



A Comparative Study on the Phytoextraction of Five Common Plants against Chromium Toxicity

B. DHEEBA and P. SAMPATHKUMAR

Department of Chemistry and Biosciences, Srinivasa Ramanujan Centre,
SASTRA University, Kumbakonam - 612 001 (India).

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ABSTRACT

Phytoextraction is a remediation technology that uses plants to remove heavy metals from soil. The success of a phytoextraction process depends on adequate plant yield (aerial parts) and high metal concentrations in plant shoots. A paper towel experiment was conducted to investigate the effects of plants sunflower (*Helianthus annuus*), maize (*Zea mays*), pearl millet (*Sorghum bicolor*), green gram (*Vigna radiata*) and ground nut (*Arachis hypogaea*). Seeds of these plants were exposed to five different concentrations (10, 20, 30, 40, 50 ppm) of Cr as potassium dichromate. The ecotoxicological effects of hexavalent Chromium (Cr) on germination, early seedling growth and chlorophyll content of five plants were investigated. Cr accumulating capacity of those plants was compared. Different species showed different levels of tolerance to Cr pollution. Total chlorophyll content declined progressively with increasing concentrations of heavy metals. The maximum chromium accumulation capacity of roots was in the order of *Zea mays* > *Sorghum bicolor* > *Helianthus annuus* > *Arachis hypogaea* > *Vigna radiata* and the amount is 5.56, 3.65, 3.56, 0.8 and 0.08 ppm respectively.

Key words: Phytoremediation, Hexavalent chromium, Root and shoot length, Chlorophyll.

INTRODUCTION

In recent decades there has been increasing concern with heavy metal contamination, because of their toxicity to microorganisms, plants and animals. Unlike most organic contaminants are non-biodegradable and can accumulate in living tissues (Srivastava *et al.*, 2003). A variety of anthropogenic sources, such as mining processing, electroplating, wood preservation, iron and steel production, pigment manufacture, smelters, power station industry, production and application of metal-containing pesticides, can lead to soils acquiring heavy metal

contents substantially in excess of natural levels. Some of these metals, at relatively low concentrations, may stimulate the biological life (Yamaji *et al.*, 2001; He *et al.*, 2005), while increased concentrations in the environment can be detrimental to a variety of living species (Chatterjee and Chatterjee, 2000; Naimo, 1995). The toxic potential of heavy metals in soil depends on soil composition, particularly on amount and type of clay minerals, organic matter and iron and manganese oxides (Covelo *et al.*, 2007). All these properties influence metal mobility and availability, and therefore, influence their release and their interaction with other components of the ecosystem,

such as plants. Chromium is an important metal that is usually encountered in the environment at oxidation states of (+III) and (+VI) (Kotas and Stasika, 2000). Each of these oxidation states has very different biological and toxicological properties (Kimbrough *et al.*, 1999). Hexavalent chromium has a high solubility, being a well-established carcinogen based upon animal, human, and *in vitro* assessment data (Costa, 1997; Gheju, 2005). On the contrary, the reduced form of chromium, Cr(III), is much less toxic and usually precipitates as hydroxides (Rai *et al.*, 1987)

Increased world population has resulted in the pollution of the environment. The main factors responsible for pollution and other type of environmental degradation in any community caused by combined effects of effluents and due to modern technology (Meadows *et al.*, 1992) Chromium enters the food chain through consumption of plant material. A high concentration of Cr has been found to be harmful to vegetation. As the chromium concentration in plants increases, it adversely affects several biological parameters. Ultimately there is loss of vegetation, and land some times becomes barren (Dube *et al.*, 2003). In recent years, contamination of the environment by chromium has become a major concern. Chromium is used on a large-scale in many different industries, including metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production, and pulp and paper production (Zayed and Terry, 2003). These industries have become especially large contributors of Cr pollution, which can ultimately have significant adverse biological and ecological effects. Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma *et al.*, 1995). There are many studies on Cr toxicity in crop plants. Chromium significantly affects the metabolism of plants such as barley Ali *et al.*, 2004), citrullus (Dube *et al.*, 2003), cauliflower (Chatterjee and Chatterjee, 2000), vegetable crops (Zayed *et al.*, 1998), wheat (Sharma *et al.*, 1995) and maize (Sharma and Sharma, 1994).

Chromium is highly toxic non-essential element for microorganism and plants (Cervantes

et al., 2001). The source of chromium in environment are both natural and anthropogenic, natural source include burning of oil and coal, petroleum from ferro chromate refractory material, chromium steels, pigments oxidants, catalyst and fertilizers. This element is also used in metal plating tanneries and oil well drilling (Abbassi *et al.*, 1998). Sewage and fertilizers are also the sources of chromium (Pillay *et al.*, 2003). Chromium has its effect on certain enzymes such as catalase, peroxidase, a cytochrome oxidase, which have iron as constituent. Agarwala and Kumar (1962) has reported that stimulation of catalase activity at excess supply of chromium occurred. Marked toxicity of chromium was found with respect to photosynthetic pigment, photosynthesis, nitrate reductase activity and protein content of some alga (Rai *et al.*, 1992). The direct interaction of metal with cellular components can initiate variety of metabolic responses finally leading to a shift in the development of the plant (Assche Van and Clijsters, 1990). Chromium toxicity produces chlorosis and necrosis in plants (Cervantes *et al.*, 2001). Several polluting metal and compounds are discharged into the water streams by tanneries.

