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Poultry manure induce biotransformation of hexavalent chromium in soil

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Abstract: *Chromium (Cr) 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 lipid metabolism in humans and animals. In contrast, hexavalent Cr (Cr VI) is a highly toxic carcinogen and may cause death to animals and humans if ingested in large doses. 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. In the presence of organic amendments (poultry manure) and microbial inoculants (Pseudomonas fluorescens and Trichoderma viride) the chromium (VI) reduction was observed in maximum in the poultry manure treatment (91%) whereas poultry manure with Pseudomonas fluorescens reduced the hexavalent chromium up to 90 %.*
Key Words: *Bioremediation, Cr (VI), Poultry manure, Trichoderma viride, Pseudomonas fluorescens, detoxification*

I. Introduction

Increasing levels of heavy metals in the environment pose serious threats to water quality, soil eco system, 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) as a Group 1 (human carcinogen) (WHO 2004). Some cities in Iran have high amount of hexavalent chromium content in ground water resources.

In recent year's contamination of the environment by Cr, especially hexavalent Cr has become a major area of concern. Dissemination of Cr in the environment is due to many different industries including metallurgical, electroplating, paints and pigments, tanning, wood preservation, Cr chemicals production and pulp and paper industries. Often, wastes from such industries (e.g., sludge, fly ash, slag, etc.) are used as a fill material at numerous locations to reclaim marshlands, tank dikes, and 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 a large contributor of Cr pollution to water resources. Chandra *et al.* (1997) estimated that in India alone about 2000 to 3200 tonnes 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}.

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.

Inexpensive remediation technologies are needed for Cr (VI) by reduction or biotransformation is a promising approach. In the present study, we studied the detoxification of Cr (VI) from the contaminated soil using poultry manure, earthworm and microbe. The earthworm population and its survival were examined.

II. Materials and Methods

A pot experiment was conducted to examine the effect of poultry manure, 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 TNAU (Farm Eastern Block Field No. 37), Coimbatore. The samples were air dried at 25^{o}C and sieved (< 2 mm) and the soil was used in this study. Syntax pots with a capacity of 1500 cm^3 were used. One kg of soil was weighed and transferred into each pot. The soil was added 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, poultry manure (@ 10 t ha^{-1}) was added in as per the treatment schedule and uniformly mixed. The rate of application of poultry manure 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 size ten number of earthworm species (20 cm length of *Eudrilus eugenia* and 10 cm length of *Eisenia foetida* which was 0.2 g and 1g each respectively) 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).

Treatment details

T_1 : Control - Soil* (No amendment)
T_2 : Soil* + Poultry manure
T_3 : Soil*+ Poultry manure + *Eisenia foetida*
T_4 : Soil*+ Poultry manure + *Eudrilus eugeniae*
T_5 : Soil*+ *Pseudomonas fluorescens*
T_6 : Soil*+ Poultry manure + *Pseudomonas fluorescens*
T_7 : Soil* + Poultry manure + *Pseudomonas fluorescens* + *Eisenia foetida*
T_8 : Soil*+ Poultry manure + *Pseudomonas fluorescens* + *Eudrilus eugeniae*
T_9 : Soil* + *Trichoderma viride*
T_{10} : Soil* + Poultry manure + *Trichoderma viride*
T_{11} : Soil* + Poultry manure + *Trichoderma viride* + *Eisenia foetida*
T_{12} : Soil* + Poultry manure + *Trichoderma viride* + *Eudrilus eugeniae*
Soil * **-** Soil spiked with 300 mg Cr (VI) kg^{-1}

The Cation Exchange Capacity of the soil sample was determined by neutral ammonium acetate solution method (Jackson, 1973).

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 organic carbon content of the samples was determined by the wet digestion method (Walkley and Black, 1934).

The concentration of Cr (VI) (water soluble fraction) was determined as per the method outlined by USEPA (1979b).

