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Original Research Paper

Phytostabilization of Chromium by Organic Amendments in Sunflower (*Helianthus annus* L.) Field Soil

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ABSTRACT

Chromium, in the trivalent form (Cr III), is an important component of a balanced human and animal diet and its deficiency causes disturbance to the glucose and lipids metabolism in them. But the concentration above the permissible limit causes the crop failure and human health impacts. In contrast, hexavalent Cr (Cr VI) is highly toxic, carcinogen and may cause death in animals and humans if ingested in large doses. Recently, concern about Cr as an environmental pollutant has been escalating due to its buildup to toxic levels in the environment as a result of various industrial and agricultural activities. In our study, a reduction of chromium of about 87 per cent was recorded in sunflower field. At the same time reduction of only 33.8 per cent from the initial concentration of Cr in the surface soils under sunflower was observed due to the addition of organic amendments. Such reduction is attributed to the formation of either organo-chromic complexes (Immobilization) or chelates. Phytostabilization utilizes the plant production of compounds, which immobilize contaminants at the interface of roots and soil, or root and water. An example of this method is where root exudates cause the precipitation of metals, reducing their bioavailability.

INTRODUCTION

The industrial activity accelerates pollution of the biosphere, especially the soil. Nowadays soil pollution is getting considerable public attention since the magnitude of this problem is growing rapidly. Heavy metals are the most dangerous substances in the environment due to their high level of durability and toxicity to the biota (Alkorta 2004). Heavy metals will tend to adsorb very firmly to the soil matrix, and once released to the environment, they will not degrade like organics by microbial activity or through chemical oxidation. Human activities such as mining, smelting, electroplating, etc. can result in contamination of soil with heavy metals.

Global industrialization over the past century has resulted in widespread contamination of the environment with persistent organic and inorganic wastes. Compounding this problem is the fact that traditional physico-chemical methods of clean up are often expensive, difficult and inefficient. Those methods that are applied to soils and sediments may also be of high impact; hence, detrimental to soil structure and fertility (Negri & Hinchman 1996, Salt et al. 1995, Baker et al. 1994). Current research in biotechnology now includes investigations that utilize plants to facilitate reclamation, the technique being known as phytoremediation. Phytoremediation is a developing technology which uses plants and their associated microbes for the remediation of soil contamination. This process is cost effective without creating disturbance to the landscape (Itanna & Coulman 2003).

This alternative approach has arisen because plants have a remarkable ability to extract, concentrate and metabolize materials from air, water and soil (Bollag et al. 1994). Baker (1981) proposed that plants respond to the presence of soil contaminants in three ways. They can act as contaminant accumulators, indicators or excluders, based on the way they take up and translocate constituents to above ground biomass. Accumulators are plants that survive despite concentrating contaminants in their aerial tissues. Indicator plants have a mechanism that controls the translocation of contaminants from the roots to the shoots. Their composition often reflects that of the parent soil (Kleinhamp & Koteff 1960). Phytostabilization utilizes the plant production of compounds, which immobilize contaminants at the interface of roots and soil, or roots and water. An example of this method is where root exudates cause the precipitation of metals, reducing their bioavailability.

MATERIALS AND METHODS

Site description and sample preparation: A field experiment was conducted in a farmer's field at Valaiyampattu village in Vaniyambadi Taluk of Vellore district which is highly contaminated with chromium (due to tannery wastes disposal) to examine the phytoremediation potential of sunflower. Two amendments (poultry manure and vermicompost) with and without *Pseudomonas fluorescens* and *Trichoderma viride*. The poultry manure (10 t ha^{-1}) and vermicompost (5 t ha^{-1}) were applied to the field and incorporated along with *Pseudomonas fluorescens* and *Trichoderma* (2.5 kg ha^{-1}). T_1 : Control, 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}).

Laboratory determination: Physical and chemical characteristics of soil such as cation exchange capacity (CEC), soil reaction (pH), electrical conductivity (EC), organic matter (OM) and extractable chromium were measured before and after the test. Soil pH and EC were measured in 1:2.5 extract (soil:water). Total chromium in soil samples was determined by aquaregia (APHA 1998). Soil OM was determined as in Walkley and Black, and CEC by Jackson (1973).

Statistical analysis: The experimental results were statistically scrutinized as suggested by Panse and Sukhatme (1985). The critical difference was worked out at 5 per cent (0.05) probability levels.

Total chromium: One gram of sample was weighed in a acid washed 100 mL conical flask and added 15 mL of aquaregia (HCl : HNO_3 @ 3:1). The samples were digested on a hot plate at 110°C for about 2 h. After obtaining a white slurry, the flasks were cooled, added 5 mL of distilled water and boiled for few minutes. The volume of the contents was made to 50 mL and kept overnight. Then the contents were filtered through Whatman No. 1 filter paper and the Cr concentration was measured using an Atomic Absorption Spectrophotometer with air-acetylene flame. A wavelength of 357.9 nm was used with a spectral slit width of 0.2 nm (U.S.EPA 1979).

