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Enhanced Phytoremediation Technology for Chromium contaminated Soils using Biological Amendments

Sunitha, R[1], S. Mahimairaja[1], A. Bharani[1] and P. Gayathri[2]
[1]Department of Environmental Science, Agriculture College and Research Institute,
Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India.
[2]Department of Biotechnology, Indian Institute of Technology, Karagpur, West Bengal, India.

ABSTRACT

The chromium contamination in soil is an environmental concern because, the accumulated metals may have adverse affects on soil ecology, agricultural production, animal and human health as well as groundwater quality. Phytostabilization involves the reduction of the mobility of heavy metals in soil. Immobilization of metals can be accomplished by decreasing wind-blown dust, minimizing soil erosion, and reducing contaminant solubility or bioavailability to the food chain. The addition of soil amendments, such as organic matter, phosphates, alkalizing agents, and biosolids can decrease solubility of metals in soil and minimize leaching to groundwater. The mobility of contaminants is reduced by the accumulation of contaminants by plant roots, absorption onto roots, or precipitation within the root zone. In some instances, hydraulic control to prevent leachate migration can be achieved because of the large quantity of water transpired by plants. Within these new approaches, in-situ immobilization and phytoremediation are becoming increasingly attractive remediation options. Thus a bioremediation technology was developed by integrating organic amendments and microbial strains. The effectiveness and feasibility of the bioremediation technology was evaluated by conducting field experiment in the 'Hot-spots' of Cr contaminated area in Vellore district. This study examined the effects of two organic soil amendments; poultry manure and vermicompost and microbial cultures on Cr accumulation in maize (*Zea mays* L.) in soil contaminated with tannery wastes. The Cr content of the maize plants were harvested and determined with Atomic Adsorption Spectrophotometer (AAS). It was found that total Chromium uptake by maize decreased with the application of organic amendment towards contaminated soil samples. The results demonstrated that the bioremediation of Cr contaminated soil has resulted in significant improvement in yield of maize. The yield increase over control was about 53.6 to 118 percent in maize, due to the application of poultry manure and vermicompost, with or without microbial strains. The addition of poultry manure or vermicompost with or without microbial strains significantly (60 %) reduced the Cr content and uptake by maize due to manure-induced Cr immobilization in soil. This is explained in terms of *in-situ* immobilization of the metals, due mainly to the phosphorus content of the organic matter that was provided for the soil from the amendments.

Keywords: *Contaminated soil, Chromium, Organic amendments, Zea mays and Immobilization.*

I. INTRODUCTION

Indiscriminate disposal of industrial and domestic wastes and large scale application of agricultural chemicals had resulted accumulation of heavy metals in soils. Continuous accumulation of these heavy metals not only leads to deterioration of soil health, but also affects the quality of surface and ground water. The heavy metals considered as threats to environment are: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). They are regarded to be cytotoxic, mutagenic and carcinogenic. Direct discharge of industrial effluent and sludge into rivers and lakes has led to a series of well-documented ecological disasters in India. For example, several million people are at risk from drinking arsenic-contaminated water in West Bengal (Mahimairaja *et al*., 2005). Similarly soil contamination resulting from tannery wastes and wastewater discharge in Vellore district and textile industries in Tirupur district of Tamil Nadu has resulted in the deterioration of soil and environmental health.

Currently, it has been estimated that more than 50,000 ha of productive agricultural lands have been contaminated with Cr alone is due to the disposal of tannery wastes in Tamil Nadu, where more than 60 percent of Indian tanneries are located. Assessment of Cr levels in contaminated soils in Vellore district showed that the soils surrounding tannery industries are severely contaminated with Cr. Large amounts of Cr (16731-79865 mg kg-1) were found in surface and subsurface soils in Vellore districts, where large number of tanneries exist (Mahimairaja *et al.*, 2000a). The Cr concentration in ground waters is also markedly higher than the average background value reported in different parts of India (Mahimairaja *et al.*, 2000b). The accumulation of Cr in soil is of concern because of its possible phytotoxicity or increased movement of metals into the food chain and the potential for surface and groundwater contamination. Chromium contamination in soil and water has drastically reduced the crop yields (25 to 40%) over the years and decreased significantly.

In contaminated soil, Cr is subjected to biological / chemical transformation processes which determine its Cr species and their toxicities. For example, tannery waste is rich in Cr (III) which is relatively less toxic. In soil Cr (III) is oxidized to Cr (VI) which is highly toxic, carcinogenic and mutagenic to animals as well as humans and is associated with decreased plant growth and changes in plant morphology. These ions cause physical discomfort and sometimes life-threatening illness including irreversible damage to vital body system (Malik, 2004). With greater public awareness of Cr poisoning in animal and human, there has been growing interest in developing regulatory guidelines and remediation technologies for mitigating Cr-contaminated soil and water ecosystems. Remediation of soils contaminated with Cr is an important issue which not only helps sustaining agriculture, but also minimizes adverse environmental impacts.

