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Effect of vermicompost on biotransformation and bioavailability of hexavalent chromium in soil

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Abstract: *Chromium is released into the environment by a number of processes such as steel manufacturing, pulp processing, wood preservation, leather tanning, metal plating, metal cleaning and processing and alloy formation, mostly with-out proper treatment. As a consequence, elevated concentrations of chromium become a major threat to the environment. Among the different forms of chromium, hexavalent chromium is highly soluble in water, and mutagenic and carcinogenic. Recently, concern about Cr as an environmental pollutant has been escalating due to its build up to toxic levels in the environment as a result of various industrial and agricultural activities. In the present study, the hexavalent chromium was reduced into trivalent chromium from chromium contaminated (300 µg g^{-1}) soil. The vermicompost and microbial cultures (Pseudomonas fluorescens and Trichoderma viride) were used for chromium detoxification studies. The chromium (VI) reduction was observed in best treatment like vermicompost alone reduced the chromium up to 85 per cent and vermicompost along with Pseudomonas fluorescens reduced the hexavalent chromium up to 84.6 per cent. The large amount of hexavalent chromium was detoxified due to application of vermicompost. The chromium hexavalent reduction was confirmed with maize plant uptake. The plants grown on the control soil (T_1) had the highest content of Cr (39.2 µg g^{-1}) and the plants grown on the soil with the application of Trichoderma viride (T_9) had the lesser value of Cr content. These biological materials were reduced the toxicity of chromium and bioavailability to the maize plant uptake.*

Key words: *Bioremediation, biotransformation, Cr (VI), Vermicompost, Pseudomonas fluorescens*

I. Introduction

In recent years, contamination of the environment by Cr, especially hexavalent Cr, has become a major area of concern. Chromium is used on a large scale in many different industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, Cr chemicals production, and pulp and paper production. Often wastes from such industries (e.g., sludge, fly ash, slag, etc.) are used as a fill material at numerous locations to reclaim marshlands, for tank dikes, and for backfill at sites following demolition (Salunkhe et al., 1998). At many such sites, leaching and seepage of Cr (VI) from the soils into the groundwater poses a considerable health hazard. The tanning industry is an especially large contributor of Cr pollution to water resources; Chandra et al. (1997) estimated that in India alone about 2000 to 3200 tones of elemental Cr escape into the environment annually from the tanning industries, with a Cr concentration ranging between 2000 and 5000 mg L^{-1} in the effluent compared to the recommended permissible limit of 2 mg L^{-1}.

Increasing levels of heavy metals in the environment pose serious threats to water quality, soil ecosystem, human health and living organisms (An et al., 2001; Wingenfelder et al. 2005; Vinodhini and Narayanan, 2008). Cr, Ni, Zn, Cu and Cd are considered as priority metals from the point of view of potential health hazards to human. Hexavalent chromium has high toxicity for humans and animals (McBride 1994; Ayuso et al., 2003; Babel and Opiso, 2007) and commonly interferes with beneficial use of effluents for irrigation and industrial applications. They are also the groundwater contaminants at industrial installations (Mier et al., 2001; Malakootian et al., 2009). In epidemiological studies, an association has been found between exposure to Cr (VI) by the inhalation and lung cancer. IARC has classified chromium (VI) in Group 1 (human carcinogen) (WHO 2004). Some cities in Iran have high amount of hexavalent chromium contents in ground water resources.

Conventional method for industrial effluent treatment is physicochemical treatment including ion exchange, vacuum evaporation, solvent extraction and membrane technologies (Applegate 1984; Sengupta and Clifford 1986; Kentish and Stevens 2001). Among these, ion exchange is one of the most effective and economical methods (Tran et al., 1999; Nameni et al., 2008). Use of various sorbents such as bentonite (Zvinowanda et al., 2009), chitosan (Jha et al., 1988), perlite (Mathialagan and Viraraghavan 2002), coal (Karabulat et al., 2000), and activated carbon (Fan and Anderson 2005; Gueu et al., 2007) have been reported

for the removal of heavy metals including chromate from aqueous solutions. The present study deals with hexavalent chromium detoxification used with vermicompost and microbial strains.