Plant species have different responses to heavy metal pollution of soils. Although it may exist a relationship between heavy metal accumulation and plants tolerance, many plant species grow on contaminated soils and yet do not accumulate metals (Gabberelli *et al.*, 1990). Plants that possess the ability to tolerate, uptake and accumulate high levels of metals in their biomass are termed as hyperaccumulators (Brown *et al.*, 1995). The technique in which plants are used for the removal of heavy metals is known as phytoremediation. With these aspects, the present investigation was made to study the effect of different concentrations of chromium on the growth, chlorophyll content and phytoremediation property of *Zea mays*, *Helianthus annuus*, *Sorghum bicolor*, *Vigna radiata* and *Arachis hypogaea*.

MATERIALS AND METHODS

Test plants

The seeds of *Zea mays*, *Helianthus annuus*, *Sorghum bicolor*, *Vigna radiata* and *Arachis hypogaea* were collected from local agricultural

centre and authenticated by Dr. S.Kalavathy, Associate professor of Botany, Bishop Heber college, Trichy. Seeds were surface sterilized in 0.5 % sodium hypochlorite solution for 20 minutes and washed thoroughly with distilled water.

Experimental setup

Potassium dichromate solution was used as a source of Cr(VI). The experimental design was completely randomized with 6 replications. The treatments were randomized in order to eliminate experimental bias. Five Cr levels ranging from 10,20,30,40 and 50 ppm including distilled water (control) were selected. Applications of chromium up to 50ppm had nominal effect, while there was no plant survival beyond 50 ppm therefore Cr levels selected for this study were 0(control)10,20,30, 40 and 50ppm. The paper towels were made by using filter papers in the size of 15X15 cm and wet with distilled water. Surface sterilized seeds (6) were placed in the middle of the paper and rolled by having a plastic sheet in same size for support. Then rolled towel is kept in 20ml of different concentrations of Cr in a cup and seedling were allowed to grow for 12days, after which they were removed from paper towel. The physical parameters such as root length, shoot length and fresh weight were analysed by using appropriate methods.

Plant analysis

Chlorophyll (a, b, and total) analysis

Pigment contents of 12 days old plants were extracted by using the formula of Arnon, 1949. The leaves were chopped into small pieces that were extracted with 80%acetone. The absorbance was measured at 645 nm and 663 nm for chlorophyll a,b and total chlorophyll respectively by using spectrophotometer (Hitachi Model-U 2001 Japan). Then chlorophyll a, b & total chlorophyll were calculated. (Lichtenthaler and Wellburn, 1983)

$$\text{Chl a (mg g}^{-1} \text{ leaf fresh weight) = } [12.7(\text{OD}_{663}) - 2.69 (\text{OD}_{645})] \times V/1000 \times W.$$

$$\text{Chl b (mg g}^{-1} \text{ leaf fresh weight) = } [22.9(\text{OD}_{645}) - 4.68 (\text{OD}_{663})] \times V/1000 \times W.$$

$$\text{Total Chl (mg g}^{-1} \text{ leaf fresh weight) = } [20.2(\text{OD}_{645}) - 8.02(\text{OD}_{663})] \times V/1000 \times W.$$

where OD = Optical Density, V = Volume of sample. W = Weight of sample.

Extraction of metal from plant samples

The harvested plant was separated out as root and shoot, crushed into powder and incinerated at high temperature. The ash obtained from the incinerated plant samples were treated with concentrated HCl and the metals analyzed using Atomic Absorption Spectrometer (AAS) (APHA, 1990).

RESULTS AND DISCUSSION

Physical parameters

Shoot length

Under various experimental conditions, the shoot lengths of *Zea mays*, *Helianthus annus*, *Sorghum bicolour*, *Vigna radiata* and *Arachis hypogaea* were analysed. The shoot lengths of three plant species (*Helianthus annus*, *Vigna radiata* and *Arachis hypogaea*) were reduced by more than 50% when compared to control (without Cr). In *Vigna radiata* and *Arachis hypogaea* plants, root and shoot growth were similarly sensitive. For example, the shoot lengths of all plants are represented in the following graph which supports significant alteration. In particular it is reduced more than 60% when it is compared the other treatments with controlled treatment. (Fig. 1).

Further, the \bar{X} - chart, R-chart for shoot length in *Arachis hypogaea* under 6 treatments are obtained. The - chart shows that the shoot lengths

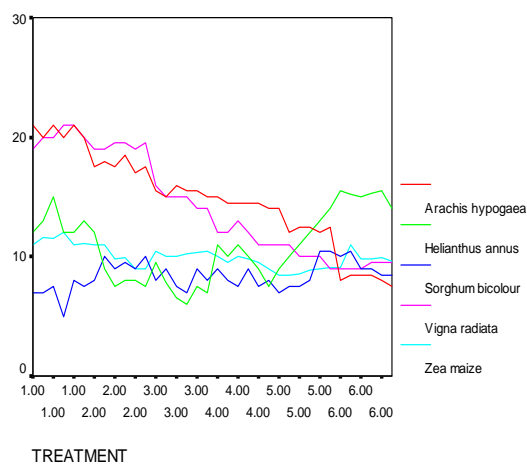


Fig. 1. The shoot length (cm) of experimental plants under various experimental conditions.

