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Phytoremediation of Chromium contaminated soil using Sorghum plant

Revathi.K[1], Haribabu.T.E[2], Sudha. P.N[3]

1- Part – Time Research Scholar, SCSVMV University, Enathur, Kanchepuram, Tamil Nadu, India

2- Part – Time Research Scholar, Bharathiar University, Coimbatore, Tamil Nadu, India

3- PG & Research Department of Chemistry, D.K.M College for Women, Vellore, Tamil Nadu, India

parsu8@yahoo.com

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ABSTRACT

Industrial waste is one of the most important sources of contamination in the surface environment. The impact of heavy metals in soils, plants, animals and humans is due to the unabated expansion of toxic effects. The toxic wastes generated are treated by physico-chemical processes in which "Bioremediation" is the microbial clean up approach. Plant-based remediation (Phytoremediation) is an emerging biotechnological tool, for cleaning metal polluted or contaminated soil. The present study deals with phytoremediation of Chromium metal polluted soil of Ranipet Tanneries utilizing Sorghum plant, which is one of the best "Hyper-accumulators" for the phytoremediation of metal polluted sites. The impact of chromium on the biomass and chlorophyll content and also the effect of addition of biosolids like vermicompost on the bioaccumulation efficiency of the plant were investigated. The results indicated that there is significant reduction of biomass of the plant with increased dosage of chromium. It is also observed that addition of vermicompost to the contaminated soil improves the biomass of the plants thus making room for more bioaccumulation. Pytoremediation is found to be cost-effective and highly efficient in remediating the heavy metal polluted sites.

Keywords: Phytoremediation, bioaccumulation, sorghum, vermicompost, Chromium

1. Introduction

Industrial waste is one of the most important sources of contamination in the surface environment. Numerous studies of environmental contamination due to industrial wastage activities have been undertaken to further the understanding of the impacts of heavy metals and metalloids in soils, plants, animals and humans. The unabated expansion of chemical industries and technologies indeed left a trial of anxiety and global concern.The toxic metals may be absorbed by plants grown in contaminated soil which then accumulate in animals eating those plants perhaps reaching to chronic toxic levels. Most heavy metals become quite insoluble in soil at pH 6 or more. Cadmium, being highly soluble than other heavy metals, is a frequent contaminant. Other metals such as Ni, Cu, Mo and Zn are also soluble but to a lesser extent (Mathur et al., 1977).

The various conventional remediation technologies that are used to clean heavy metal polluted environments are soil vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification and stabilization electro kinetic systems. Each of the conventional remediation technology has specific benefits and limitations (MADEP, 1993). Metallic contaminants like Hg, Pb, Zn, As, Cd, Cr, Na, K, Cu etc, destroy bacteria and

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beneficial microorganisms in the soil. Heavy metals tend to precipitate phosphatic compounds and catalyze their decomposition. These metals are considered to be indestructible poisons and their accumulation in soil for a long period may be highly fatal to living organisms. Hence in the present investigation heavy metal like Chromium was chosen for further studies.

Chromium (Cr) is abundant in the earth's crust, with both the hexavalent (Cr[VI]) and more predominant trivalent (Cr[III]) forms readily found in nature. Chromium concentrations in soil can range from 0.1 to 250 ppm Cr, and in certain areas, soil content may be as high as 400 ppm Cr (Langard and Norseth, 1979). Overall, most soils have been shown to contain on average 50ppm Cr (Hartel, 1986). Chromium in ambient air originates primarily from industrial sources (i.e., steel manufacturing and cement production) and the combustion of fossil fuels. The content in coal and crude oil varies from 1 to 100 μg Cr/L and from 0.005 to 0.7μg Cr/L, respectively (Pacyna, 1986). Hexavalent Cr compounds can penetrate the skin more readily than trivalent forms, and uptake is enhanced with increases in the pH of the Cr-containing substances.