II. Materials and Methods

1.1 Hexavalent detoxification study with different amendments

A pot experiments was conducted to examine the effect of vermicompost, Earthworms (*Eisenia foetida* and *Eudrilus eugeniae*) and microorganisms (*Pseudomonas fluorescens* and *Trichoderma viride*) in reducing the toxic Cr (VI) in soil. Bulk soil samples were collected at 0 to 30 cm depth from Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The samples were air dried at 25^{o}C and sieved (< 2 mm) and the soil was used in this study. One kg of soil was weighed and transferred into each pot. The soil was then artificially contaminated with Cr (VI) at a rate equivalent to 300 mg kg^{-1}, by using a standard solution of $K_2Cr_2O_7$. The Cr solution was added and thoroughly mixed with the soil. After a day of equilibration, vermicompost (at a rate of 5 t ha^{-1}) were added in as per the treatment schedule and uniformly mixed. The rate of application of vermicompost was based on TNAU recommendation (Crop production guide, 2009). The microbial strains viz., *Pseudomonas fluorescens* and *Trichoderma viride* were introduced into the soil (@ 2.5 kg ha^{-1}). After 3 days of incubation, uniform sized (20 cm length of *Eudrilus eugenia* and 10 cm length of *Eisenia foetida* which was 0.2 g and 1g each respectively) ten number of earthworm species were introduced into the pots and incubated at field capacity moisture (0.52 g g^{-1}). The moisture loss was compensated by adding distilled water once in three days and the moisture content was maintained throughout the experimental period. At fortnight intervals, soil samples were removed from each pot and analysed for Cr (VI).

After 60 days incubation, the seeds of maize (CO_3 variety – 15 seeds per pot) were sown. One week after sowing, three uniform sized healthy seedlings were allowed to grow in each pot. Periodically water was added uniformly to all pots to compensate moisture loss. The plants were grown up to active vegetation stage and harvested to examine the reduction of Cr (VI) in soil and Cr accumulation in the plants. Soil and plant samples were collected at the end of the experiment for important parameter (Cr VI) analysis.

Treatment details

T_1 : Control - Soil* (No amendments)
T_2 : Soil* + Vermicompost
T_3 : Soil*+ Vermicompost + *Eisenia foetida*
T_4 : Soil*+ Vermicompost + *Eudrilus eugeniae*
T_5 : Soil*+ *Pseudomonas fluorescens*
T_6 : Soil*+ Vermicompost + *Pseudomonas fluorescens*
T_7 : Soil* + Vermicompost + *Pseudomonas fluorescens* + *Eisenia foetida*
T_8 : Soil*+ Vermicompost + *Pseudomonas fluorescens* + *Eudrilus eugeniae*
T_9 : Soil* + *Trichoderma viride*
T_{10} : Soil* + Vermicompost + *Trichoderma viride*
T_{11} : Soil* + Vermicompost + *Trichoderma viride* + *Eisenia foetida*
T_{12} : Soil* + Vermicompost + *Trichoderma viride* + *Eudrilus eugeniae*

Soil * - Soil spiked with 300 mg Cr (VI) kg^{-1}

1.2 Characterization of vermicompost

Vermicompost samples were collected from the compost Unit of the Department of Farm management, TNAU, Coimbatore. The vermicompost was analysed for the initial characters like pH, EC and organic carbon, etc. The microbial cultures viz., *Pseudomonas fluorescens* and *Trichoderma viride* were obtained from the Biofertilizers Unit of the Department of Plant Pathology. These two cultures were also identified in the Cr contaminated soils of Vellore district.