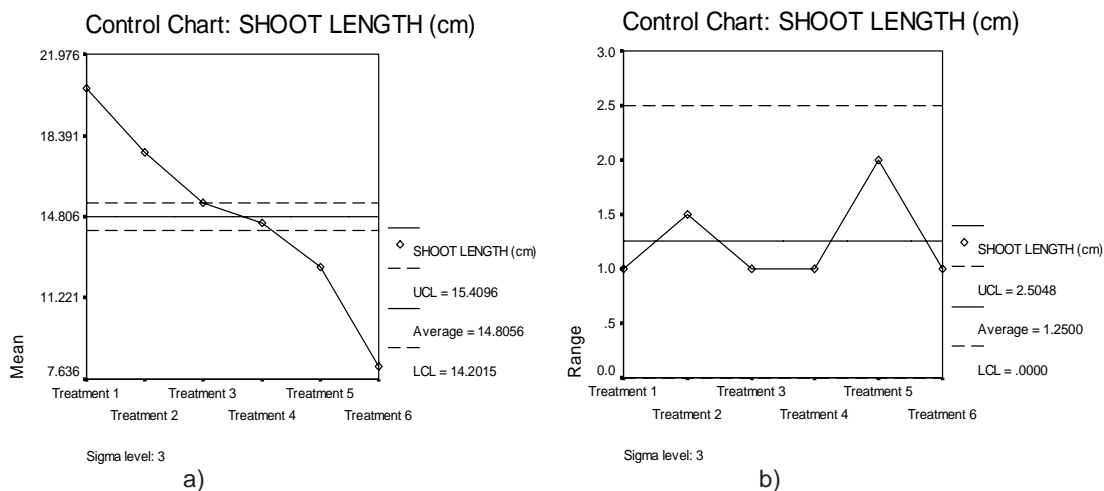


Fig. 2. Mean chart (a) and Range chart (b) of shoot length of experimental plants under various experimental conditions.

are not control but the R-chart indicated that the ranges are within the control limits. (Fig 2 a and b)

Moreover, the ONE WAY ANOVA for Shoot length (cm) is obtained which is shown in the Table 1. The calculated F value is 427.692. But the tabulated F value at (5, 30) degrees of freedom at 1% and 5% level of significance is 3.70 and 2.53 respectively. Hence calculated F Value > tabulated F value at both level of significances. Therefore, the difference between the shoot lengths is highly significant. Also the Karl Pearson's correlation coefficient and Spearman's rank correlation coefficients between the treatments and shoot lengths are -0.973 and -0.987 respectively. Hence they are negatively correlated and correlation is significant at the 0.01 level (2-tailed).

Root length

The root lengths of *Zea mays*, *Helianthus annuus*, *Sorghum bicolor*, *Vigna radiata* and *Arachis hypogaea* in different experimental conditions

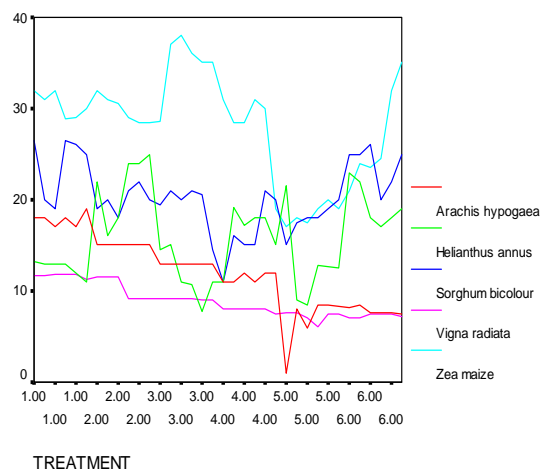


Fig. 3. The root length(cm) of experimental plants under various experimental conditions

decreased when Cr concentration is increased. In *Arachis hypogaea* the root length is reduced to 66% and shoot length is also markedly reduced to 60 %

Table 1: ONE WAY ANOVA for Shoot length (cm) of experimental plants under various experimental conditions.

	Sum of Squares	Degrees of freedom	Mean Square	F
Between Groups	540.556	5	108.111	427.692
Within Groups	7.583	30	.253	
Total	548.139	35		