RESULTS AND DISCUSSION

The soil collected from Vaniyambadi belongs to Vellore district. The data on initial soil characteristics of the experimental site are presented in Table 1. The total chromium content of the soil was 691.6 mg kg^{-1}. Considerable populations of beneficial microorganisms were also enumerated in the experimental field soil.

The soil samples were collected from sunflower experimental field at 15 cm depth and total Cr was analysed (Table 2). The highest total Cr content was found in control (683 mg kg^{-1}) than the other amended soil treatments at initial day sample. The Cr was significantly immobilized with soil due to the application of organic amendments (poultry manure and vermicompost) on 30 days, 45 days, 70 days and 90 days and ranged from 369.5 to 574.2, 182.6 to 525.4, 92.8 to 505.5 and 88.6 to 493.2 mg kg^{-1} respectively. The highest Cr was observed in poultry manure amended with *Pseudomonas fluorescens* (574.2 mg kg^{-1}), and the lowest was in control (369.5 mg kg^{-1}) at vegetative stage. At harvesting stage soil samples were analysed and the highest Cr was accumulated in poultry manure amended with *Pseudomonas fluorescens* (493.2 mg kg^{-1}), and the lowest value was found in control (88.6 mg kg^{-1}) due to plant uptake. The reduction in Cr content and uptake by sunflower 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, Bolan et al. 2003). Though, large reduction in the Cr VI was observed due to poultry manure and vermicompost in the pot experiment, 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.

Table 1. Initial characteristics of field experimental soil.

S. No.	Parameters	Sunflower
1.	pH	8.36
2.	EC (dSm^{-1})	1.41
3.	Organic carbon (%)	1.70
4.	Bulk density (mg m^{-3})	1.18
5.	Soil texture	Sandy loam
6.	Cation exchange capacity (cmol (p^+) kg^{-1})	61.23
7.	Available N (kg ha^{-1})	276
8.	Available P (kg ha^{-1})	18
9.	Available K (kg ha^{-1})	703
10.	Exchangeable sodium (mg kg^{-1})	147
11.	Exchangeable Ca (mg kg^{-1})	2053
12.	Exchangeable Mg (mg kg^{-1})	1264
13.	Chloride (mg kg^{-1})	1064
14.	Sulphate (mg kg^{-1})	40
15.	Total chromium (mg kg^{-1})	691.6
16.	Bacteria (× 10^6 cfu g^{-1})	16
17.	Fungi (× 10^5 cfu g^{-1})	11
18.	Actinomycetes (× 10^2 cfu g^{-1})	21

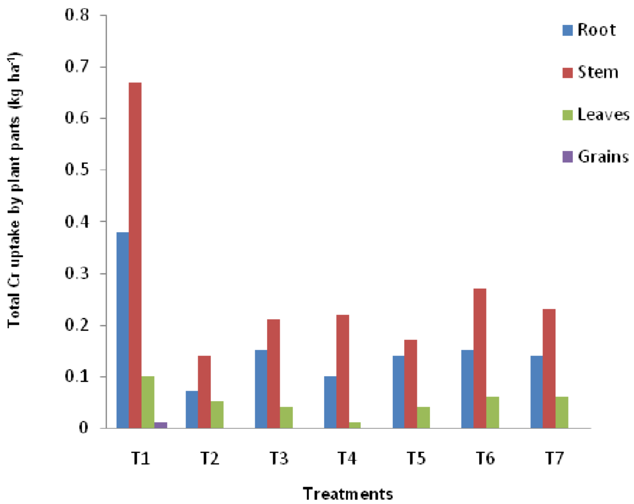

Fig. 1: Effect of bioremediation on Cr content of sunflower.

The chromium was analysed in different plant parts of the sunflower crop and data presented in Table 3. The total Cr was highly accumulated in roots than the shoots followed by leaves. Moral et al. (1996) reported that the Cr (III) was accumulated in roots up to 100 mg kg^{-1} than the shoots in tomato plant. Srivastava et al. (1994) studied in onion crop that 70-90 % of Cr was accumulated in roots. A recent study was observed in sunflower (Shahandeh & Hossner 2010a) plant in which Cr (VI) was highly accumulated in the root system up to 500 mg kg^{-1}.

Relatively large amount of Cr was found accumulated in roots, than in stem and leaves of sunflower. The distribution of Cr in different parts of maize and sunflower is depicted in Fig. 1. Both maize and sunflower, grown on control soil, have shown greater accumulation of Cr. Whereas, these plants when 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, 63 to 88 per cent reduction in sunflower was recorded due to the application of organic amendments with or without microbial strains. Large accumulation of Cr in the roots of maize and sunflower could be due to the fact that 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 & Fulekar 2008). However, no such difference was observed between poultry manure and vermicompost in sunflower.