1.3 Hexavalent chromium analysis in soil by DPC method

One gram of moist soil was shaken in end-over-end shaker with 25 ml of distilled water for 2 h in a polypropylene centrifuge tube. The suspension was centrifuged at 8000 rpm for 10 min. and filtered through Whatman No.40 filter paper. The concentration of Cr (VI) in the water soluble fraction was determined as per the method outlined by USEPA (1979). A known quantity of water extract was added with 10 ml of 1N H_2SO_4, 0.4 ml of H_3PO_4 and 4 ml of 1,5-diphenyl carbazide reagent. After making the contents to desired volume, the samples were allowed to stand for 20 min for purple-violet colour development. The absorbance of the colour was measured at 540 nm using UV-Vis Spectrophotometer and compared reagent with that of standard Cr solutions (0-2 mg L^{-1}). The 1,5-diphenyl carbazide reagent was prepared by dissolving 0.25 g of 1,5-diphenyl carbazide and 4 g of pthalic anhydride in 100 ml of 95 per cent ethyl alcohol.

1.4 Chromium uptake by maize plants

The hexavalent detoxification study was continued up to 60 days. In 60 days incubation the chromium hexavalent deduction was observed by DPC method. After 60 days the maize seeds were sown in the same pot and allowed to grow for 25 days. After 25 days the plants were harvested and note down the shoot length, root length and total chromium up taken by plants.

The whole plant samples were cleaned with water and the samples were kept in paper covers and shade dried and later oven dried (70^oC). After recording the dry weight, each sample was ground in a Wiley mill and sub-samples were obtained for laboratory analysis. One gram of plant samples were weighed and digested with aquaregia ($HCl:HNO_3$ @ 3:1). The digested samples were determined for chromium analysis by Atomic Absorbance Spectrophotometer.

1.5 Statistical analysis

The experiment was carried out using by completely randomized design and the 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 using AGRES software.

III. Results and Discussion

Large amount of animal and poultry manure or farmyard manure (FYM) and composts are used in agriculture for sustaining soil health. These organic amendments are also helpful in remediating the heavy metal contaminated soil. It has been widely reported that the organic amendments are capable of reducing toxic Cr VI to Cr III, as they form a source of electron donor. The organic amendments also immobilize Cr mainly by forming organic-metallic complexes (Harrison, 1992). The effect of vermicompost with or without microbial strains (*Pseudomonas fluorescens* and *Trichoderma viride*) and earthworms (*Eisenia foetida* and *Eudrilus eugenia*) on reduction of Cr VI and its subsequent impact on plant growth and Cr uptake was examined. The soil and vermicompost was characterized and data presented in Table 1.

2.1 Effect on Cr VI reduction

The effect of vermicompost and its combined application with microbial strains and earthworms on Cr (VI) reduction is presented in Table 2. Application of vermicompost and its combined application with earthworms resulted in a significant reduction of Cr (VI) concentration. Initially, the concentration of Cr VI ranged from 226 to 298 mg kg^{-1}; whereas it was from 44 to 227 mg kg^{-1} at the end of 60 days incubation. In the control soil (T_1) a concentration of 288 mg kg^{-1} was observed initially, which was found reduced to 227 mg kg^{-1} during 60 days incubation. The result corroborates with the findings of Bolan et al. 2003. The reduction in the concentration of Cr VI in soil amended with organic amendments could be attributed to various reasons. The supply of carbon and protons, and stimulation of microorganisms that are considered to be the major factors enhancing the reduction of Cr VI to Cr III (Losi et al., 1994; Bolan et al., 2003).

Comparing the two earthworms, the Cr VI reduction was relatively more due to *Eisenia foetida* than *Eudrilus eugeniae*, particularly due to its combined application with vermicompost and microbial strains. Application of *Pseudomonas fluorescens* (T_5) or *Trichoderma viride* (T_9) alone resulted in significant reduction of Cr (VI) concentration. Such effect was enhanced due to their combined application with vermicompost and earthworms. The percent reduction of Cr VI due to vermicompost with or without microbial strains and earthworm is depicted in Figure 1. Since the earthworms were found dead due to Cr VI toxicity within few days of introduction into the soil, the earthworm effect on Cr VI reduction has not been reflected significantly. Therefore, they have been eliminated from further studies. The maximum reduction of Cr VI (85 %) was occurred at the end of 60 days due to the application of vermicompost and *Eudrilus eugenia* (T_4). Reduction of Cr VI to Cr III in soils has often been found to be rapid, reaching the maximum within a relatively short time (Ross et al., 1981).