Remediation of Cr contaminated soil involves physical, chemical and biological approaches that may achieve either the partial / complete removal of Cr from soil or reduction of its bioavailability in order to minimize its toxicity (Wang *et al*., 2001). A large variety of methods has been developed to remediate Cr contaminated sites. The selection and adoption of these technologies depend on the extent of contamination, type of soil, cost involved, availability of materials and relevant regulations. Physical and chemical remediation technologies are expensive and may not be effective or feasible due to complex characteristics of contaminated soil environment (Lan *et al*., 1997).

The application of farm yard manure (FYM) or compost or biological waste materials is a common practice and traditionally followed in Indian agriculture. This technology can also be exploited for the remediation of Cr-contaminated soils. The in-situ immobilization of Cr using these composts or biological waste materials to remediate contaminated soil is a viable and cheap option. Large quantities of compost or organic matter can be added to contaminated soil with the aim of immobilizing Cr as stable complexes with organic colloids. During the decomposition of organic matter, compounds such as citric acid or gallic acid are formed which have the potential for chelating Cr (III) or reducing Cr (VI), and thereby reducing the toxicity of Cr (James and Bartlett, 1983).

Application of organic amendments such as cow dung, bermuda grass, and yeast extract have been found effective in the reduction and immobilization of Cr (VI) in contaminated soils (Cifuentes *et al*., 1996; Losi *et al*., 1994a). According to Cifuentes *et al*. (1996) yeast extract- amended soil removed more Cr (VI) than grass and manure amendments. The addition of an easily degradable substrate with a low C:N ratio stimulated more Cr (VI) reducers compared with the less-easy-to-degrade manure. They also suggested that although manure encourages reduction of Cr (VI), more readily decomposable substrates are better for the remediation.

Application of composts rich in the chemical factors can inactivate toxic Cr (VI) and reduce its potential for phytotoxicity and mammalian toxicity and therefore could offer great potential for soil remediation. In addition, compost would also remediate the severe infertility of metal contaminated sites, phytotoxicity caused by co-contaminants and provide improved soil physical properties and organic-N which would facilitate development of a remediated ecosystem at a contaminated site (Chaney and Oliver, 1996).

Okieimen *et al*. (2011) reported that immobilization and phytoremediation are becoming increasingly attractive remediation options. They studied three organic soil amendments namely poultry manure, cow dung and sludge from natural rubber processing in soil contaminated with chromated-copper-arsenate. It was found that total metal uptake by maize decreased with increasing loads of organic amendment to the contaminated soil samples. They concluded that in-situ immobilization of the metals as the reason for the decreased uptake of heavy metals by maize plants.

Mahimaraja *et al*. (2011) conducted laboratory experiments to examine the potential of biological wastes in remediating Cr contaminated soils. Their results revealed that 61% (in clay loam soils) and 75% (silt clay loam soils) reductions in the concentration of bioavailable fractions (soluble plus exchangeable) of Cr resulted from the application of coir pith, while reductions of 62.3% (clay loam) and 68% (silt clay loam) were observed due to poultry manure addition. From these experiments they concluded that the application of biological wastes, namely coir pith and poultry manure were found to be effective for reducing the bioavailable fractions of Cr, mainly through the formation of organic complexes, demonstrating their great potential in the bioremediation of Cr-contaminated soil.

Therefore, in the current study, a bioremediation technology was developed by integrating maize plant, microbes (*Pseudomonas fluorescens* and *Trichoderma viride*) and biological amendments (poultry manure and vermicompost) and evaluated its potential in remediating the Cr contaminated soil.

II. MATERIALS AND METHODS

Indiscriminate disposal of tannery wastes resulted in Cr contamination of soil and waters in Vellore district, Tamil Nadu. More than 50,000 ha of productive agricultural lands were affected and this necessitates an urgent need for developing remediation technologies for the Cr contaminated soils. Such remediation technologies will not only help sustaining agriculture, but also minimizes the adverse environmental impacts. A field experiment was conducted to develop a bioremediation technology by integrating crops, microbes and organic amendments. The materials used and methods adopted in various experiments are presented in this chapter.

Site description, Sample preparation

The experimental soil was a sandy loam and belongs to *Fluventic Haplustepts* in USDA classification. The experimental field was ploughed well, leveled and divided into 21 plots of 20 m^2 of area. The statistical design adopted for the study was a randomized block design, where in the following treatments was replicated thrice.