III. Results and Discussion

Large amount of animal and poultry manure / farmyard manure (FYM) and organic amendments are used in agriculture for sustaining soil health. Along with these 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). Therefore, a series of laboratory experiment was conducted to evaluate poultry manure and microbial cultures for developing a bioremediation technology for Cr contaminated soil. Initially the soil and poultry manure was characterised and data presented in Table 1.

The effect of poultry manure, microbial strains (*P. fluorescens* and *T. viride*) and earthworms (*Eisenia foetida* and *Eudrilus eugeniae*) on reduction of Cr VI is presented in Table 2. Initially Cr VI (300 mg kg^{-1}) was taken for treatment along with poultry manure, microbial strains and earthworms. From the initial day to 60 days of incubation, the Cr VI concentration range was drastically reduced from 226 to 298 mg kg^{-1} to 25 to 224 mg kg^{-1}. Although in control soil (T_1) the concentration was significantly decreased from 15 to 60 days of incubation. Application of poultry manure alone (T_2) markedly reduce the Cr VI concentration (25 mg kg^{-1}) at all days of incubation. The percentage of Cr VI reduction on various amendments is given in Figure 1. The higher rate of Cr VI reduction was seen not only treating with poultry manure but also along with other amendments (T_3 and T_4). Among the worms, *Eisenia foetida* was found relatively more effective in reducing the Cr VI than *Eudrilus eugeniae* were seen at all days of incubation. Mortality of the earthworms was observed due to Cr (VI) toxicity within few days of introduction into the soil, though the earthworm effect on Cr (VI) reduction has not been reflected significantly. Therefore, they have been eliminated from further studies. Among the all treatments the poultry manure amended with *Eisenia foetida* soil treatment (T_3) was reduced the chromium up to 24 per cent in all the incubation period (15 days to 60 days) interval followed by Poultry manure with *Pseudomonas fluorescens* (T_6) and poultry manure alone (T_2) treatment. The maximum reduction of Cr VI (90.7%) occurred at the end of 60 days due to the application of poultry manure and *P. fluorescens* (T_2). Reduction of Cr (VI) to Cr (III) in soils has often been found to be rapid and reaching the maximum within a relatively short time (Ross *et al*., 1981). The supply of carbons 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). The result corroborates with the findings of Bolan *et al.* 2003. Losi and co workers (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.

It has been shown that the addition of microbial strains (*P. fluorescens* / *T. viride*) alone resulted in marked reduction (66.3 to 75.7 %) in Cr VI (Figure 1). Only a small increase in the reduction of Cr (VI) was observed when *P. fluorescens* alone applied along with organic amendments. Losi and co workers (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 and co workers (2003) showed that the treatment with manure 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.

The effect of poultry manure, microbial strains and earthworms on soil organic carbon (SOC) content is presented in Table 3. Initially the SOC ranged from 9.6 to 18.2 g kg^{-1}. Soil with the application of *P. fluorescens* alone recorded the lowest value of SOC; whereas, the soil with the application of poultry manure (T_2) had the highest amount of SOC. Irrespective of treatments, the SOC was found decreased gradually during the incubation. At the end of 60 days of incubation, the SOC ranged between 3.2 and 6.7 g kg^{-1}. Due to microbial respiration and decomposition of soil organic matter (SOM), the SOC was found reduced gradually after the treatment. However, the combined effect of poultry manure, earthworms and microbial strain has not produced any clear cut trend on SOC content. There was a significant linear relationship between the extent of Cr (VI) reduction and DOC. DOC has been identified to facilitate the reduction of Cr (VI) to Cr (III) in soils (Jardine *et al*., 1999; Nakayasu *et al*., 1999; Bolan *et al*., 2003). Theoretically, it is estimated that 1.00 mg of organic carbon is required to cause a reduction of 5.78 mg Cr (VI) based on the following reaction (Adriano, 2001).

$$3CH_2O \text{ (organic matter)} + 2Cr_2O_7 + 16\,H^+ \rightarrow 4Cr^{3+} + 3CO_2 + 11H_2O$$