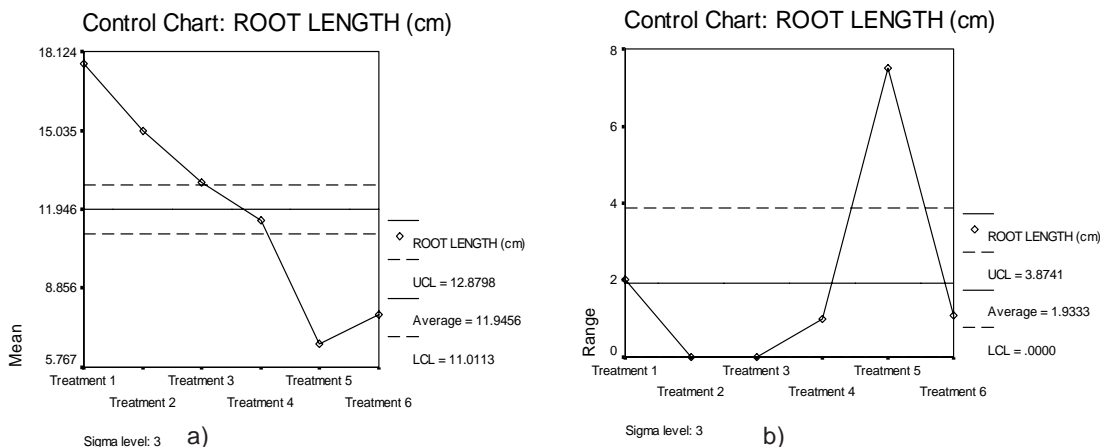


Fig. 4. Mean chart (a) and Range chart (b) of root length of experimental plants under various experimental conditions.

at 50 ppm Cr concentration when compared to control plants. Among five plants *Zea mays* is more tolerant to Cr toxicity followed by *Sorghum bicolor* in which only upto 13% reduction in root and shoot length was noticed in 50 ppm Cr concentration when compared to control. For example, the root lengths of *Arachis hypogaea* are represented in the following graph which shows that the shoot lengths are decreased gradually with respect to the treatments. In particular it is reduced more than 60% when it is compared the other treatments with controlled treatment. (Fig. 3)

Further, the - chart, R-chart for root length in *Arachis hypogaea* under 6 treatments are obtained. The - chart shows that the root lengths are not control but the R-chart indicated that the ranges are with in the control limits. (Fig. 4 a and b)

The one way anova for root length (cm) is obtained which is shown in the Table 2. The calculated F value is 63.507. But the tabulated F

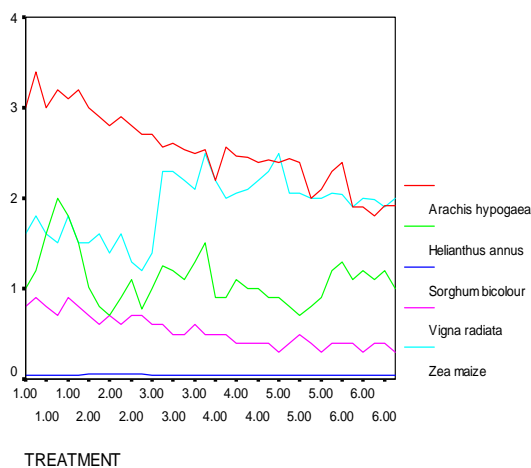


Fig. 5. The fresh weight (g) of experimental plants under various experimental conditions

value at (5, 30) degrees of freedom at 1% and 5% level of significance is 3.70 and 2.53 respectively. Hence calculated F Value > tabulated F value at

Table 2: ONE WAY ANOVA for root length (cm) of experimental plants under various experimental conditions.

	Sum of Squares	Degrees of freedom	Mean Square	F
Between Groups	528.104	5	105.621	63.507
Within Groups	49.894	30	1.663	
Total	577.998	35		

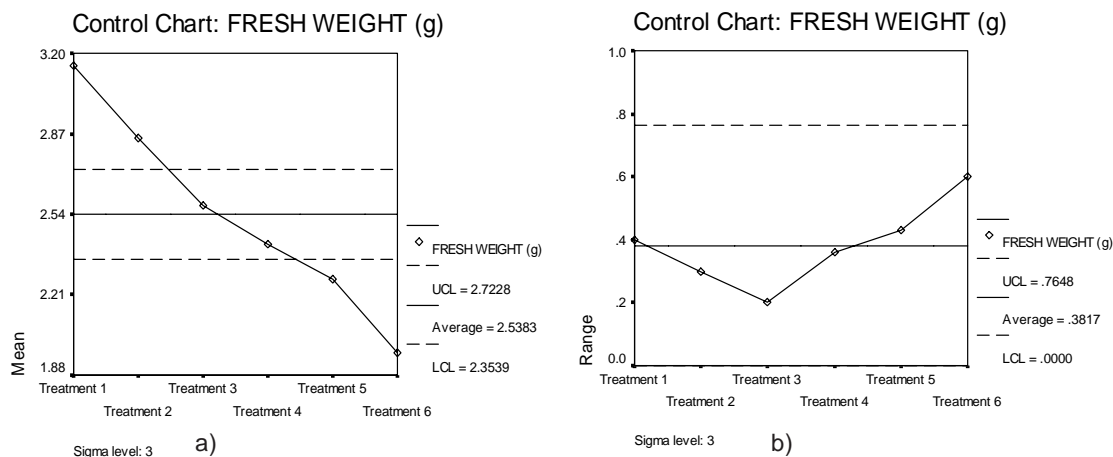


Fig. 6. Mean chart (a) and Range chart (b) of fresh weight of experimental plants under various experimental conditions.

both level of significances. Therefore, the difference between the root lengths is highly significant. The Karl Pearson's correlation coefficient and Spearman's rank correlation coefficients between the treatments and root lengths are also -0.922 and -0.967 respectively. Hence they are negatively correlated and correlation is significant at the 0.01 level (2-tailed). But, the Karl Pearson's correlation coefficient and Spearman's rank correlation coefficients between the shoot lengths and root lengths are 0.844 and 0.943 respectively. Hence they are positively correlated and correlation is significant at the 0.01 level (2-tailed).