Phytoremediation is the use of certain plants to clean up soil, sediment, water and polyaromatic hydrocarbons (PAHs). Phytoremediation is an aesthetically pleasing mechanism that can reduce remedial costs, restore habitat and clean up contamination in place rather than entombing it in place or transporting the problem to another site. Phytoremediation can be used to clean up contamination in several ways such as Phytovolatilization, Microorganism stimulation, Phytostabilization, Phytoaccumulation or extraction and Phytodegradation by plants (Cunningham et al., 1997; Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Ensley, 2000). Chaney, (1983) was the first to suggest using these "hyperaccumulators**"** for the phytoremediation of metal-polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Cunningham et al., 1995; Comis, 1996; Ebbs et al., 1997). By definition, a hyperaccumlator must accumulate at least 100 mg g^{-1} (0.01% dry wt (Reeves and Baker, 2000; Wantanabe, 1997). Vetiver grass (*Vetiveria zizanio ides L*. Nash) has been one among the well-documented grasses to have strong resistance to the execrable environment and to be able to survive in high concentrations of heavy metals (Xia et al., 1999). The sorghum plant selected in this study is a local species, easily available and is found to be tolerant to both the Cadmium and Chromium metals.

Sorghum (*Sorghum bicolor*) is a grass (Family *Poaceae*) whose seeds are used to make a flour and as cattle feed. It is an important food crop in Africa, Central America, and southern Asia and is the fifth major cereal crop grown in the world (470,000 km^2 harvested in 1996). The largest producer is the United States. Therefore, in the present work an attempt has been made on the study of impact of heavy metal Chromium on the biomass and chlorophyll content of the plant sorghum bicolor. The heavy metal accumulation efficiency of the sorghum plant and also effect of addition of biosolids like vermicompost on the bioaccumulation efficiency of the plant have been investigated. The various metal bioaccumulated plants were subjected to phytoextraction and the percentage of metal extracted from the plants was also evaluated.

2. Materials and Method

2.1 Collection of heavy metal polluted soil

Top soil upto 15 cms depth, were collected from the tannery polluted agricultural lands of Ranipet Industrial Town, India**.** The samples were air dried, crushed to powder and sieved in 0.5 mm mesh. The sieved soil samples were stored in polythene bags. The various physico-chemical factors were analyzed using the soil extracts (Jackson, 1958). Analar sample of Potassium dichromate was used as source of chromium. Vermicompost was prepared with cow dung using earthworm species *Eurdilus euginae* (Ismail, 1997). Sorghum (SSV – 84) (*Sorghum bicolor L.)* seeds were procured from Tamil Nadu Agricultural University, Coimbatore, India. The seeds were cold treated (10^oC) for 3 days to break dormancy and synchronize germination. Seeds were germinated in roll towels and germinating seedlings of similar size were sown in the control and experimental pots and watered. On sixtieth day the plants were harvested for experiments.

2.2 Experimental setup

The seedlings were exposed to different concentrations of heavy metal Chromium to find the toxicity**.** Chromium at high concentrations of 200 and 300 mg/ Kg showed high toxicity that the plants died**.**

The various experimental setups used for the present study are listed below.

1. Garden soil + sorghum plant
2. 2. 50% garden soil + 50% vermicompost + sorghum plant
3. Garden soil + 50 mg/kg chromium + sorghum plant
4. 50% garden soil + 50% vermicompost + 50 mg/kg Cr + sorghum plant
5. Garden soil + 150 mg/kg chromium + sorghum plant
6. 50% garden soil + 50% vermicompost + 150 mg/kg Cr + sorghum plant
7. Heavy metal polluted soil + sorghum plant
8. 50% heavy metal polluted soil + 50% vermicompost + sorghum plant

Plants were harvested and roots washed with running tap water. Roots, shoots and leaves were then separated and oven dried for 3 days at 80^oC. The sample was then ground into fine powder and used for metal analysis.

2.3 Physico-chemical characteristics of soil samples

Physico-chemical factors such as pH, Electrical conductivity (EC), moisture, porosity, specific gravity, calcium, nitrogen, phosphorous, potassium (NPK values) were analyzed for soil as per the method of Jackson (1958).

2.4 Heavy metal analysis of soil samples

Soil samples were air dried and crushed to pass through 0.2 mm sieve and stored in polythene bags for analysis (Bhide and Sundaresan, (1983). Representative samples from this lot were used throughout the study. 10 grams of each soil was treated with 2ml of perchloric acid and 5 ml of con. HNO_3 and stored in vials for further analysis.

