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BIOTRANSFORMATION AND BIOAVAILABILITY OF CHROMIUM CONTAMINATED SOIL AND THE EFFECT OF POULTRY MANURE AND PSEUDOMONAS

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ABSTRACT: Biological and/or chemical transformation and the chemical species determine the biotoxicity of Cr in soil. Such information is needed for developing effective remediation techniques. In the current study the transformation of Cr in soil and the effect of poultry manure with and without Pseudomonas were examined in Laboratory closed incubation experiment. The concentration of Cr (VI) was markedly increased up to 15 days and thereafter found decreased due to oxidation of Cr (III) and subsequent reduction of Cr (VI) in this soil. Increase in the rate of application of poultry manure significantly reduced the concentration of Cr (VI) and the effect was more pronounced when Pseudomonas was applied along with poultry manure. About 43% reduction in Cr (VI) concentration was recorded within 15 days when poultry manure was added at a rate of 18 t ha^{-1}. Whereas, about 80% reduction in Cr (VI) was achieved by the combined application of poultry manure and Pseudomonas. Such effect of poultry manure and Pseudomonas was also reflected on H_2O – Cr and KNO_3. A complete removal of KNO_3 – Cr in soil was observed due to the application of poultry manure and Pseudomonas. Therefore, the study demonstrates the potential of poultry manure in reducing the bioavailability of Cr in the contaminated soil and this can effectively be used in the bioremediation of Cr contaminated soil.
Key words: Bioavailability, transformation, Hexavalent Cr, Poultry manure, *Pseudomonas*

INTRODUCTION

Many industrial activities, including electroplating, electro power production, the leather and pulp industries, and ore and petroleum refining generate waste products that contain solid and aqueous forms of chromium (Cr) Frostner and Wittman (1981). The land disposal of such wastes has resulted in extensive contamination of soils and groundwater with Cr in many countries including India. Tannery wastes (both sludge and effluent) typically contain high concentration of Cr and sodium (Na) salts (chlorides and sulphates etc). Mahimairaja *et al.,* (2000a) reported severe contamination of Cr in soils around tanning industries in Vellore district. Chromium accumulation ranged from 569 to 79865 mg kg^{-1} on surface and subsurface soils, due to the indiscriminate disposal of tannery wastes.

The transformation (biological and chemical) and the chemical species determine the toxicity of Cr in soil. Such information helps to develop appropriate remediation techniques for Cr contaminated soil. A number of studies have shown that addition of organic manures enhances the immobilization of metals and thus reduces their bioavailability. In the Cr contaminated soil, Mahimairaja *et al.,* (2000b) showed that the application of coir pith and poultry manure reduces the concentration of soluble plus exchangeable – Cr which represent toxic form of Cr in soil.

Chromium exists in both anionic and cationic state and the nature of the species may vary with the changes in the soil environmental conditions. Such conditions may be those that occur following the disposal of wastes, with fluctuations in soil moisture levels, pH, redox potential, etc. Of the two forms or species commonly exist in the environment, hexavalent Cr [Cr (VI)] is of concern because of its solubility, high mobility and toxic effect on plants and animals. Hexavalent Cr species are anionic (i.e. $HCrO_4^-$ and CrO_4^{2-}) and are generally mobile in most neutral to alkaline conditions. Under acidic conditions, Cr (VI) is generally removed from the solution phase by adsorption reactions involving positively charged sites on oxidic surfaces (Bartlett and James 1988). Trivalent Cr follows the general reductions of cationic heavymetals, i.e. complexation with humic matter, cation exchange on humic acids or clay minerals, and formation of hydroxyl compounds by hydrolysis. Since Cr (VI) is both toxic and mutagenic Adriano (1986) many researchers have been working on techniques for remediating water and soils contaminated with Cr. In the current study, the transformation of Cr in soil and the effect of poultry manure and Pseudomonas were examined by conducting a closed laboratory incubation experiment.