The higher concentration of effluent having heavy metals decrease enzyme dehydrogenase activity that is considered as one of the biochemical change which may have disrupt germination and seedling growth (Murkumar and Chauan, 1987). Studies on heavy metal tolerance in plants indicate that root growth is particularly sensitive to heavy metals (Punz and Sieghardt, 1993). Copper toxicity affected the growth of *Alyssum montanum* (Ouzounidou, 1994) and *Brassica juncea* (Singh and Tiwari, 2003). Reduction in root growth due to heavy metals has also been reported in wheat seedlings (Oncel *et al.*, 2000). The number of leaves and branches, root and shoot length and biomass decreased as concentration of Cr increased in egg plant and tomato (Purohit *et al.*, 2003) and in barley (Arey and Rana, 2003). Reduced growth in terms of root and shoot lengths at increasing doses of

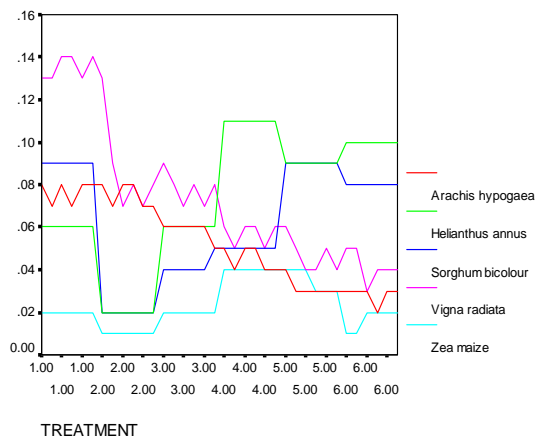


Fig. 7. The chlorophyll a (mg/g) of experimental plants under various experimental conditions.

chromium might be due to adverse effect of this metal on auxin synthesis in plants more during early stages of their growth. Increasing concentrations of chromium caused significant reduction in root length and shoot length.

Fresh Weight

Fresh weight of plants are not much affected at 10 and 20 ppm Cr concentration but affected at higher concentration of Cr in all five species of plants. When compared to control plants at 50ppm, fresh weight of *Vigna radiata* and *Arachis hypogaea* plants were reduced to 50%. In all plants at 5ppm concentration, fresh weight is increased. Presence

Table 3: ONE WAY ANOVA for fresh weight (g) of experimental plants under various experimental conditions.

	Sum of Squares	Degrees of freedom	Mean Square	F
Between Groups	5.279	5	1.056	48.151
Within Groups	.658	30	.022	
Total	5.937	35		

of Zn at higher concentrations retarded the growth and development of plants by interfering with certain important metabolic processes (Alia., 1995). The following are the sample for the analysis in *Arachis hypogaea*. (fig 5).

Moreover, the calculated F value is 48.151. But the tabulated F value at (5, 30) degrees of freedom at 1% and 5% level of significance is 3.70 and 2.53 respectively. Hence calculated F Value > tabulated F value at both level of significances. Therefore, the difference between the fresh weights is highly significant. (Table 3)

Plant pigments

Increasing concentrations of chromium caused significant reduction in Chl a, b and total chl. In all five variety of plants pigment levels significantly decreased at 10, 20, 30, 40 and 50 ppm of chromium as compared to control. In *Zea mays*, *Sorghum bicolor* and *Helianthus annuus* plants chlorophyll a is reduced at higher

concentration of Cr than chlorophyll b. In *Vigna radiata* and *Arachis hypogaea* group both chlorophyll a and chlorophyll b are reduced more than 50% at higher concentration of chromium treatment when compared to control group plants.(Fig 8 -12).

Photosynthesis was reduced significantly by increasing Cr(VI) concentrations in the growth medium. The modification of total chlorophyll content and the chl *a/b* ratio is noted under toxic conditions. chlorophyll *a* and *b* content decreased significantly in the 50 ppm, showing the negative effect of Cr(VI) on processes of chlorophyll biosynthesis. The increase in chlorophyll *a* and *b* content at the 10ppm Cr(VI) dose was noticed and that suggested that low concentrations of Cr(VI) increases chlorophyll synthesis in all five plant species. The decrease in chlorophyll content with increasing Cr(VI) dose has been described in the literature repeatedly (Liu *et al.*, 2008) It is thought to be related to direct inhibition of chlorophyll pigment synthesis. Other