The sunflower had small amount of Cr in seeds (8 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 seeds. The Cr uptake by sunflower stalk varied significantly. About 64.9 to 74.6 per cent reduction in Cr uptake by sunflower stalk was recorded due to the application of organic amendments and microbial strains. However, the vermicompost treatments recorded higher reduction of Cr content and uptake by sunflower.

The mobility of Cr from the polluted soil into the roots of sunflower 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/stalks (mg kg^{-1})/Cr in roots (mg kg^{-1})

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

The ability of sunflower 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 sunflower. Therefore, it may not be considered as Cr hyper-accumulator. Heavy metal tolerance with high TF and low BCF value was suggested for phytoaccumulator for contaminated soil (Yoon et al. 2006). However, sunflower plant 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 bioremediating the Cr contaminated soil.

CONCLUSION

The study has demonstrated that the bioremediation technology, developed by integrating organic amendments, (poultry manure, vermicompost) and microbial strains (*Pseudomonas fluorescens*, *Trichoderma viride*), is very effective in remediating the Cr-contaminated soil using maize or sunflower. The poultry manure (10 t ha^{-1}) or vermicompost (5 t ha^{-1}) along with any microbial strains (preferably *Pseudomonas fluorescens*) may be recommended for the Cr-contaminated soils in Vellore. The organic amendments and microbes are effective in reducing the bioavailability of Cr and thus its biotoxicity on crops. The bioavailability and biotoxicity of Cr is mainly achieved by immobilization of Cr in soil which restricts the Cr uptake by crops. The immobilization of Cr in soil occurs through complexation

Table 2: Effect of organic manures on total chromium (mg kg^{-1}) in soil at different stages of sunflower (soil at 15cm).

Treatments	Initial	30 DAS	45 DAS	70 DAS	90 DAS
T_1- Control	683.2	369.5	182.6	92.8	88.6
T_2- Poultry manure (10 t ha^{-1})	623.1	561.0	521.0	504.6	490.0
T_3- Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	632.4	574.2	525.2	505.5	493.2
T_4- Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	609.5	546.2	398.4	473.9	460.3
T_5- Vermicompost (5 t ha^{-1}) alone	653.1	557.2	525.4	421.2	462.3
T_6- Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	652.1	552.9	513.7	482.4	488.1
T_7- Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	666.5	556.9	522.6	468.8	442.1
Mean	645.7	531.1	450.7	426.2	417.8
	SEd	CD (0.05)			
T	6.133	12.238**			
S	5.183	10.343**			
T × S	13.713	27.365**			

Table 3: Effect of organic amendments and microbial strains on chromium content and uptake by sunflower.

Treatments	Root (mg kg^{-1})	Leaves (mg kg^{-1})	Stem (mg kg^{-1})	Stalk uptake (g ha^{-1})	Seeds (mg kg^{-1})	Seed uptake (g ha^{-1})
T_1- Control	298	92	198	599	8	8.7
T_2- Poultry manure (10 t ha^{-1})	35	34	31	161	bdl	Bdl
T_3- Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	68	29	51	264	bdl	Bdl
T_4- Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	52	32	49	246	bdl	Bdl
T_5- Vermicompost (5 t ha^{-1}) alone	66	25	34	152	bdl	Bdl
T_6- Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	76	34	42	210	bdl	Bdl
T_7- Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	66	32	41	199	bdl	Bdl
Mean	94	40	64	262	1	1
SEd	8.99	2.29	5.92	11.89	-	-
CD (0.05)	19.60	5.00	12.91	24.22	-	-

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

Treatments	Sunflower		
	BCF	TF	EF
T_1- Control	0.44	0.66	2.24
T_2- Poultry manure (10 t ha^{-1})	0.06	0.88	0.06
T_3- Poultry manure (10 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	0.11	0.75	0.10
T_4- Poultry manure (10 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	0.09	0.93	0.11
T_5- Vermicompost (5 t ha^{-1}) alone	0.10	0.51	0.07
T_6- Vermicompost (5 t ha^{-1}) and *Pseudomonas fluorescens* (2.5 kg ha^{-1})	0.12	0.56	0.09
T_7- Vermicompost (5 t ha^{-1}) and *Trichoderma viride* (2.5 kg ha^{-1})	0.09	0.62	0.09
Mean	0.14	0.70	0.39

(formation of organo-chromic complexes), chelation, precipitation of chromic hydroxide, adsorption of Cr (Cr III & Cr VI) and reduction of Cr VI to Cr III in soil. The organic amendments also improve the fertility and physical properties of the Cr-contaminated soils. However, field experiments are needed for confirmatory results.

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