MATERIALS AND METHODS

Laboratory Incubation Experiment – Bioavailability of Chromium

The biotransformation and bioavailability of Cr in soil was examined by conducting a laboratory closed incubation experiment. Five hundred grams of air dried, powdered and sieved (<2mm) soil was weighed in a plastic containers (750 cm^3). Some important characteristics of the soil are presented in Table.1. The soil was artificially contaminated with Cr. Chromium was added as [$Cr_2(SO_4)_3$] at a rate equivalent of 5000 mg Cr kg^{-1} and thoroughly mixed with the soil. Water was added to attain a final moisture content equivalent to that of field moisture capacity (= 0.37 g g^{-1} soil). After a week of incubation, poultry manure (collected from TNAU poultry shed) was added at a rate of 6, 12 and 18t ha^{-1}. In one set of treatment, Pseudomonas fluorescens (Pf1- commercial grade obtained from the Dept. of Pathology, TNAU) was introduced into the soil. The following were the treatment

T_1 – Soil*
T_2 - Soil* + Poultry manure 6 t ha^{-1}
T_3 - Soil* + Poultry manure 12 t ha^{-1}
T_4 Soil* + Poultry manure 18 t ha^{-1} **T_5** - Soil* + Pseudomonas
T_6 - Soil* + Poultry manure 6 t ha^{-1} + Pseudomonas
T_7 - Soil* + Poultry manure 12 t ha^{-1} + Pseudomonas
T_8 - Soil* + Poultry manure 18 t ha^{-1} + Pseudomonas
* spiked with Cr

Whenever necessary required quantity of distilled water was added to achieve a final moisture content equivalent to of field capacity (0.37 g g^{-1}). The plastic containers were covered with polyethylene bags containing small pin-sized holes to permit aeration. Three replicates of each treatment were prepared, randomly placed and incubated in the laboratory at 25±2°C for 30 days. Based on the weight loss distilled water was added to the container to maintain the moisture content throughout the incubation experiment. At fortnight intervals 100g of sample was removed from all the treatments and analysed for pH, Cr (VI) and various fraction of Cr.

The transformation of Cr in soil was examined by determining the concentration of different species of Cr following a sequential fractionation procedure Noble and Hughes. (1991). One gram of fresh sample was shaken end-over-end with 25 cm^3 of Millipore water (two times) at 20±2°C, for 2 hrs in a 50 cm^3 polypropylene centrifuge tube. The tubes were centrifuged at 8000rpm for 10 min and filtered through Whatman No. 40 filter paper.

The soluble Cr in the water extract was determined. To the residue in the tube 25cm^3 of 0.5M KNO_3 was added, shaken for 16 hrs, centrifuged and filtered. This was followed by extraction with 0.5 M NaOH for 16 hrs and 0.5 M Na_2 EDTA for 6 hrs. Finally residue was digested with concentrated HNO_3. The Cr in the respective extracts was determined in an Atomic Absorption Spectrophotometer (Varian spectrAA 200) with air acetylene flame at 257.9 nm USEPA, (1977). The amount of Cr extracted by each extractant was computed by the following equation:

$$\text{Cr extracted} = C \times (E + M) - (C' \times M) / \text{Weight of soil}$$

Where C is the concentration of Cr in the extraction solution, E is the mass (g) of extractant, C' is the concentration of Cr in the extraction solution in the preceding step of the sequence, and M is the mass (g) of the entrained solution carried over from the previous extraction. The hexavalent Cr was determined by a diphenyl carbazide method using a UV- Vis spectrophotometer (USEPA 1979)

RESULT AND DISCUSSION

Characteristics of Soil and Poultry Manure

Some important characteristics of soil and poultry manure are given in Table 1 and 2. The soil was a calcareous black soil, which belongs to Periyanaickenpalayam series and *Vertic Ustropept* in USDA classification. It was sandy clay loam in texture. The pH was 8.42 with an EC of 0.56 dSm^{-1}. The soil organic carbon content was low (5.1 g kg^{-1}). With regard to nutrient status, the soil was low in N and P, but medium in K contents. The pH and EC of the poultry manure were 7.51 and 8.30, respectively. It has 15 g kg^{-1} of organic carbon with high level of NPK content.