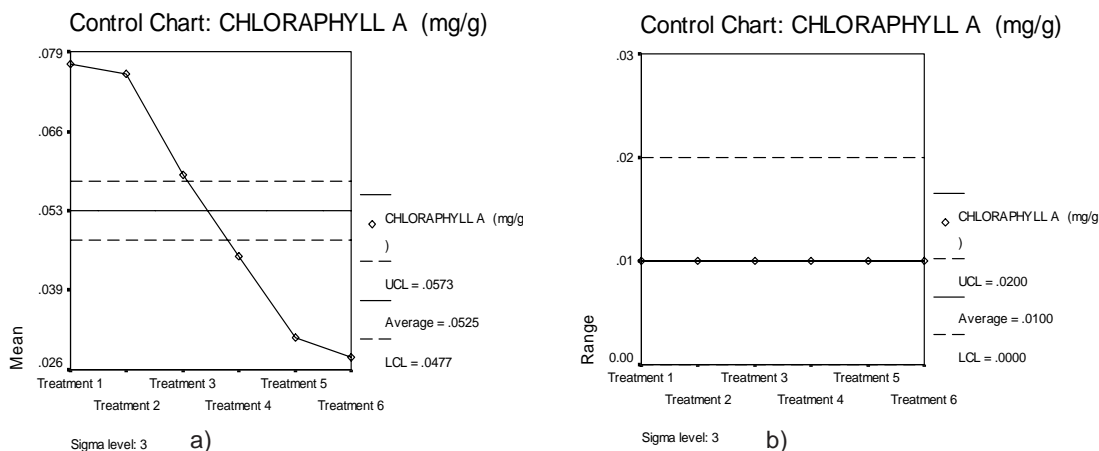


Fig. 8. Mean chart (a) and Range chart (b) of chlorophyll a of experimental plants under various experimental conditions

authors have reported a decrease in chlorophyll content correlated with metal concentration (Panda and Choudhury, 2005; Scoccianti *et al.*, 2006). Chromium possesses the capacity to degrade δ -aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting δ -aminolevulinic acid (ALA) utilization; this results in the buildup of ALA and reduction of the level of chlorophyll (Vajpayee *et al.*, 2000). Chromium, mostly in its hexavalent form, can replace Mg ions from the active sites of many enzymes. Cr(VI) also causes Fe deficiency in stressed plants, disrupting chlorophyll biosynthesis (Liu *et al.*, 2008). The chl *a/b* ratio decreased when the plants were exposed to 40 μ M and 100 μ M Cr(VI), perhaps due to faster breakdown or decreased synthesis of chl *a* as compared to chl *b*, although chl *b* also decreased (Vajpayee *et al.*,

2000).

The decrease in the concentrations of chlorophyll *a* and *b* and total chlorophyll in plants exposed to excess supply of Cr is similar to the effects of other pollutant elements (Lee *et al.*, 1993). The decrease in chlorophyll concentration may be the result of an inhibited photosynthetic electron transport (Bohner *et al.*, 1980) and decomposition of the chloroplast membrane with excess Cu (Sandmann and Boger, 1980). The adverse effects of heavy metals in excess in plants may be because these metals interfere in the formation of chlorophyll either through the direct inhibition of an enzymatic step or through the induced Fe deficiency (Van Assche and Clijsters, 1990). Heavy metal induced reduction in total chlorophyll has been reported in pigeon pea due to Cd and Ni stress (Shoran *et al.*, 1990) and in *Brassica juncea* due to Cd stress (Singh and Tiwari, 2003). Reduction in chlorophyll content due to its degradation and unfavourable effect on photosynthetic electron transport has been reported due to copper toxicity in sensitive species of *Silene compecta* and *Thalpsi ochrolucum* (Ouzoudou, 1994).

Chromium accumulation in plants

The maximum chromium concentration in shoot was found in 50 ppm chromium treated *Zea mays* plants *i.e.* 2.86 ppm and the lowest amount is in the shoot of *Vigna radiata* plants (0.061 ppm) in 50 ppm concentration.

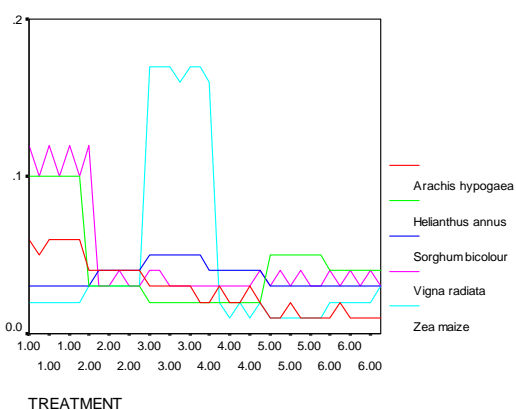


Fig. 9. The chlorophyll b (mg/g) of experimental plants under various experimental conditions.

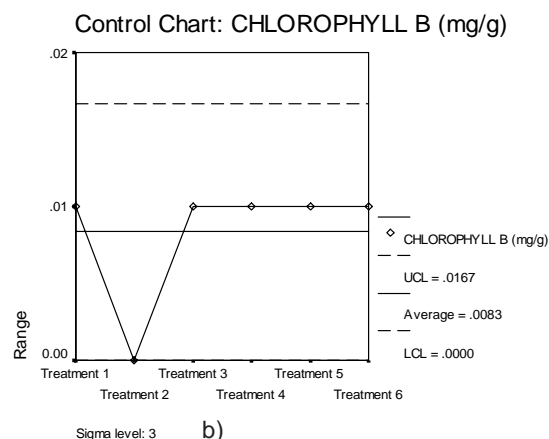
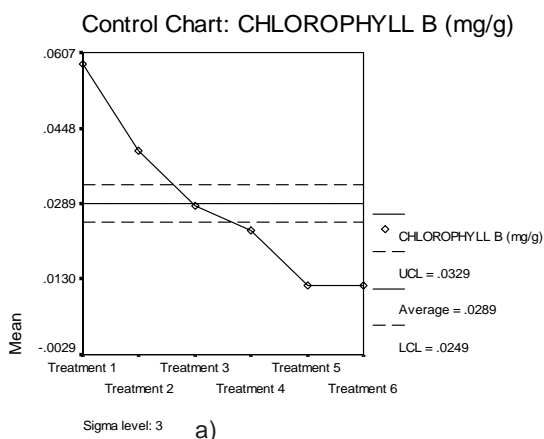