The results have been shown that the addition of microbial strains (*Pseudomonas fluorescens* / *Trichoderma viride*) alone resulted in marked reduction (70.7 to 71.7 %) in Cr VI. Only a small increase in the reduction of Cr VI was observed when the microbial strains, particularly *Pseudomonas fluorescens*, were applied along with organic amendments. Losi et al (1994) reported that the addition of organic manure compost increased Cr VI reduction both under sterile (i.e., abiotic) and non-sterile (i.e., biotic) conditions. However, Bolan et al (2003) reported that the manure additions caused a large increase in biotic than abiotic Cr VI reduction. This may suggest that the supply of microorganisms was more important than the supply of organic carbon in enhancing the reduction of Cr VI with the addition of organic compost. Losi et al. (1994) have shown that the addition of manure compost caused a larger increase in the biological reduction than the chemical reduction of Cr VI, which suggests that the supply of microorganisms is more important than the supply of organic carbon in enhancing the reduction of Cr VI with the addition of organic compost. In many studies, for

example Bolan et al. (2003), it has been observed that the extent of Cr VI reduction was found increased with increasing levels of easily oxidizable carbon, and dissolved organic carbon (DOC) content added through the organic amendments. There was a significant linear relationship between the extent of Cr VI reduction and DOC. The DOC has been identified to facilitate the reduction of Cr VI to Cr III in soils (Jardine et al., 1999; Nakayasu et al., 1999).

2.2 Growth of maize and uptake of chromium

The data on maize growth and Cr uptake as affected due to application of vermicompost, with or without microbial strains and earthworms are presented in Table 3. The results showed that the growth parameters viz., shoot and root length and biomass of maize were significantly lower when grown on the soil (control) without any amendments (T_1). The maize grown on soil amended with the vermicompost, with or without microbial strains and earthworms, had shown greater biomass, and root and shoot growth. The biomass of maize ranged from 8.7 to 16.2 g pot^{-1}. While the plants on the control soil (T_1) had the lowest biomass, the plants on the soil with the application of vermicompost alone had the highest biomass of maize (T_4), which was on par with the treatment (T_7) consisted of vermicompost along with *Pseudomonas fluorescens* and *Eisenia foetida*, and followed by T_3 consisted of vermicompost with *Eisenia foetida*.

Both the Cr content and uptake by maize varied significantly due to different treatments (Table 3). The Cr content in plants varied from 8.5 to 39.2 µg g^{-1}. The plants grown on the control soil (T_1) had the highest content of Cr (39.2 µg g^{-1}) and the plants grown on the soil with the application of *Trichoderma viride* (T_9) had the lesser value of Cr content. The Cr uptake by maize ranged between 112 and 341 µg pot^{-1}. While the plants in the control treatment (T_1) recorded the highest uptake of Cr (341 µg), the plants with the application of vermicompost, with or without microbial strains and earthworms recorded the lowest uptake of Cr.

The results of the pot experiments showed that maize growth and Cr uptake were significantly affected due to application of organic amendments. The plants grown on soil without any amendments had lesser biomass. The application of vermicompost with or without microbial strain resulted in a significantly greater biomass of maize. In the absence of any organic amendments, the maize growth and biomass yield were decreased due to the toxicity of Cr VI. Significant variation in Cr content and uptake by maize was observed (Fig. 2). The plants grown on the control soil had higher concentration of Cr. Similarly, the plant Cr concentration was found relatively higher due to the application of *Pseudomonas fluorescence* or *Trichoderma viride*, but without any organic amendments. This effect was also reflected on uptake of Cr by maize. The application of vermicompost was very effective in reducing the Cr concentration in plant tissue and its uptake. The biomass yield was found decreased with increasing concentration of Cr in plants as well as Cr uptake (Fig. 2).