2.5 Digestion of the sample

0.5 grams of each sample was treated with 3ml of Merk Hydrofluoric Acid, 1 ml of Merk Perchloric Acid and 7 ml of 65% Suprapur Merk Nitric acid. The water used for washing and dilution was milli Q element distilled water from Millipore. This mixture was digested in Ethoplus high performance microwave lab station. The digested sample was made upto 25 ml and analyzed for the metal chromium by Atomic Absorption Spectroscopy (Varian AAA 220 FS). The whole analysis was conducted in clean air room of class 10,000 (APHA, 1990).

2.6 Estimation of Chlorophyll

The leaves were analyzed for chlorophyll content by homogenizing 200mg of leaves in 80% acetone and the extract was centrifuged and the supernatant was analyzed for chlorophyll content in a spectrophotometer (spectronic 20), at wavelength 645nm for chlorophyll with 80% acetone as blank.

2.7 Extraction of metals from plant samples

The harvested plant was 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)** according to APHA (1990).

3. Results and Discussions

Table 1: Physico-chemical characteristics of the various soil samples

Physico-chemical factors	Garden soil	50% garden soil + 50% vermicompost	Polluted soil	50% polluted soil + 50% vermicompost
pH	7.4± 0.02	7.9± 0.02	4.8± 0.02	5.3± 0.02
Electrical conductivity (mmho/cm2)	0.249± 0.001	0.924± 0.001	1.12± 0.001	1.35± 0.001
Porosity	2.03± 0.09	2.82± 0.09	0.92± 0.09	1.49± 0.09
Specific gravity	2.0± 0.76	2.4± 0.16	1.12± 0.21	1.74± 0.14
Organic matter (%)	5.6± 0.06	17.2± 0.13	5.2± 0.11	12.4± 0.34
Total Nitrogen (mg/kg)	7527± 0.007	8215± 0.017	6432± 0.103	9396± 0.010
Phosphorus (mg/kg)	416.8± 0.02	521.8± 0.02	312.4± 0.01	354.2± 0.12
Potassium (mg/kg)	110± 0.02	450± 0.02	248.1± 0.02	812.9± 0.02
Sodium (mg/kg)	400 ± 0.09	367 ± 0.09	1710 ± 0.02	1200 ± 0.02
Calcium (mg/kg)	280± 0.07	320± 0.09	424± 0.06	725± 0.045
Salinity (mg/kg)	21.6± 0.07	23.2± 0.079	143± 0.08	70± 0.06
Chromium (mg/kg)	7.9± 0.001	4.2± 0.021	105.63± 0.021	82.3± 0.011