T_1 : Control (Recommended NPK)
T_2 : Poultry manure (10 t ha^{-1}) alone

T_3 : Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})
T_4 : Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})
T_5 : Vermicompost (5 t ha^{-1}) alone
T_6 : Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})
T_7 : Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})

The calculated amount of partially decomposed poultry manure (20 kg per plot) and Vermicompost (10 kg per plot) were applied to the field plots and incorporated into the soil manually. After three days of equilibration, the microbial strains viz., *Pseudomonas fluorescens* and *Trichoderma viride* (4 g per plot) were inoculated over the surface soil and uniformly incorporated. Two weeks after incorporation, the maize seeds were sown (Hybrid C.P.818.) on 3rd August 2011 by adopting a seed rate of 20 kg ha^{-1} and a spacing of 60 cm x 20 cm. Life irrigation was given to all plots immediately after sowing. The entire dose of recommended P and K was applied with four applications (125:100 g of NPK per plot) as basally. The N (120 per plot) was applied in four equal splits, one basally and three top dressing (25, 50 and 75 DAS) in all the treatments.
All intercultural operations viz., weeding and plant protection measures against pest and diseases were carried out as per the TNAU recommendation (CPG, 2009). The crop was harvested on 23.11.2011.

Soil samples were collected from 0 to 15 cm depth both at the time of sowing and harvesting. The soil samples were air dried, gently powdered with a wooden mallet and sieved through a 2 mm nylon sieve.

The plant samples were collected by uprooting the whole plants during vegetative, tasseling, silking and harvesting stages. The whole plant was washed carefully first with 1 per cent HNO_3 and then with distilled water. The plant was dissected into root, shoot and leaves. All the plant materials were air dried for 3 days and then oven dried at 80°C until the samples had reached consistency in weight. The dry tissues were weighed for calculating biomass and uptake. Dried plant materials were ground in a Wiley Mill to pass a 40 mesh screen and stored in plastic containers until further analysis.

Laboratory Determinations

Cation exchange capacity: Ten gram sample was added with 50 ml of neutral ammonium acetate solution and kept overnight. The sample was then transferred to the filter paper (Whatman No.3) and leached with CH_3COONH_4 for 6-8 times. After that, a pinch of ammonium chloride was added and leached with 60 % alcohol until the filtrate runs free of chloride. The sample along with the filter paper was transferred to the distillation flask and added 500 ml of distilled water and 10 ml of 40% NaOH. The NH_3 evolved during distillation was collected in 25 ml of 0.1 N H_2SO_4 added with 2 drops of methyl red indicator. The excess acid was titrated against 0.1 N KOH and CEC was calculated (Jackson, 1973).

CEC = (V-V1) x 0.1 x 100/10 c.mol kg-1 soil
V- Volume of 0.1 N H_2SO_4
V1- Volume of 0.1 N KOH consumed

Moisture: The moisture content of soil was determined using Gravimetric method(AOAC, 1962).

Chemical Properties

The soil samples were air dried at 25° C and sieved (< 2mm) and stored in polythene bags, until further analysis.
pH and Electrical Conductivity: The pH and the electrical conductivity of the samples (EC) were measured using a combined electrode pH meter and Conductivity Bridge, respectively (Jackson, 1973).

Total Chromium

One gram of sample was weighed in a acid washed 100 ml conical flask and added 15 ml of aqua-regia (HCl : HNO_3 @ 3:1). The sample was digested in a hot plate at 110° C for about 2 hours. After obtaining white slurry, the flask was cooled, added 5 ml of distilled water and boiled for few minutes. The volume of the content was made to 50 ml and kept over night. Then the content was filtered through Whatman No. 1 filter paper and the Cr concentration was measured using an Atomic Absorption Spectrophotometer (AAS) with air- acetylene flame (VARIAN, AA240). A wave length of 357.9 nm was used with a spectral slit width of 0.2 nm (USEPA, 1979a).

Crude protein and reducing sugars analysis

To assess the seed protein content, seeds were analysed for total N content by micro-kjeldahl method (Yoshida *et al*., 1971) and this N fraction was multiplied by the factor 6.25 (Dubtez and Wells, 1968) to arrive at the crude protein content of the seed. Reducing sugar was estimated by the method of Omemu *et al*., (2004).

Statistical analysis

The experimental results were statistically scrutinized as suggested by Panse and Sukhatme (1985) to find out the influence of various treatments on the soil properties and on the mobility of metals through soil. The critical difference was worked out at 5 per cent (0.05) probability levels.