There exists a negative correlation (R^2 = - 0.752) between the biomass yield and water soluble fraction of Cr in soil. This suggests that with increasing concentration of Cr both in soil and plant, Cr exerted phytotoxicity on plants. The phytotoxicity threshold concentration of Cr in plant tissue corresponding to 50% growth retardation (PT_{50}) was found to be only 6.2 mg kg^{-1} for Cr VI (Chang et al., 1992). The PT_{50} value varied between plant species and metal species. The PT_{50} value of 5.9 mg kg^{-1} was suggested for maize (Mortvedt and Giordano, 1975). In all the treatments, the Cr concentration in plant exceeded the threshold value of PT_{50}. Similar result was reported by Bolan et al. (2003).

IV. Conclusions

Addition of vermicompost with or without microbial strains and earthworms resulted in a significant reduction in the concentration of Cr VI. Since the earthworms were found dead due to Cr VI toxicity within few days of introduction into the soil, the earthworm effect on Cr VI reduction has not been reflected significantly. A maximum reduction of Cr VI (85.3%) was occurred at the end of 60 days due to the application of vermicompost and *Eudrilus eugenia*. Addition of microbial strains (*Pseudomonas fluorescens* / *Trichoderma viride*) alone resulted in marked reduction (70.7 to 71.7 %) in Cr VI. Only a small increase in the reduction of Cr VI was observed when the microbial strains, particularly *Pseudomonas fluorescens*, were applied along with vermicompost. The large amount of hexavalent chromium was detoxified due to application of vermicompost. The plants grown on the control soil had the highest content of Cr (39.2 µg g^{-1}) and the plants grown on the soil with the application of *Trichoderma viride* had the lesser value of Cr content. Vermicompost was reduced the toxicity of chromium and bioavailability to the plant uptake. This organic amendment can recommend for hexavalent chromium contaminated soil. Results from this study indicate that the addition of biological materials like vermicompost and microbial strains to Cr (VI) contaminated mineral soil enhanced the reduction of Cr (VI) to Cr (III), thereby reducing the bioavailability of Cr for plant uptake.

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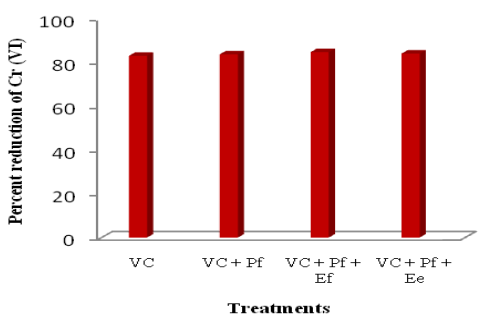

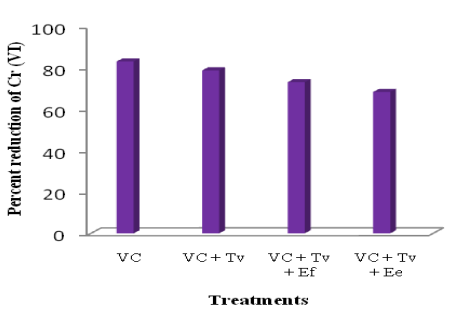

VC : Vermicompost
Pf : *Pseudomonas fluorescens*
Tv : *Trichoderma viride*
Ef : *Eisenia foetida*
Ee : *Eudrilus eugenia*

Fig. 1 Percent reduction of Cr (VI) with vermicompost, earthworms and microbial strains

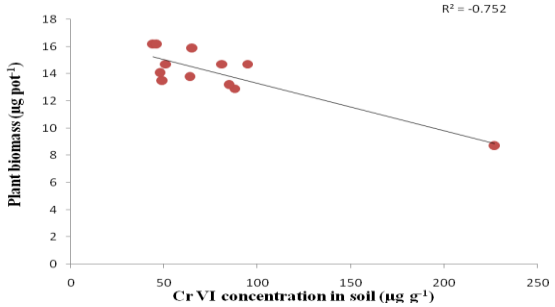

Fig. 2 Correlation between plant biomass and Cr VI concentration in soil

Table 1 Initial characteristic of soil and vermicompost

Parameters	Soil	Vermicompost
pH	7.80	7.25
EC (d Sm^{-1})	0.36	1.35
Organic Carbon (%)	0.98	16.0
Available N (kg ha^{-1})	72.80	1.10 [Total N (%)]
Available P (kg ha^{-1})	14.07	0.45 [Total P (%)]
Available K (kg ha^{-1})	180.00	0.72 [Total K (%)]
CEC (m.eq / 100g)	14.50	