Values expressed as mean ± SE of six individual values

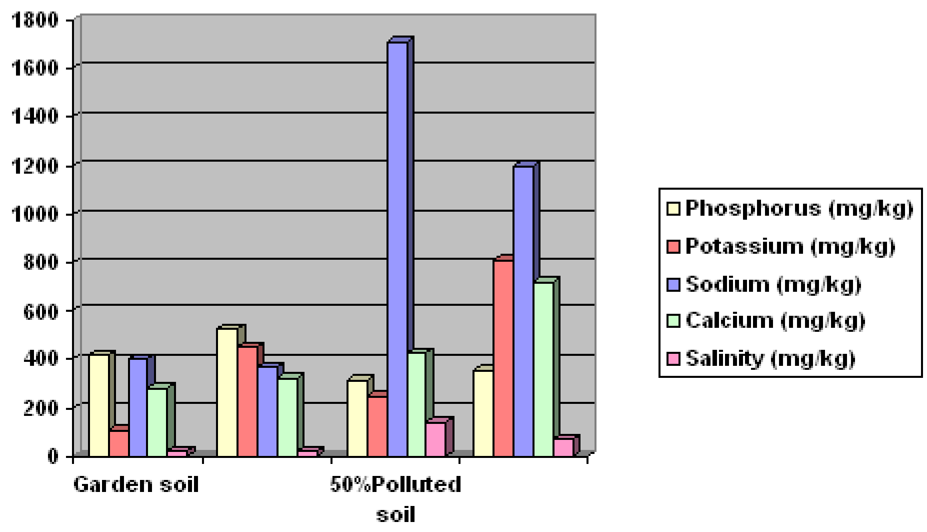

Figure 1: A few physico-chemical characteristics of the various soil samples

Table – 1 and Figure – 1 represent the physico-chemical characteristics of the Garden soil, 50% Garden soil (GS) + 50% vermicompost (VC), heavy metal polluted soil, 50% heavy metal polluted soil (PS) + 50% VC. As the polluted soil was collected from tannery polluted agricultural lands, the pH of the soil is acidic (4.8). Electrical conductivity of the polluted soil and 50% mixture of PS and VC were found to be 1.12 mmho/cm^2 and 1.35 mmho/cm^2 where as the EC of GS and GS + VC were recorded 0.249 and 0.924 mmho/cm^2. The porosity of the 50% GS + 50% VC was recorded the highest (2.82).

The organic matter of the polluted soil was found to be almost the same range as that of GS. This is because this soil is contaminated by tannery effluents which are rich in hair, flesh and blood of the sheep and goat. Vermicompost is a complex mixture of humic acid, fulvic acid and many other organic acids. Hence, addition of vermicompost increases the organic matter content of both GS and PS.

Total nitrogen recorded 7,527 mg/kg, 8215 mg/kg, 6432 mg/kg and 9396 mg/kg in GS, GS + VC, PS and PS + VC respectively. The salinity was found to be the highest in the polluted soil. This is because tanneries handled large volumes of salted hides and skins of cattle, which contaminate the nearby water and soil environs. Similarly the levels of sodium were the highest in the polluted soil (1710 mg/kg) as common salt is used as a preservative for hides and skins.

The heavy metal chromium was found to be in appreciable amount in the polluted soil and 50% PS + 50% VC. The pH is raised (5.3) by the addition of VC. All the factors such as electrical conductivity, percentage of organic matter, porosity, specific gravity, total nitrogen, total phosphorus, total potassium and calcium have been increased in the garden soil and heavy metal polluted soil by the addition of vermicompost.

This shows that soil amendment with humus material like vermicompost improves the soil properties showing improvement in the fertility of the soils for the cultivation of crops. The organic matter content had increased uniformly in all the soil samples due to the addition of organic substrates like vermicompost.

Table 2: Growth performance (Biomass) of sorghum plant and chlorophyll content of sorghum leaves exposed to the heavy metal contaminated soils

Sample	Biomass (gms)	Chlorophyll (mg/kg)
Garden soil (GS)	380± 0.14	54.0 ± 0.4
50% GS + 50% Vermicompost (VC)	412± 0.23	64.1 ± 0.3
GS + 50 mg/kg Cr	210± 0.10	36.3 ± 0.3
GS + 150 mg/kg Cr	100± 0.05	23.0 ± 0.8
50% GS + 50% VC + 50 mg/kg Cr	246± 0.12	40.5 ± 0.3
50% GS + 50% VC + 150 mg/kg Cr	126± 0.41	34.8 ± 0.4
Polluted soil (PS)	180± 0.32	34.0 ± 0.9
50% PS + 50% VC	245± 0.11	39.4 ± 0.2