Fig. 10. Mean chart (a) and Range chart (b) of chlorophyll b of experimental plants under various experimental conditions

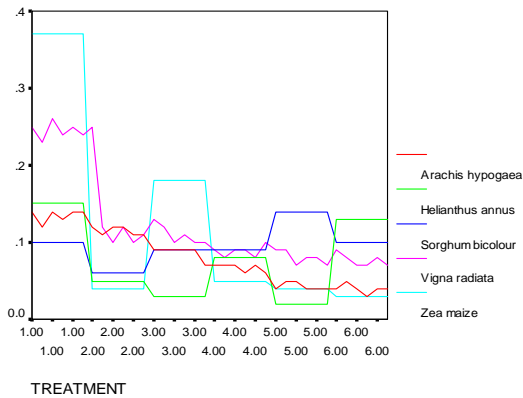


Fig. 11. The total chlorophyll (mg/g) of experimental plants under various experimental conditions

In all five varieties of plants the accumulation of chromium is higher in roots than shoots this is because of poor translocation from root to shoot. While comparing five group of plants *Zea mays*, *Sorghum bicolor* and *Helianthus annuus* plants uptake more chromium than other two species. The maximum chromium accumulation capacity of roots was in the order of *Zea mays* > *Sorghum bicolor* > *Helianthus annuus* > *Arachis hypogaea* > *Vigna radiata* and the amount is 5.56 , 3.65, 3.56, 0.8 and 0.08 ppm respectively. (Fig 13 - 16)

Chromium produced a significant reduction in the growth of *P. juliflora*. The effect was

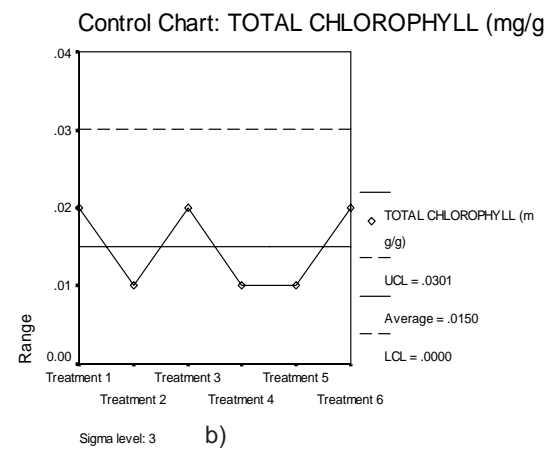
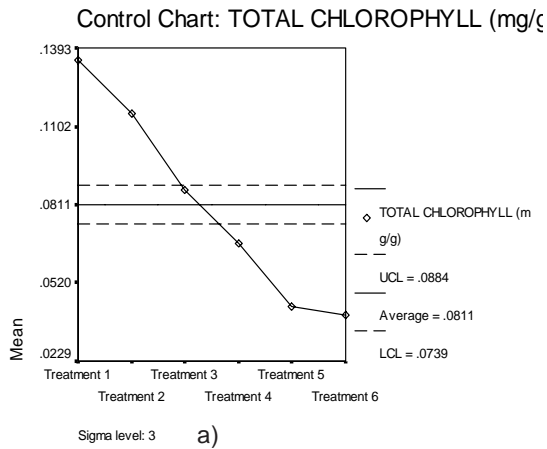


Fig. 12. Mean chart (a) and Range chart (b) of total chlorophyll of experimental plants under various experimental conditions.

mainly observed on root and shoot and to a lesser extent on seedling length. The inhibition of growth by chromium could be mainly due to the accumulation of chromium in the roots. Bishoni *et al.*, (1993) observed that hexavalent chromium applied as potassium di chromate did not affect the percentage germination of pea seed but the growth of radicles and plumule were significantly suppressed above 20 ppm concentration and the deleterious effect of chromium was more pronounced on the growth of roots than on shoots. The present study showed the deleterious effect of chromium on roots due to its accumulation in the roots which was extremely limited in the shoots .Accumulation of chromium in roots was 100 fold higher than that by shoot regardless of the

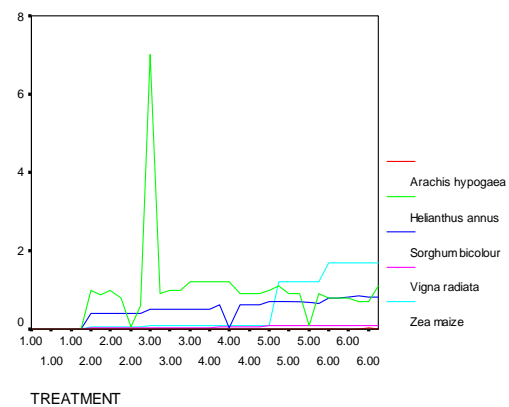


Fig. 13. Shoot Chromium (mg/g) content of experimental plants under various experimental conditions