IV. Conclusions

Addition of organic amendments with or without microbial strains and earthworms resulted in a significant reduction of Cr (VI) in soil. Since mortality of earthworms were found due to Cr (VI) toxicity within few days of introduction into the soil, the effect of earthworm on Cr (VI) reduction has not been reflected significantly. Application of organic amendments increases the soil pH which directly increases the reduction of Cr (VI). A maximum reduction of Cr VI (90.7%) occurred at the end of 60 days due to the application of poultry manure and *P. fluorescens*. Addition of microbial strains (*P. fluorescens* / *T. viride*) alone resulted in marked reduction (66.3 to 75.7 %) in Cr (VI). The large amount of hexavalent chromium was detoxified due to application of poultry manure and there by the crops can uptake the nutrients supplied by these amendments. These particular amendments can be recommended for hexavalent chromium contaminated soil. Our findings indicate that the addition of organic amendments 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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Table 1. Initial characteristics of soil and Poultry manure

Parameters	Soil	Poultry manure
pH	7.80	7.20
EC (dsm^{-1})	0.36	1.45
Organic Carbon (%)	0.98	19.00
Avail. N (kg ha^{-1})	72.80	
Avail. P (kg ha^{-1})	14.07	
Avail. K (kg ha^{-1})	180.00	
CEC (m.eq / 100g)	14.50	
Total N (%)		3.10
Total P (%)		2.10
Total K (%)		2.80

Table 2. Effect of poultry manure, 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*	294	269	250	231	224
T_2 –Soil* + Poultry manure	268	149	125	120	32
T_3- Soil*+ Poultry manure + *Eisenia foetida*	241	168	94	82	25
T_4– Soil* + Poultry manure + *Eudrilus eugeniae*	240	149	104	56	44
T_5 - Soil* + *Pseudomonas fluorescens*	236	188	153	139	101
T_6 - Soil* + Poultry manure + *Pseudomonas fluorescens*	226	139	75	68	28

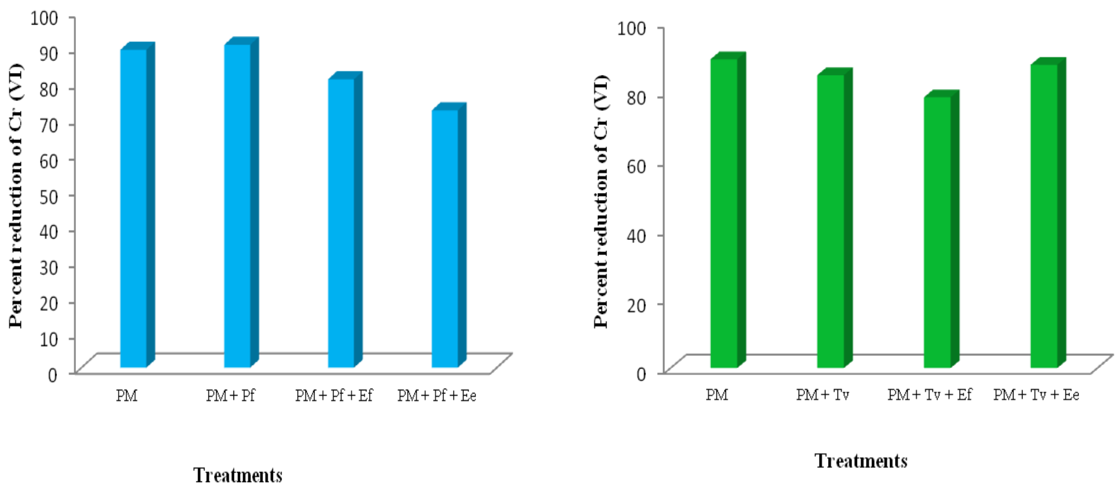
T$_7$ - Soil* + Poultry manure + *Pseudomonas fluorescens* + *Eisenia foetida*	245	130	97	80	57
T$_8$ - Soil* + Poultry manure + *Pseudomonas fluorescens* + *Eudrilus eugeniae*	263	197	156	101	83
T$_9$ - Soil* + *Trichoderma viride*	283	149	128	93	74
T$_{10}$ - Soil* + Poultry manure + *Trichoderma viride*	231	120	66	54	46
T$_{11}$- Soil* + Poultry manure + *Trichoderma viride* + *Eisenia foetida*	298	135	100	82	65
T$_{12}$ - Soil* + Poultry manure + *Trichoderma viride* + *Eudrilus eugeniae*	260	101	78	53	37
Mean	259	175	127	102	80
	SEd			**CD (0.05)**	
T	12.72			25.36**	
S	7.60			15.16**	
T x S	28.43			NS	