III. RESULTS

Long term disposal of tannery wastes has led to extensive contamination of soil and water in many parts of Vellore district, where more than 550 tanning industries are located. In the current study, the 'Hot-spots' of Cr contamination were delineated and characterized. Based on the results of laboratory and pot experiments a bioremediation technology, by integrating organic amendments and microbial strains, for the Cr-contaminated soil was developed and evaluated by conducting field experiments using maize. The results obtained from various experiments are discussed under the following titles:

Initial characteristics of experimental field soil samples

The experimental field in the Cr contaminated area (Valaiyampattu) was a sandy loam (black soil) and belongs to *Fluventic Haplustepts* in USDA classification. Some important characteristics are given in Table 1. The soil was saline (EC = 2.19 dS m^{-1}) with a pH of 8.25. It had a SOC content of 1.07 per cent, but surprisingly a high CEC (21.1 cmol (p+) kg^{-1}) was observed. With regard to fertility status, the soil was medium in N (280 kg ha^{-1}) and P (21 kg ha^{-1}), but high in K (464 kg ha^{-1}) nutrients. The bacteria, fungi and actinomycetes population of the soil were 12 x 10^6, 8 x10^5 and 18 x10^2 CFU g^{-1} of soil, respectively. The concentrations of other cations in the soil followed: Ca>Mg>>Na. However, the exchangeable sodium percentage (ESP) of the soil was only 3.40. Among the anions, Cl concentration (5680 mg kg^{-1}) was greater than SO_4 (35 mg kg^{-1}). The soil had relatively higher concentration of Cr (669 mg kg^{-1}) which is far exceeding the threshold limit prescribed by several Environmental Protection Agencies.

Table 1 Initial Characteristics of soil

S. No	Soil Parameters	Results
1.	Bulk density (Mg m^{-3})	1.12
2.	Soil texture	Sandy loam
3.	USDA classification	*Fluventic Haplustepts*
4.	pH (1:2.5 water)	8.25
5.	EC (dS m^{-1})	2.19
6.	Organic carbon (%)	1.07
7.	Cation exchange capacity (cmol (p^+) kg $^{-1}$)	21.10
8.	$KMnO_4$-N (kg ha^{-1})	280
9.	$NaHCO_3$-P (kg ha^{-1})	21
10.	NH_4OAc- K (kg ha^{-1})	464
11.	Exchangeable Na (mg kg^{-1})	325
12.	Exchangeable Ca (mg kg^{-1})	3320
13.	Exchangeable Mg (mg kg^{-1})	2616
14.	ESP (%)	3.40
15.	Chloride (mg kg^{-1})	5680
16.	Sulphate (mg kg^{-1})	35
17.	Total Chromium (mg kg^{-1})	669
18.	Bacteria (x 10^6 cfu g^{-1})	12
19	Fungi (x 10^5 cfu g^{-1})	8
20.	Actinomycetes (x 10^2 cfu g^{-1})	18

Effect of bioremediation on maize yield

The effect of bioremediation of Cr contaminated soil on maize yield is presented in Table 2. The grain yield ranged from 4200 to 9173 kg ha^{-1} and the stover yield from 6675 to 15389 kg ha^{-1}. In both the cases the lowest yields were recorded in the control plots (T_1). The application of poultry manure or vermicompost with and without microbial strains had significantly increased the grain and stover yield. The highest grain yield (9173 kg ha^{-1}) was obtained due to the application of vermicompost alone (T_5), which was closely followed (8612 kg ha^{-1}) by the combined application of vermicompost and *Pseudomonas fluorescens* (T_6). The application of poultry manure (T_2) and its combined application with *Pseudomonas fluorescens* (T_3) or *Trichoderma viride* (T_4) resulted in a marked improvement in grain and stover yield; however the poultry manure based remediation was found relatively lesser effective than vermicompost. Significant increase in grain yield was observed due to the microbial strains, applied with the poultry manure. However, such effect on grain yield was not evident in the presence of vermicompost as small reduction in yield was recorded.

Effect on crude protein and reducing sugars

Some important quality indices namely crude protein content and reducing sugars of maize grain were determined. The results have shown that the crude protein content ranged from 14.9 to 17.8 per cent (Table 2). The plants from control treatment (T_1) have had the lowest content (14.9 %) of crude protein. Significant increase in crude protein content was observed due to the application of poultry manure or vermicompost with and without microbial strains. Only a small variation was observed with poultry manure and vermicompost. Similar effect was also observed in reducing sugar content of maize grain. The concentration of reducing sugars ranged from 1.0 to 1.4 per cent. The application of poultry manure (10 t ha^{-1}) alone (T_2) recorded the highest value (1.4%) of reducing sugars. However, its combined application with microbial strain was found to have reduced the reducing sugar content, only a small increase in reducing sugar was observed due to the application of microbial strains with vermicompost.