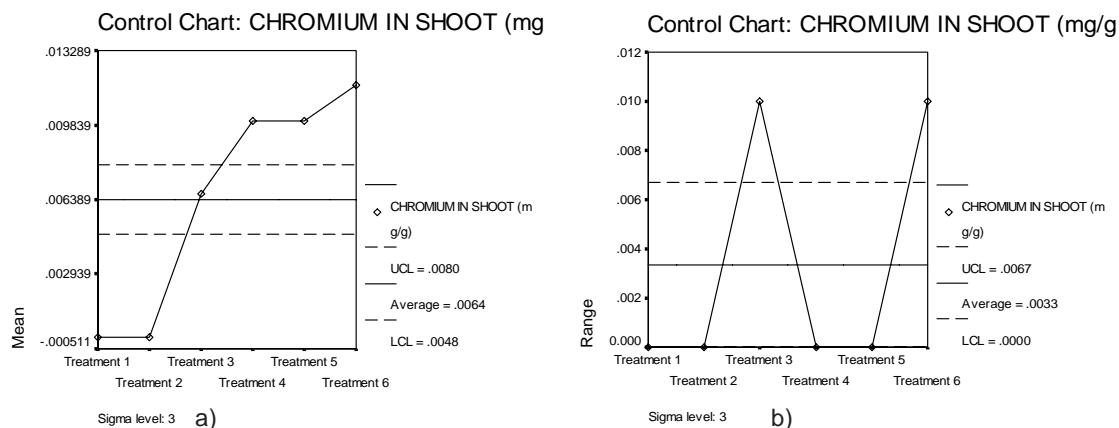


Fig. 14. Mean chart (a) and Range chart (b) of shoot chromium content of experimental plants under various experimental conditions.

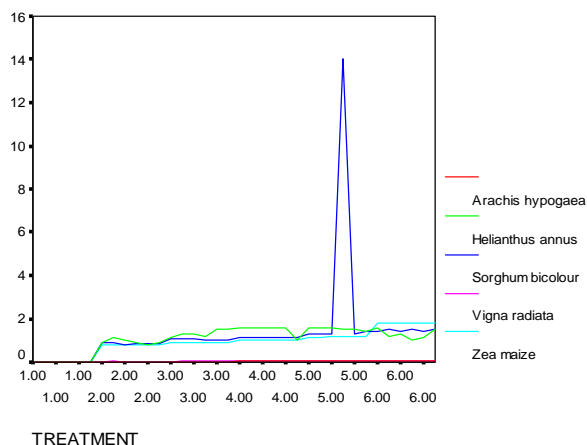


Fig. 15. Root Chromium (mg/g) content of experimental plants under various experimental conditions

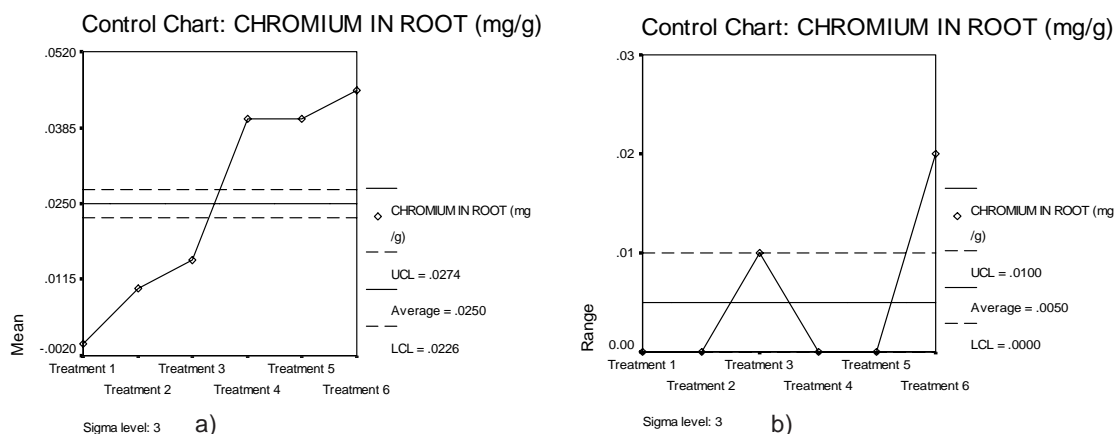


Fig. 16. Mean chart (a) and Range chart (b) of root chromium content of experimental plants under various experimental conditions.

chromium supplied. Low concentration of chromium was stimulatory to the plant growth while other workers contradicted it and observed that chromium is inhibitory and toxic to plant growth. Chromium toxicity manifested itself in plants by inhibiting the growth more or less, showing chlorosis with small brownish-red or purple leaves and necrotic lesions. High chromium concentration inhibits photosynthesis and greatly inhibits the root growth. It is therefore evident that chromium affects the plant growth mainly by damaging the root while its translocation into other parts of the plant is of minor importance.

Deleterious effect of chromium on plants suggests that chromium is very much toxic in nature and considered inhibitory to plant (Sharma and Arey, 2000). It is concluded that among the five plants studied *Zea mays* and *Sorghum bicolor* shown better chromium accumulation when compare with other plants with reference to growth. Hence these plants can be suggested for phytoremediation with further studies.

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