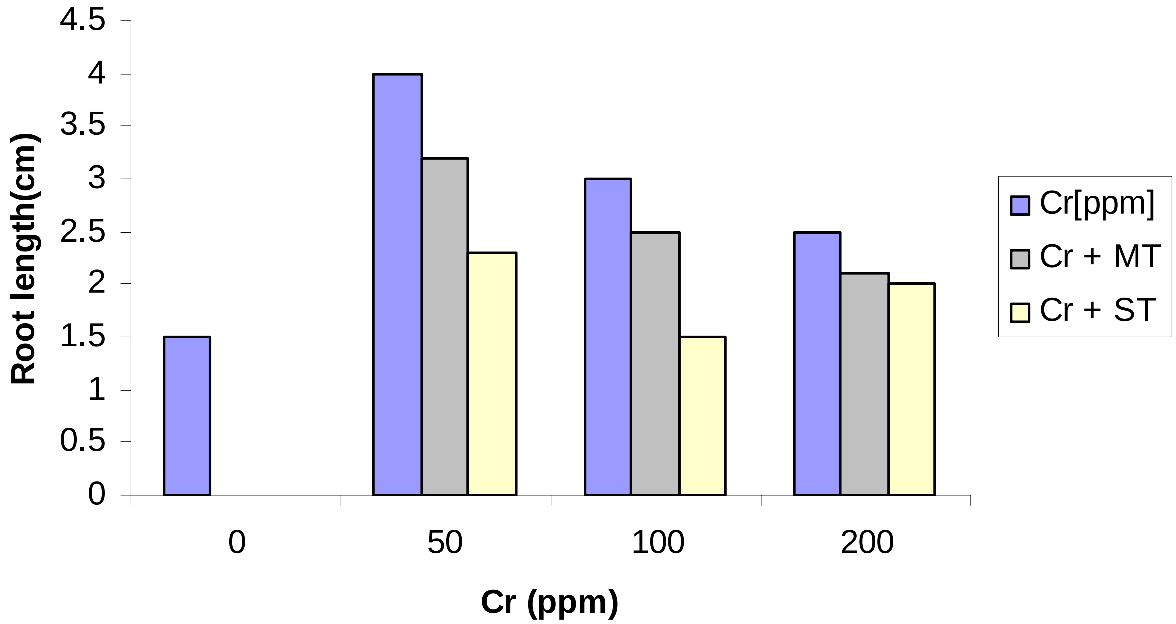

Fig. 2. A plot of effect of tea waste on root length of *Vigna radiata* in Cr contaminated soil.

An increase in concentrations of the phosphorus (Table 1) in different parts of the seedling was observed at low concentration of Cr (50ppm) which gradually lowered with an increase in concentration of Cr as compared to the control plants ($p<0.005$). It is known that P and Cr are competitive for surface sites. Hence, it is possible that Cr effectively competed with this element to gain rapid entry into the plant system. The reduction in K and P at elevated concentration could be due to the reduced root growth and impaired penetration of the roots into the soil due to Cr toxicity (Biacs *et al*., 1995). The magnitude of the content of P (Table 1) in tissues increased at lower concentration and its uptake decreased with increasing levels of Cr in the soil (Tsvetkova & Georgiev, 2003). It is concluded that added P alleviates the deleterious effect of Cr in the soil and improves the growth and the dry matter of shoot in been plants. Poor translocation of Cr to the shoots could be due to sequestration of most of the Cr in the vacuoles of the root cells to render it non-toxic which may be a natural toxicity response of the plant. It must be noted that Cr is a toxic and nonessential element to plants, and hence, the plants may not possess any specific mechanism of transport of Cr. The reduction in nitrogen compounds, K and P could be due to the reduced root growth and impaired penetration of the roots into the soil due to Cr toxicity (Azmat & Khanum 2005) which is successfully controlled by solid tea surface.

Results reported in the Table 2 showed that proline contents increases with the increase in concentration of Cr because the proline is the only amino acid that accumulates to a greater extent in the leaves of many plants under stress. Accumulation of proline starts under mild water stress and the magnitude of accumulation is proportional to the severity of stress (Ganesh *et al*., 2009; Kaushalya *et al*., 2005). In the present investigation, higher proline content was observed at 100 ppm (0.669) of Cr and the decrease may be related with the reduced growth of plant (Figs. 1 & 2). Thus proline accumulation under such condition may also be operative as usual in osmotic adjustment while accumulation of proline in tissues can be taken as a dependent marker for stress. The higher proline content was reduced (0.039) by application of solid tea surface and plant observed normal growth under metal stress because proline has multiple functions such as osmoticum, scavenger of free radicals, protective role of cytoplasmic enzymes, source of nitrogen and carbon for post-stress growth, stabilizer of membranes, machinery for protein synthesis and a sink for energy to regulate redox potential. Proline acts as a cytoplasmic osmoticum as it accumulates to a higher degree under stress conditions, which may play an adaptive role for any stress tolerance (Table 2) (Vartika *et al*., 2004). Proline increases the stress tolerance of plants through such mechanisms as osmoregulation, protection of enzymes against denaturation and stabilization of protein synthesis (Kaushalya *et al*., 2005).