Table 2 Effect of Bioremediation on maize yield and quality parameters

Treatments	Yield parameters		Quality parameters	
	Grain yield (kg ha^{-1})	Stover yield (kg ha^{-1})	Crude protein (%)	Reducing sugars (%)
T_1- Control	4200	6675	14.9	1.1
T_2- Poultry manure (10 t ha^{-1})	6452	12367	16.7	1.4
T_3- Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	7460	13228	16.0	1.3
T_4- Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	8056	13890	17.4	1.0
T_5- Vermicompost (5 t ha^{-1}) alone	9173	15389	17.8	1.1
T_6- Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	8612	14098	17.2	1.2
T_7- Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	7950	12996	16.9	1.3
Mean	7415	12663	16.7	1.2
SEd	164.03	278.17	0.11	0.03
CD (p=0.05)	357.39	606.09	0.24	0.07

Chromium content and uptake by maize

The distribution of Cr in different parts of maize is depicted in Table 3. Relatively higher concentration was observed in roots than in stem and leaves. In roots, the Cr content ranged from 79 to 214 mg kg^{-1}; whereas, in stem and leaves it was from 43 to 196 mg kg^{-1} and 22 to 82 mg kg^{-1}, respectively. In general maize grown on the control soil had greater amount of Cr in root, followed by stem and leaves. Application of poultry manure, and vermicompost with and without microbial strains resulted the significant reduction in Cr content of maize. The poultry manure addition with and without *Pseudomonas fluorescens* (T_3) and *Trichoderma viride* (T_4) resulted in significantly higher reduction in Cr content of maize root and stem, than the vermicompost with and without *Pseudomonas fluorescens* (T_6) and *Trichoderma viride* (T_7). Among the two strains, *Pseudomonas fluorescens* was found better in reducing the Cr content of maize. The application of poultry manure and *Pseudomonas fluorescens* (T_3) resulted in the lowest content of 79, 43 and 22 mg kg^{-1} respectively, in roots, stem and leaves. Maize grains from control soil (T_1) were found to have higher concentration of Cr (3 mg kg^{-1}). However, maize grown on soil with poultry manure and vermicompost with and without microbial strains recorded no Cr in grains.

The Cr uptake by maize grain and stover is presented Table 3. As the Cr concentration was not detectable in all other treatments, the uptake was observed in the control plot (12.6 g ha^{-1}). The Cr uptake by maize stover ranged from 689 to 1308 g ha^{-1}. Similar to content, the uptake of Cr by maize stover was the highest in plants that grown on the control soil (T_1). The application of poultry manure and vermicompost with and without microbial strains significantly reduced the Cr uptake by maize stover. The lowest value (569 g ha^{-1}) of Cr uptake was observed in plants that were treated with poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens*.

Table 3 Effect of organic amendments and microbial strains on chromium content and uptake by maize

Treatments	Cr content (mg kg^{-1})				Cr uptake (g ha^{-1})	
	Root	Leaves	Stem	Grains	Grain uptake	Stem Cr uptake
T_1- Control	214	82	196	3	12.6	1308
T_2- Poultry manure (10 t ha^{-1})	85	29	47	bdl	bdl	581
T_3- Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	79	22	43	bdl	bdl	569
T_4- Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	84	23	55	bdl	bdl	764
T_5- Vermicompost (5 t ha^{-1}) alone	88	22	58	bdl	bdl	892
T_6- Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	96	29	61	bdl	bdl	860
T_7- Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	99	25	53	bdl	bdl	689
Mean	106	33	73	bdl	bdl	809
SEd	4.74	2.15	5.40	-	-	2.52
CD (p=0.05)	10.33	4.67	11.77	-	-	5.10

bdl = below detectable limit

IV. DISCUSSION

The maximum stover yield (15389 kg ha^{-1}) was obtained with the application of vermicompost (5 t ha^{-1}) (T_5), which was closely, followed by the application of vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^1) (T_6). The effect of microbial strains on stover yield was more pronounced in the presence of vermicompost than poultry manure. Similar to grain yield, the stover yield was also found reduced due to the application of *Pseudomonas fluorescens* and *Trichoderma viride*.

Bioremediation of Cr contaminated soil has resulted in significant improvement in yield of maize. In the control plots, where only recommended dose of NPK fertilizers alone added, the grain yield of maize was only 4200 kg ha^{-1}. Due to the application of poultry manure and vermicompost, with or without microbial strains, the grain yield was significantly increased. The grain yield increase over control was about 53.6 to 118 percent. Similarly, an increase of 85.7 to 130 percent in maize stover over the control treatment was recorded. Under the Cr contamination, the role of organic amendments and microbes are reducing the toxicity of Cr. The improvement in yield could be attributed mainly due to the reduction in the bioavailability and thus biotoxicity of Cr in the soil. The bioavailability of metal in the soil environment has been defined as the fraction of the total metal in the interstitial pore water (i.e. soil solution) and soil particles that is available to the receptor organism (Naidu *et al*, 2008). More specifically, it refers to the biologically available fraction (or pool) that can be taken up by an organism and can react with its metabolic machinery (Tessier and Turner, 1999). The bioavailability in soil can be reduced by immobilization of metals. It has often been shown that the addition of organic amendments increases the immobilization of metal through adsorption reactions, formation of organo-chromic complexes or reduction of toxic in soil or by chelation.