Values expressed as mean ± SE of ten individual values

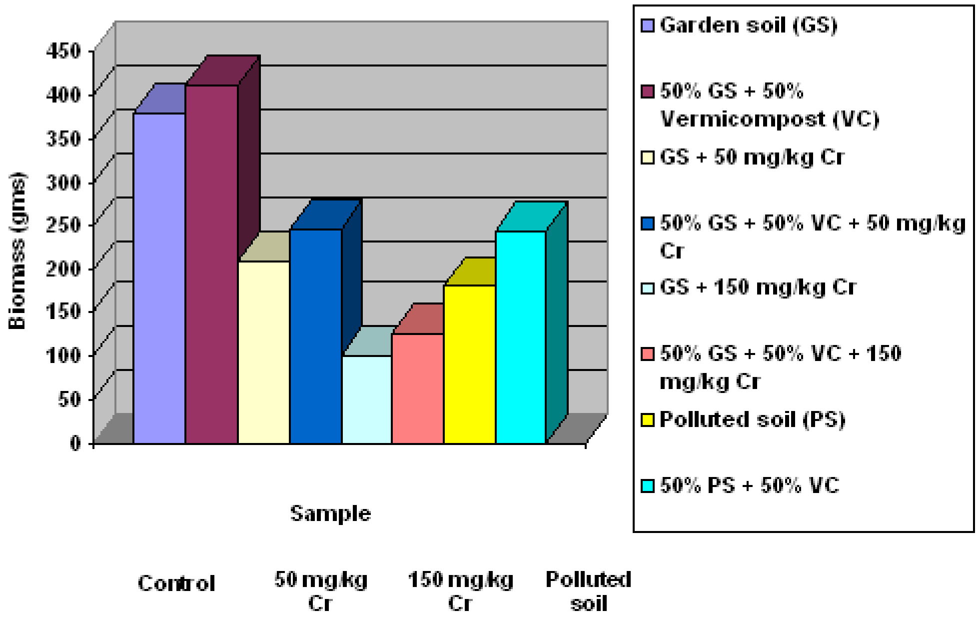

Figure 2.a: Growth performance (Biomass) of sorghum plant exposed to the chromium metal contaminated soils

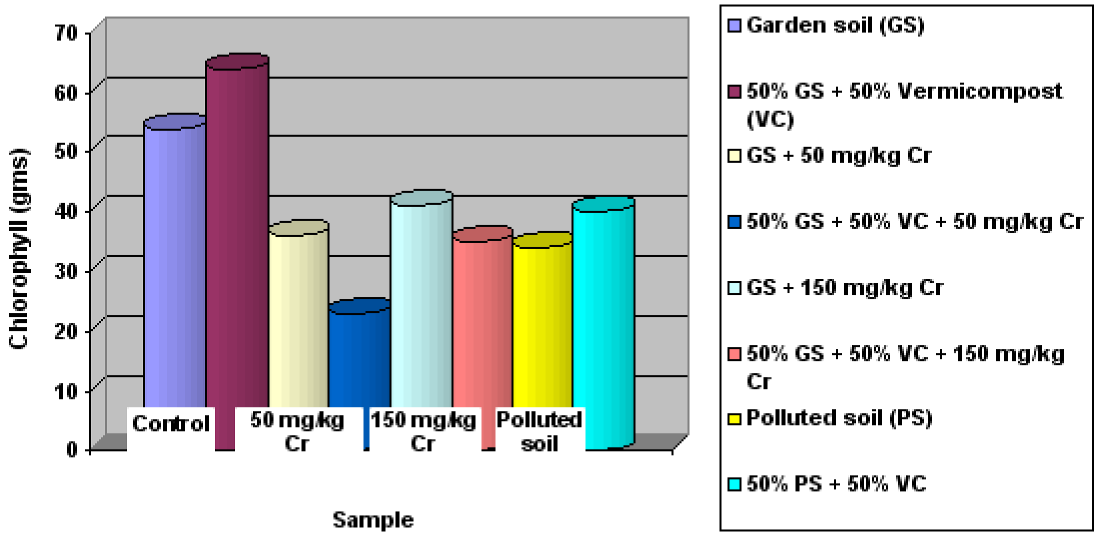

Figure 2.b: Chlorophyll content of sorghum leaves exposed to the chromium metal contaminated soils

Table-2 and Figures –2.a & 2.b represent the impact of heavy metal Cr contaminated soils on the biomass and chlorophyll content of Sorghum bicolor whole plant and leaves respectively. The biomass of sorghum plant exposed to GS + VC recorded the highest (412 gms). GS + 150 mg/kg chromium exposed plant showed the lowest biomass (100 gms) indicating the stress and toxicity exerted by chromium. Similarly GS + VC + 150 mg/kg chromium exposed plant had a biomass of 126 gms.

From the experimental values, the biomass of the plant is reduced with the increased dosage of chromium. It is also observed that addition of vermicompost to the contaminated soil improves the biomass of the plants thus making room for more bioaccumulation. When conducting phytoremediation to remove heavy metals from soil, the first factor to be considered is to select the species with high biomass and then that with greater uptake ability**.** Furthermore, plants having a larger biomass could yield better covering and revegetating benefits (Xia et al., 1999). Thus with the increase in the concentration of Cr and also in the 100% polluted soil containing lower concentration of chromium the biomass of the plants decreased.