Table 1. Characteristics of Experimental soil

S.No.	Characters	Values
1.	pH (1: 2.5 soil water suspension)	7.42
2.	EC (dSm^{-1}) (1: 2.5 soil water extract)	0.56
3.	Cation Exchange Capacity (cmol(+) kg^{-1})	17.9
4.	Organic carbon (g kg^{-1})	5.10
5.	NH_4- N (mg kg^{-1})	93
6.	NO_3- N (mg kg^{-1})	56
7.	$NaHCO_3$ - P (mg kg^{-1})	5
8.	NH_4OAc- K (mg kg^{-1})	183
9.	EDTA – Mn (mg kg^{-1})	220
10.	Bacteria (x10^6 CFU g^{-1} of soil)	13
11.	Fungi (x10^4 CFU g^{-1} of soil)	5
12.	Actinomycetes (x10^3 CFU g^{-1} of soil)	4
13.	Phosphatase activity(µg p-nitrophenol g^{-1}of soil hr^{-1})	5.60
14.	Dehydrogenase activity (µg TPF g^{-1} of soil hr^{-1})	6.26
15.	Urease activity (µg NH_4-N g^{-1} of soil hr^{-1})	44

Table 2. Characteristics of Poultry Manure

S.No.	Characters	Values
1.	pH (1: 2.5 soil water suspension)	7.51
2.	EC (dSm^{-1}) (1: 2.5 soil water extract)	8.30
3.	Organic carbon (g kg^{-1})	15
4.	NH_4- N (mg kg^{-1})	284
5.	NO_3- N (mg kg^{-1})	1204
6.	$NaHCO_3$ - P (mg kg^{-1})	5524
7.	NH_4OAc- K (mg kg^{-1})	14200
8.	Calcium (mg kg^{-1})	22400
9.	Sulphate (mg kg^{-1})	543

Changes in soil pH

The pH of Cr contaminated soil was 7.05 which was significantly increased due to incorporation of different levels of poultry manure (Table.3). It didn't change much due to pseudomonas application immediately. During the incubation, the pH was found markedly increased at 15th day, and thereafter decreased at 30th day. Similarly, the soil pH was significantly increased due to poultry manure up to 15 days and decreased thereafter. The increase in pH was mainly due to the production of NH_4 and addition of base ions from the poultry manure. Significant reduction in pH was probably be due to organic acids produced during the decomposition of poultry manure (Mahimairaja *et al.,* 1995)

Table.3. Effect of Poultry Manure and Pseudomonas on pH in soil during 30 days of incubation

Treatments	Incubation period (days)			
	0	15	30	Mean
T1- Contaminated soil	7.05	7.16	6.92	7.04
T2- Soil+ PM (6 t ha^{-1})	7.17	8.15	7.98	7.77
T3- Soil+ PM (12 t ha^{-1})	7.25	8.15	7.92	7.74
T4- Soil+ PM (18 t ha^{-1})	7.34	8.12	7.9	7.79
T5- Soil+ Pseudomonas	7.03	8.22	7.97	7.88
T6- Soil+ Pseudomonas+ PM (6 t ha^{-1})	7.23	8.18	7.98	7.80
T7- Soil+ Pseudomonas+ PM (12 t ha^{-1})	7.17	8.1	7.92	7.73
T8- Soil+ Pseudomonas+ PM (18 t ha^{-1})	7.1	8.08	7.76	7.65
	SEd		CD (0.05)	
Treatment (T) Incubation Period (P) T x P	0.05 0.03 0.08		0.10 ** 0.06 ** 0.18**	

Effect of Poultry Manure and Pseudomonas on hexavelent Cr

The change in the concentration of hexavelent Cr in the soil during 30 days of incubation is shown in Table.4. In the Cr spiked soil, initially the concentration of Cr (VI) was only 0.74 mg kg^{-1}. It was markedly increased up to 15 days, but found decreased at 30 days. The increase in Cr (VI) provides evidence for the oxidation of Cr III into Cr VI in this soil.

Table.4. Effect of Poultry Manure and Pseudomonas on hexavelent Chromium (mg kg $^{-1}$) in soil during 30 days of incubation

Treatments	Incubation period (days)			
	0	15	30	Mean
T1- Contaminated soil	0.74	4.31	3.96	3.00
T2- Soil+ PM (6 t ha-1)	0.56	3.09	2.91	2.19
T3- Soil+ PM (12 t ha-1)	0.35	2.61	2.56	1.84
T4- Soil+ PM (18 t ha-1)	0.35	2.43	2.25	1.68
T5- Soil+ Pseudomonas	0.48	2.13	1.86	1.49
T6- Soil+ Pseudomonas+ PM (6 t ha-1)	0.38	1.72	1.28	1.13
T7- Soil+ Pseudomonas+ PM (12 t ha-1)	0.26	1.32	1.14	0.91
T8- Soil+ Pseudomonas+ PM (18 t ha-1)	0.24	1.20	0.76	0.73
	SEd		CD (0.05)	
Treatment (T) Incubation Period (P) T x P	0.02 0.01 0.04		0.04 ** 0.02 ** 0.08**	