The organic amendment induced retention of metal is attributed due to an increase in surface charge and the presence of metal binding compounds. For example Bolan *et al*. (2003) observed that the addition of compost increased the surface charge of the amended soils, which is attributed to the higher pH and surface charge of the compost. Addition of organic amendment like animal and poultry manure, biosolid and composts has often been shown to reduce the bioavailability of Cr (Losi *et al*, 1997; Bolan *et al.,* 2003; Ano and Ubochi, 2007; Shenbagavalli and Mahimairaja, 2010). The reduction in the bioavailability could also be due to either formation of organo-chromic complexes or reduction of toxic, soluble Cr VI higher valency to non-toxic, less soluble Cr III and subsequent precipitation as chromic hydroxide in soil (Park *et al* 2011). During the decomposition of organic matter in the poultry manure and vermicompost compounds such as citric acid and gallic acid are formed which have the potential for chelating Cr III and thereby reducing the toxicity of Cr (James and Bartlett, 1983).

Organic manure has the ability to form stable metal chelates, in which metal ions are held between atoms of the complex organic molecules, thereby reducing toxicity (Aery and Tiagi, 1985). Significant increase in yield of maize and sunflower may also be due to improvements in nutrients availability and uptake, conducive chemical and biological environment (microbial and enzyme activity) and better physical properties in soil amended with organic amendments (Mahimairaja *et al.*, 1999; Belay *et al.*, 2001; Saranappa, 2002; Agyenim *et al.,* 2006; Ana *et al.,* 2008).

Application of microbial strains viz., *Pseudomonas fluorescens* and *Trichoderma viride* was found to markedly enhance the grain yield of maize. In general, the application of organic manures increases the microbial and enzymatic activities in soil. Therefore, the organic amendments could have provided nutrients and energy (carbon) for the activity of *Pseudomonas fluorescens* and *Trichoderma viride*, which might have reduced the biotoxicity of Cr and thus increased the yield.

However, the combined application of microbial strains with vermicompost reduced the yield slightly. The application of vermicompost (5 t ha^{-1}) besides the recommended dose of NPK fertilizer recorded the highest grain yield of maize; whereas, the application of poultry manure was found better in improving the stover yield of maize. The difference in the efficiency of poultry manure and vermicompost is attributed mainly to the differential nutrients and organic matter contents. The higher yield obtained with poultry manure could be attributed to either an increase in the availability of nutrients or to reduced bioavailability of Cr in soil, leading to increased nutrient uptake. Such effect has also been reflected on crude protein and sugar contents of maize. The crude protein content was significantly higher in maize field soil amended with organic amendments than the control soil.

Chromium content and uptake by maize

Relatively large amount of Cr was found accumulated in roots, than in stem and leaves of maize. The distribution of Cr in different parts of maize is depicted in Fig 1. The maize crop grown on control plot have shown greater accumulation of Cr. Whereas, the plants those grown on soil amended with organic amendments and microbial strains appeared to have significantly lesser amount of Cr in roots, leaves and stem. In general 60 to 78 per cent reduction of Cr in maize were recorded due to the application of organic amendments with or without microbial strains. Large accumulation of Cr in the roots of maize control plots could be due to the roots were the specialized absorptive organs so that they were affected earlier and subjected to accumulation of more Cr than any of the other organs (Xiong, 1998; Jadia and Fulekar, 2008). Relatively lesser amount of Cr was found accumulated in roots of maize due to poultry manure application than vermicompost application.