Chlorophyll content of the sorghum plant in GS + VC was the highest (64.1 mg/kg) and GS + 150 mg/kg chromium recorded the lowest (23 mg/kg). Thus the chlorophyll content of the leaves of the sorghum plant increased with increase in the concentration of the metals. Application of VC increased the chlorophyll concentration. Maximum chlorophyll was observed (64.1mg/Kg) in the leaves of control soil + VC exposed plant. This is in corroboration with the results of Panda and Patra (2000). According to them chromium can reduce the growth of the plants. It can cause ultrastructural changes in the chloroplast leading to inhibition of photosynthesis. Such alteration in the chloroplasts has been observed in the case of plants like L*emna Minur, Pistia sp., Taxithelium nepalense (*Bassi et al., 1990). Chromium possesses the capacity to degrade ∂-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting ∂-aminolevulinic acid utilization (Vajpajee et al., 2000). Chromium mostly in its hexavalent form can replace Mg ions from the active sites of many enzymes and deplete chlorophyll content (Vajpayee et al., 2000).

At the same time, the plants seem to survive and accumulate the metals. Addition of vermicompost increases this efficiency. We know heavy metals like Cr, Cd, Zn, Fe, Al, Pb, As are highly reactive and toxic to living cells. Some heavy metals, particularly Cu, Zn and Fe are essential micronutrients involved in various physiological processes but become toxic above certain threshold concentrations (Panda and Choudhury, 2005). By accumulating these metals, plants have developed complex mechanisms by which they control the uptake and accumulation of heavy metals (Cobbett and Goldsbrough, 2002). These mechanisms involve chelation and sequestering of metals ions ligands such as phytochelations (PC) and metallothionins (MT) (Cobbett, 2000; Cobbett and Goldsbrough, 2002). MTs have a possible role of Cr detoxification in plants and it has been reported for sorghum that MT-like proteins are expressed under Cr-stress (Shankar et al., 2004).

Bioaccumulation of metals in roots and shoots of sorghum plant

Irrespective of the concentration of the metals and the presence or absence of vermicompost the bioaccumulation of the selected metal was higher in the roots than shoots. It was 140% and 101% respectively in 150 mg/kg chromium exposed sorghum plant. Addition of vermicompost decreased the bioaccumulation percentage. **(Table 3 and Figure 3).**

Table 3: Percentage bioaccumulation of chromium by Sorghum bicolor.L

Sample	50 mg/kg chromium		150 mg/kg chromium		50%Polluted soil	50% polluted soil + 50% vermicompost
	Garden soil	50% GS + 50% VC	Garden soil	50% GS + 50% VC		
Root	39	31	112	98	101.1	81.2
Shoot	31	29	101	78	89	78.1