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

Table 3 Effect of poultry manure, microbial strains and earthworms on soil organic carbon (g kg^{-1})

Treatments	Initial	15th day	30th day	45th day	60th day
T$_1$ -Control - Soil*	9.6	9.2	7.7	5.6	4.6
T$_2$ –Soil* + Poultry manure	18.2	12.8	8.0	7.3	6.7
T$_3$- Soil*+ Poultry manure + *Eisenia foetida*	15.2	11.2	9.8	5.9	3.5
T$_4$– Soil* + Poultry manure + *Eudrilus eugeniae*	13.7	13.3	8.6	5.3	3.2
T$_5$ - Soil* + *Pseudomonas fluorescens*	8.9	8.4	5.2	5.2	4.4
T$_6$ - Soil* + Poultry manure + *Pseudomonas fluorescens*	10.7	12.8	9.3	5.8	5.5
T$_7$ - Soil* + Poultry manure + *Pseudomonas fluorescens* + *Eisenia foetida*	11.4	12.2	9.1	7.1	4.4
T$_8$ - Soil* + Poultry manure + *Pseudomonas fluorescens* + *Eudrilus eugeniae*	11.2	12.5	9.5	6.2	4.4
T$_9$ - Soil* + *Trichoderma viride*	12.0	9.7	7.8	5.6	4.9
T$_{10}$ - Soil* + Poultry manure + *Trichoderma viride*	11.2	12.5	8.5	6.4	4.6
T$_{11}$- Soil* + Poultry manure + *Trichoderma viride* + *Eisenia foetida*	11.0	12.0	9.0	6.2	4.4
T$_{12}$ - Soil* + Poultry manure + *Trichoderma viride* + *Eudrilus eugeniae*	12.6	11.8	8.8	5.0	5.2
Mean	11.6	11.2	8.0	5.7	4.6
	SEd			**CD (0.05)**	
T	0.67			1.34**	
S	0.40			0.80**	
T x S	1.50			2.99**	

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



PM	:	Poultry manure	Ef	:	*Eisenia foetida*
Pf	:	*Pseudomonas fluorescens*	Ee	:	*Eudrilus eugenia*
Tv	:	*Trichoderma viride*			

Figure 1. Percent reduction of Cr (VI) with poultry manure, earthworms and microbial strains

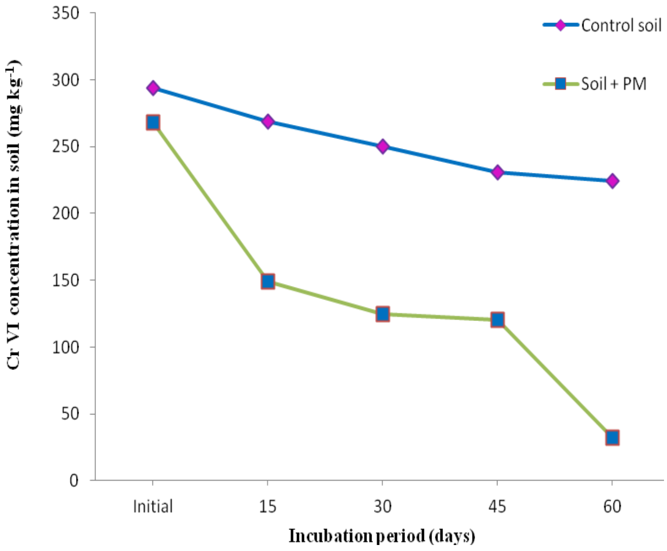


Figure 2. Effect of poultry manure on concentration of hexavalent chromium (Cr VI) in soil