Table 1. Effect of Cr and solid tea wastage as a biosorbent manure on P and K contents of *Vigna radiata*.

Cr (ppm)	Control	Set 1 $CrCl_3$	Set 2 $CrCl_3$ + Mix. Tea	Set 3 Tea + $CrCl_3$
Phosphorus (mgg^{-1})			**Leaves**	
50		0.490 ± 0.15	1.110 ± 0.13	0.338 ± 0.12
100	0.375 ± 0.02	0.570 ± 0.12	0.900 ± 0.15	1.117 ± 0.12
200		0.247 ± 0.08	1.623 ± 0.14	1.046 ± 0.13
			Stem	
50		0.740 ± 0.02	0.638 ± 0.03	1.080 ± 0.40
100	0.464 ± 0.01	0.650 ± 0.11	0.824 ± 0.20	0.454 ± 0.14
200		0.316 ± 0.12	1.000 ± 0.20	0.595 ± 012
			Root	
50		0.780 ± 0.11	0.726 ± 0.04	0.560 ± 0.01
100	0.623 ± 0.01	0.660 ± 0.2	0.832 ± 0.40	0.595 ± 0.12
200		0.242 ± 0.01	0.887 ± 0.30	0.571 ± 0.16
Potassium contents (ppm)			**Shoot**	
50		75.0 ± 12	81.0 ± 10	80.0 ±09
100	82.00 ± 16	61.0 ± 11	72.00 ± 09	64.0 ± 06
200		43.0 ± 10	51.0 ± 04	49.0 ± 05
			Root	
50		54 ± 06	92 ± 03	81.0 ± 05
100	95.3 ± 14	53 ± 04	70 ± 02	69.0 ± 02
200		29 ± 05	43.0 ± 04	39.0 ± 04

Set 1: Experimental plants, Set 2 and Set 3 treated plants with solid tea surface

Table 2. Effect of Cr^{3+} and solid tea wastage as a biosorbent manure on Proline contents and protease activity of *Vigna radiata*.

Concentration (ppm)	Control	Set 1 $CrCl_3$	Set 2 $CrCl_3$ + Mix. Tea	Set 3 Tea + $CrCl_3$
Proline contents (μgml^{-1})			**Leaves**	
50		0.150 ± 0.01	0.039 ± 0.01	0.166 ± 0.04
100	0.04 ± 0.01	0.669 ± 0.01	0.106 ± 0.01	0.060 ± 0.01
200		0.374 ± 0.02	0.035 ± 0.01	0.059 ± 0.01
			Stem	
50		0.121 ± 0.01	0.05 ± 0.01	0.121 ± 0.01
100	0.039 ± 0.01	0.669 ± 0.01	0.06 ± 0.02	0.020 ± 0.01
200		0.444 ± 0.02	0.08 ± 0.01	0.080 ± 0.01
			Root	
50		0.302 ± 0.01	0.052 ± 0.01	0.070 ± 0.01
100	0.039 ± 0.01	0.109 ± 0.01	0.059 ± 0.01	0.040 ± 0.01
200		0.166 ± 0.01	0.030 ± 0.01	0.030 ± 0.01
Protease activity (μgml^{-1})			**Shoot**	
50		0.586 ± 0.01	0.708 ± 0.01	0.824 ± 0.01
100	0.734 ± 0.07	0.575 ± 0.01	1.014 ± 0.01	0.645 ± 007
200		0.58 ± 0.05	0.722 ± 0.01	0.606 ± 0.01
			Root	
50		0.359 ± 0.01	0.719 ± 0.01	0.622 ± 0.01
100	0.480 ± 0.03	0.436 ± 0.01	0.659 ± 0.01	0.628 ± 0.01
200		0.494 ± 0.01	0.673 ± 0.01	0.649 ± 0.01

Set 1: Experimental plants, Set 2 and Set 3 treated plants with solid tea surface

Decrease in the protease activity of *Vigna radiata* under Cr stress may be related with deficiency in phosphorus contents which may be attributed with reduced nitrogenous compounds (protein) and potassium contents of seedling due to which biophysical visual symptoms on leaves appeared like leaf growth traits that might serve as suitable bio-indicators of heavy metal pollution. Primary and trifoliate leaves of bean plants due to which Cr showed a marked decrease in leaf area; trifoliate leaves were more affected by Cr than the primary leaves. Similar results are reported by Barcelo *et al*., (1985); Dube *et al*., (2003). Protease activity in tea waste amended plants increases (Table 2) which may increase the hydrolyzing capability of seedling to hydrolyze oxidative proteins for survival of plants under stress (Palma *et al*., 2002).

Conclusion

Chromium, due to its structural similarity with some essential elements, can affect mineral nutrition of plants in a complex way. Interactions of Cr with uptake and accumulation of other inorganic nutrients can be managed in the soil by adding suitable surface like tea waste which can control the mobility of Cr metal through complex formation with its constituents and also provide important mineral nutrients for the survival of the plant in contaminated environment. However more researches should be encouraged for controlling the mobility of heavy metals in the soil to safe the food chain.

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