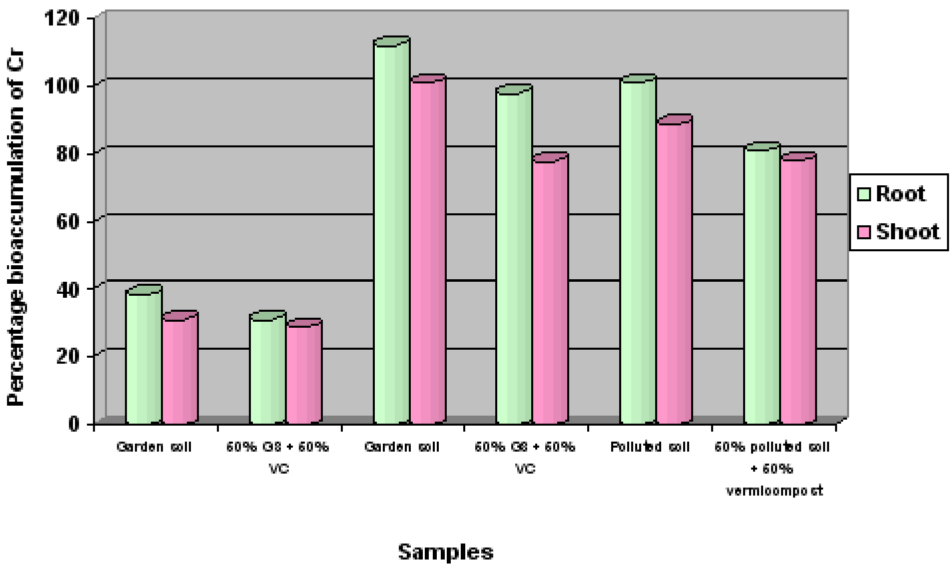

Figure 3: Percentage bioaccumulation of chromium by Sorghum bicolor.L

The roots contain organic acids that bind metals from highly insoluble forms in the soil (Rauser, 1999). The root exudates are very important agents that form complexes with trace metals and affect their redox behaviour (Hale and Grittin, 1974). Similarly in the presence of organic acids like carboxylic acid and amino acids, Cr uptake in roots is enhanced (Srivastava et al., 1999). Cr accumulation in plants is concentration, speciation and organ dependant. Plants exposed to higher concentration (150 mg / kg) accumulated more Cr than lower concentration (50 mg/kg). Under equal concentration, roots accumulated more than shoots. Although heavy metal hyper accumulation in plants was first reported in 1865 for *Thlaspi calaminare*, the study of plant heavy metal hyper accumulation is relatively recent. *Brassica juncea* is a heavy metal –accumulator plant with a high biomass, making a good candidate for application in phytoremediation strategies (Salt et al 1995, 1998). Gunther et al., (1996) conducted a laboratory study with Ryegrass (*Lolium perenne L.*). Similarly Reynolds et al., (1997) used ryegrass (*Lolum multitorum Lam*) to remediate diesel and crude oil contaminated soils. Pradhan et al., (1998) conducted laboratory study with Alfalfa (*Medicago Sativa*), switch grass (*Panicum Virgatum*) and little bluestem grass (*Selizachyrium Scoparium*) to remediate petroleum contaminated soil. Ryegrass (*Lolium annual*) and sorghum (*Sorghum bicolor L.*) were used to remediate crude oil spill site in Taxas by Gunther et al (1996).

Phytoextraction is applicable to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots. Plants being considered for phytoextraction should be tolerant of the targeted metal, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant. Ranipet industrial area is highly populated with tanneries and other chemical industries. Chromium is the metal highly found in the water and soil environs adjacent to these industries. Hence the present attempt to clean up the soil contaminated by tannery effluent is found to be efficient. Also the soil in this area is sodic with high salinity (greater chloride levels, 143 mg/kg). This will affect the osmotic pressure of the fluids of soil solution, which is an important parameter for the absorption of nutrients of this soil is effectively reduced by the addition of vermicompost while it improves the clean up process.

Table 4: Percentage extraction of heavy metals from different plant samples

50 mg/kg chromium	**150 mg/kg chromium**	**50 mg/kg chromium + Vermicompost**	**150 mg/kg chromium +Vermicompost**
50%	47%	51%	48%

Table 4 depicts the percentage of the metal extracted from the heavy metal accumulated plant samples. The ash obtained from the incinerated plant samples was treated with concentrated HCl and the metal analyzed. All the samples show 40 – 50% extraction of the accumulated metals. Thus this process helps in overcoming the bioaccumulated plant disposal problem. Further it also helps in extracting valuable metals generating revenue for offsetting the expenses of phytoremediation process.

4. Conclusions

The understanding of the basic mechanism involved in chromium uptake, transport, accumulation and detoxification in plants together with its physiological effects is necessary for the phytoremediation of the heavy metal polluted environments using molecular and genetic techniques. These approaches may include the identification of hyperaccumulators that can provide efficient phytoremediation of heavy metal polluted soils, the study of biochemical and molecular responses of these plants to the heavy metals, and the identification of genes that express PCs or MTs involved in the detoxification of the metal within the plant. These technologies will prove useful in environmental cleanup procedures and subsequent restoration of soil fertility. Hence, attempts on remediation of polluted soil by phytoremediation methods fulfills the National policy of **'Eco-friendly'** development, and create socio-economic, scientific developments among the Environmentalists, Agriculturalists and Scientists who involve in the application of this technique. Above all, this low cost technique will help the poor farmers to reuse the lands for agricultural purposes.

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