Though the oxidation of Cr III in soil is a rare process, it has been reported that the presence of manganous (Mn IV) oxide in soil favours the oxidation of Cr III (Barlett and James 1979). In the present study the soil contained about 220 mg Mn kg^{-1} and this Mn might have acted as a terminal acceptor in the soil system and thereby enhanced the oxidation of Cr III to Cr (VI). The decrease in the concentration of Cr (VI) observed at 30 day could be due to reduction of CrVI to Cr III. Though adsorption was found to be an important mechanism for the decrease in the concentration of Cr VI (Stollenwerk and Grove, 1985), the pH of the soil observed at 30th day was near neutral. Hence , at this pH the Cr VI might not have adsorbed on soil particles.

Increase in the rate of application of poultry manure progressively reduced the concentration of Cr VI, and the effect was more pronounced when Pseudomonas was incorporated along with poultry manure. About 43% reduction in Cr VI concentration was recorded on 15th day when poultry manure alone was added at a rate of 18t ha^{-1}(T4). Similar effect was observed at 30th day. The percent reduction was markedly increased in the presence of Pseudomonas. About 80% reduction in Cr VI was achieved by the incorporation of poultry manure (18t ha^{-1}) plus Pseudomonas (T8).

The reduction might be due to either formation of organo – chromic complexes (immobilization) or reduction of toxic, soluble Cr (VI) to non toxic, less soluble Cr (III) in soil. The biological wastes represent a significant reserviour of electron donors for the reduction of Cr (VI) to Cr (III). During the decomposition of organic matter in the poultry manure, compounds such as citric acid and 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). Humic substances present in the biological wastes play a major role in reduction of Cr (VI). In general it appeared that the addition of an easily degradable substrate with low C: N ratio (eg: poultry manure) might have stimulated more Cr (VI) reducers. Irrespective of treatments with the addition of pseudomonas the concentration Cr (VI) was markedly decreased towards the incubation period. The Cr (VI) reduction may just be a fortuitous reaction carried out by enzymes that have other physiological substrates and the Cr (VI) reduction may provide energy for few microbes**.**

Chromium reductase is widespread in microbes, but it is primarily present in the soluble fractions of the microbial cell. It reduced the Cr (VI) to Cr (III) with the oxidation of 3 moles of NADH per mole of Cr reduced. It has been reported that NADH could non enzymatically reduce Cr (VI to Cr (V) in the absence of enzyme. A membrane protein also appears to be important for Cr (VI) reduction by bacteria (Naidu and Kookana. 2000).

Biotransformation of Chromium

Depending upon chemical valence state and soil environmental conditions, the added Cr is subjected to mostly biological transformation. Microbial and enzyme activities play a major role in such transformation processes. The changes in the concentration of soluble Cr (H_2O-Cr), exchangeable and adsorbed Cr (KNO_3-Cr), organic form of Cr (NaOH-Cr), iron and aluminium oxide bound – Cr (EDTA-Cr) and the residual Cr or acid soluble – Cr (HNO_3-Cr) during the incubation are presented in Table. 5a, b, c.

In the spiked soil, initially, the relative distribution of Cr species followed: HNO_3-Cr >> NaOH-Cr > EDTA-Cr > KNO_3-Cr > H_2O-Cr. Only small amount of (18 $mgkg^{-1}$) of the soluble Cr was present initially, and it was markedly increased (365 $makg^{-1}$) within 15 days. There was a significant reduction in the concentration of exchangeable and adsorbed – Cr (KNO_3-Cr), and the reduction was mostly occurred during the first 15 days with the application of poultry manure and Pseudomonas, the concentration of exchangeable and adsorbed – Cr (KNO_3-Cr) in soil become negligible.

The organic – Cr remained mostly unchanged during the incubation. Only a small increase in the concentration of iron and aluminium oxide bound- Cr was observed. The acid soluble fraction (HNO_3-Cr) was found increased at 15th day but decreased at 30th day. The result showed that the added Cr (Cr III) was oxidized to Cr (VI) which is soluble in water and increased the soluble Cr in soil. The reduction in exchangeable and adsorbed Cr and the increase in Cr (VI) also provide evidence for the oxidation of Cr (III).