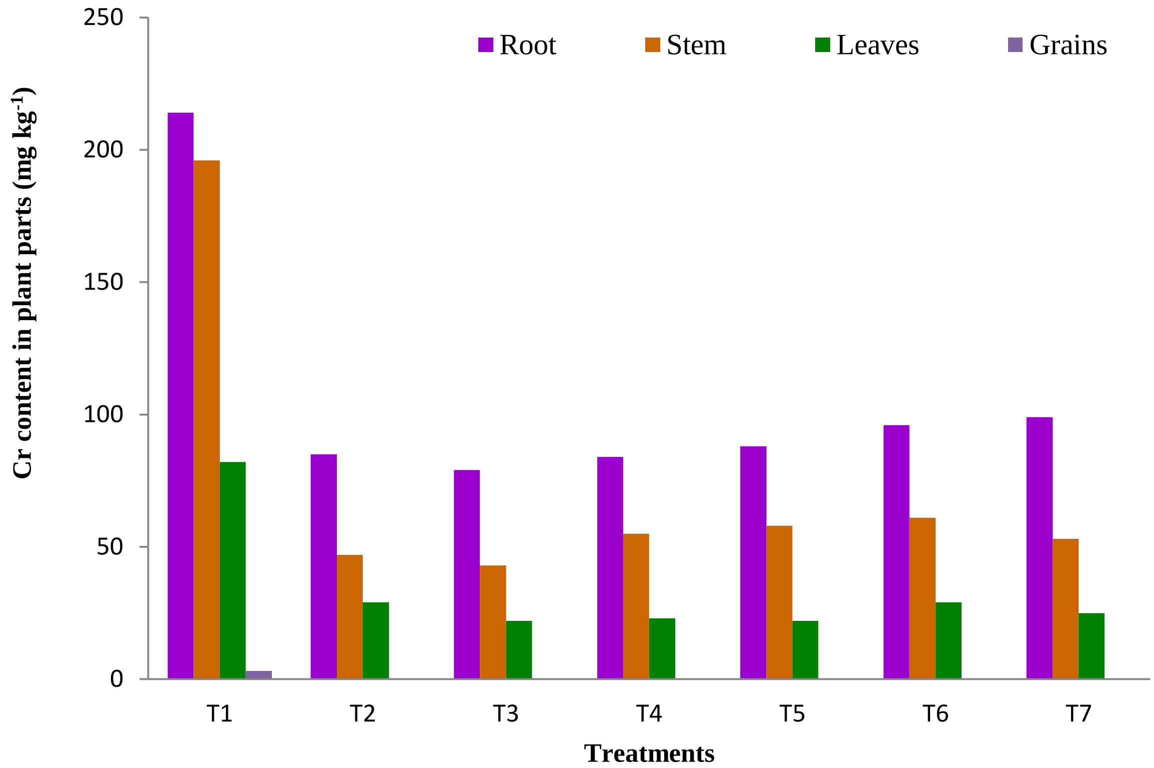

Figure 1. Effect of bioremediation on chromium content of maize

The maize had small amount of Cr in grains (3 mg kg^{-1}), when they were grown on Cr contaminated soil. The addition of organic amendments and microbial strains resulted in almost zero concentration of Cr in grains. The Cr uptake by maize grain varied significantly. About 31.8 to 56.5 per cent reduction in Cr uptake by maize stover were recorded due to the application of organic amendments and microbial strains. Though not always significant, the per cent reduction in Cr content and uptake by maize was relatively higher due to poultry manure treatments.

The reduction in Cr content and uptake by maize due to organic amendments and microbial strains was very significant. It could be attributed to the greater immobilization of Cr in soil. As has already been mentioned the application of poultry manure and vermicompost with or without *Pseudomonas fluorescens* and *Trichoderma viride*, appeared to have reduced the bioavailability of Cr in soil which may have been due to the formation of either organo-chromic complexes (immobilization) or reduction of toxic, soluble Cr VI to non toxic, less soluble Cr III in the soil (Mahimairaja *et.al*., 2011 and Bolan *et al*., 2003). Though, large reduction in the Cr VI was observed due to poultry manure and vermicompost in the pot experiment (Sunitha *et al*, 2013a & 2013b), the results of field experiment may suggest that the reduction in plant Cr may be due to the immobilization of Cr mainly by the formation of organo-chromic complexes, as only a small amount of Cr VI (< 0.2 mg kg^{-1}) was observed in the experimental soil. If oxidation of Cr III to Cr VI occurred in the soil, then the immobilization due to reduction process also could have contributed for the reduction of plant Cr. Hydroxide precipitation is the most common and effective method of treatment for heavy metal(loid)s (Bolan *et al*., 2003). Liming often increases the precipitation of metal(loid)s. For example, Lee *et al*. (2006) used granulated lime and calcium carbonate as coagulants to remove heavy metal(loid)s from contaminated water. They showed that the main removal mechanism of heavy metal(loid)s in their experiments was precipitation.

Immobilization of Cr by organic manure is also achieved through adsorption.

The addition of organic amendments has been shown to increase the cation exchange capacity of soils, resulting in increased metal adsorption (Bolan *et al*., 2010). As might be expected, the organic components of soil constituents have high affinities for metal cations because of the presence of ligands and functional groups that are capable of forming chelates with metals. The observed a negative correlations between yield and Cr content of maize (R^2 = -0.678, -0.856) which suggest that the Cr content and uptake by maize appeared to have greater role in determining their respective yields (Fig 2).