Table 2 Effect of vermicompost, microbial strains and earthworms on the reduction of hexavalent chromium (mg kg^{-1})

Treatments	Initial	15th day	30th day	45th day	60th day
T_1 -Control - Soil*	288	269	244	234	227
T_2 –Soil* + Vermicompost	250	129	119	80	51
T_3- Soil*+ Vermicompost + *Eisenia foetida*	226	154	81	69	65
T_4– Soil* + Vermicompost + *Eudrilus eugeniae*	245	168	91	68	44
T_5 - Soil* + *Pseudomonas fluorescens*	298	202	156	112	88
T_6 - Soil* + Vermicompost + *Pseudomonas fluorescens*	236	149	84	67	49
T_7 - Soil* + Vermicompost + *Pseudomonas fluorescens* + *Eisenia foetida*	264	135	91	60	46
T_8 - Soil* + Vermicompost + *Pseudomonas fluorescens* + *Eudrilus eugeniae*	269	139	94	57	48
T_9 - Soil* + *Trichoderma viride*	284	216	172	97	85
T_{10} - Soil* + Vermicompost + *Trichoderma viride*	264	120	84	71	64
T_{11}- Soil* + Vermicompost + *Trichoderma viride* + *Eisenia foetida*	288	188	163	91	81
T_{12} - Soil* + Vermicompost + *Trichoderma viride* + *Eudrilus eugeniae*	288	197	159	110	95
Mean	263	220	182	144	105
	SEd			**CD(0.05)**	
T	14.63			29.18**	
S	8.74			17.44**	
T x S	32.72			NS	

Soil - 300 mg Cr (VI) kg^{-1}*

Table 3 Effect of bioremediation inputs on growth of maize and total chromium uptake

Treatments	Shoot length (cm)	Root length (cm)	Total Biomass (g pot^{-1})	Cr content (µg g^{-1})	Cr uptake (µg pot^{-1})
T_1 -Control - Soil*	30.0	8.7	8.7	39.2	341
T_2 –Soil* + Vermicompost	39.8	16.2	14.7	12.7	186
T_3- Soil*+ Vermicompost + *Eisenia foetida*	40.5	14.9	15.9	15.2	242
T_4– Soil* + Vermicompost + *Eudrilus eugeniae*	37.3	15.4	16.2	18.6	301
T_5 - Soil* + *Pseudomonas fluorescens*	39.2	13.6	12.9	18.8	243
T_6 - Soil* + Vermicompost + *Pseudomonas fluorescens*	38.7	17.3	13.5	12.9	174
T_7 - Soil* + Vermicompost + *Pseudomonas fluorescens* + *Eisenia foetida*	31.7	12.3	16.2	16.5	267
T_8 - Soil* + Vermicompost + *Pseudomonas fluorescens* + *Eudrilus eugeniae*	41.8	13.7	14.1	17.5	247
T_9 - Soil* + *Trichoderma viride*	33.4	11.5	13.2	8.5	112
T_{10} - Soil* + Vermicompost + *Trichoderma viride*	39.3	10.1	13.8	9.6	132

T_{11}- Soil* + Vermicompost + *Trichoderma viride* + *Eisenia foetida*	59.7	11.3	14.7	11.9	175
T_{12} - Soil* + Vermicompost + *Trichoderma viride* + *Eudrilus eugeniae*	37.8	12.3	14.7	11.5	169
Mean	41.8	7.6	13.4	19.1	235
SEd	6.51	2.15	2.31	3.67	41.92
CD(p=0.05)	13.95*	4.62**	NS	7.87**	89.91**

Soil - 300 mg Cr (VI) kg^{-1}*