Effect of Poultry manure and Pseudomonas

The incorporation of poultry manure and Pseudomonas had remarkable impact on the biotransformation of Cr in the soil. In the presence of poultry manure with and without Pseudomonas, both soluble- Cr and exchangeable/ adsorbed- Cr were completely disappeared. The different rates of poultry manure didn't differ and showed that even at the lowest rate of application (6t ha^{-1}) the poultry manure was found very effective in reducing the bioavailable fraction of Cr. As has already been discussed, the bioavailable fraction of Cr (mostly Cr VI) might have microbially reduced to Cr (III) or immobilized in microbial tissues. Marked increase in the concentration of organic- Cr (NaOH- Cr) was observed due to poultry manure with and without Pseudomonas. This suggests that part of the bioavailable fraction might have microbially immobilized into microbial tissues, a process generally known as microbial adsorption. The treatment consisting of poultry manure at a rate of 18 t ha^{-1} plus Pseudomonas recorded significantly higher amount of organic Cr in soil. A small reduction in the organic – Cr was observed at 30th day, which might possibly be due to remineralization of Cr. Only a small change in the concentration of Al/Fe oxide bound Cr was observed. This may suggest that the adsorption of Cr was insignificant in this soil. The results demonstrate the potential of poultry manure in reducing the bioavailability of Cr in the contaminate soil and thus can be used in the bioremediation of Cr contaminated soil.

Table.5. Effect of Poultry Manure and Pseudomonas on different species of Cr (mg kg^{-1}) in soil

a). 0th day

Treatments	H_2O-Cr	KNO_3-Cr	NaOH-Cr	EDTA-Cr	HNO_3-Cr
T1- Contaminated soil	18	360	196	171	3457
T2- Soil+ PM (6 t ha^{-1})	16	bdl	158	160	3249
T3- Soil+ PM (12 t ha^{-1})	15	bdl	147	148	3762
T4- Soil+ PM (18 t ha^{-1})	14	bdl	126	140	3282
T5- Soil+ Pseudomonas	13	bdl	158	156	3762
T6- Soil+ Pseudomonas+ PM (6 t ha^{-1})	15	bdl	127	138	3602
T7- Soil+ Pseudomonas+ PM (12 t ha^{-1})	9	bdl	118	133	3662
T8- Soil+ Pseudomonas+ PM (18 t ha^{-1})	9	bdl	112	129	3762

b). 15th day

Treatments	H_2O-Cr	KNO_3-Cr	NaOH-Cr	EDTA-Cr	HNO_3-Cr
T1- Contaminated soil	365	210	196	191	3512
T2- Soil+ PM (6 t ha^{-1})	19	bdl	248	186	3483
T3- Soil+ PM (12 t ha^{-1})	15	bdl	238	155	3822
T4- Soil+ PM (18 t ha^{-1})	10	bdl	229	146	3488
T5- Soil+ Pseudomonas	26	bdl	225	128	3490
T6- Soil+ Pseudomonas+ PM (6 t ha^{-1})	bdl	bdl	219	150	3888
T7- Soil+ Pseudomonas+ PM (12 t ha^{-1})	bdl	bdl	202	147	3738
T8- Soil+ Pseudomonas+ PM (18 t ha^{-1})	bdl	bdl	298	138	3849

c). 30th day

Treatments	H_2O-Cr	KNO_3-Cr	NaOH-Cr	EDTA-Cr	HNO_3-Cr
T1- Contaminated soil	345	201	185	191	3112
T2- Soil+ PM (6 t ha^{-1})	bdl	bdl	235	175	3312
T3- Soil+ PM (12 t ha^{-1})	bdl	bdl	231	148	3642
T4- Soil+ PM (18 t ha^{-1})	bdl	bdl	223	140	3778
T5- Soil+ Pseudomonas	bdl	bdl	220	132	3589
T6- Soil+ Pseudomonas+ PM (6 t ha^{-1})	bdl	bdl	211	130	3798
T7- Soil+ Pseudomonas+ PM (12 t ha^{-1})	bdl	bdl	209	117	3975
T8- Soil+ Pseudomonas+ PM (18 t ha^{-1})	bdl	bdl	268	111	3743

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