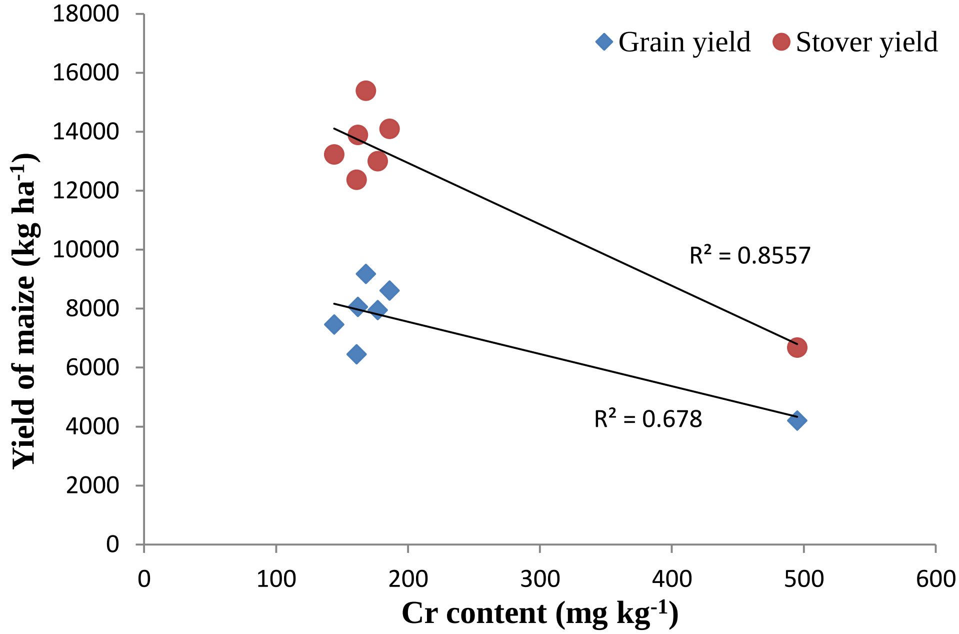

Figure 2. Correlation between yield and chromium content of maize

Bioconcentration factor and Translocation factor

The mobility of Cr from the polluted soil into the roots of maize and the ability to translocate the Cr from roots to above ground parts were evaluated by computing the Bioconcentration factor (BCF), the translocation factor (TF) and enrichment factor (EF) as follows (Lorestani *et al*., 2011);

BCF = Cr in roots (mg kg^{-1}) / Cr in soil (mg kg^{-1})

TF = Cr in stover (mg kg^{-1}) / Cr in roots (mg kg^{-1})

EF = Cr in stover (mg kg^{-1}) / Cr in soil (mg kg^{-1})

The ability of maize to tolerate and accumulate Cr is useful for phytoextraction and phytostabilization purpose. Plants with both BCF and TF greater than one have the potential to be used in phytoextraction. Besides, plants with BCF greater than one and TF less than one have the potential for phytostabilization (Yoon *et al*., 2006). The lesser values of BCF may suggest the restriction in soil-root transfer at this Cr concentration in the soil (Gafoori *et al*., 2011). The hyper accumulator plant should have EF greater than 1, or TF >1. The results obtained from the field experiment presented in Table 4 showed that the BCF, TF and EF were less than one for maize crop. Therefore, they may not be considered as Cr hyperaccumulator. Heavy metal tolerance with high TF and low BCF value was suggested for phytoaccumulator for contaminated soil (Yoon *et al*., 2006). However, these plants showed greater potential in tolerating high concentration Cr and accumulating lesser amount Cr and therefore could be integrated along with organic amendments and microbial strains for bio remediating the Cr contaminated soil.

Table 4: Bioconcentration factor (BCF), Translocation co-efficient factor (TF) and Enrichment factor (EF) for maize crop

Treatments	Maize		
	BCF	**TF**	**EF**
T_1- Control	0.32	0.92	0.29
T_2- Poultry manure (10 t ha^{-1})	0.13	0.56	0.07
T_3- Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	0.13	0.54	0.07
T_4- Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	0.13	0.65	0.08
T_5- Vermicompost (5 t ha^{-1}) alone	0.14	0.66	0.09
T_6- Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	0.15	0.64	0.09

T_7- Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	0.15	0.54	0.08
Mean	0.16	0.64	0.11

V. CONCLUSIONS

Results of this study demonstrate that both insoluble and soluble phosphate compounds are effective in Cr immobilization. Regular application of organic amendments such as poultry manure and vermicompost to agricultural soils improves the physical, chemical and biological fertility of soils. However, traditionally these organic waste products have been considered as a major source of metals input to agricultural soils. Hence organic amendments that are low in metals can be effectively utilized to remediate soils contaminated with toxic heavy metals especially Cr. Application of organic amendments reduces the bioavailability of Cr through adsorption and complexation reactions, thereby reducing their transfer through plant uptake and leaching.

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