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EFFECT OF ORGANIC AMENDMENTS ON GROWTH OF MAIZE AND CHROMIUM TRANSFORMATION IN CONTAMINATED SOIL

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ABSTRACT
Indiscriminate disposal of tannery effluent has led to extensive contamination of soil and water in many parts of Vellore district, where large number of tanneries are located. In the current study, the effect of some organic amendments on the fertility of tannery effluent contaminated soil and bioavailability of chromium and its uptake by maize was examined in order to develop a remediation technology. The potential of selective organic amendments on soil fertility and bioavailability of chromium and its uptake by maize was examined by conducting a pot experiment. Soil samples were collected and analysed for soil parameters such as electrical conductivity, pH, N, P, K, organic carbon, micronutrients and chromium. The values of all growth parameters were higher in poultry manure treatment compared to other organic amendments. The application of organic amendments was found to be effective for reducing the bioavailable fractions of chromium, mainly through the formation of organometallic complexes demonstrating their potential in the bioremediation of chromium contaminated soil.

KEYWORDS: Organic Amendments, Poultry manure, Soil fertility, Chromium contaminated soil, Tannery effluent, Organic manures.

INTRODUCTION
The leather industry is an important foreign exchange earner in India. The leather industry is one of the major sources of pollution in Tamil Nadu. The effluent and sludge disposed from these industries into rivers and onto land has led to extensive degradation of productive land. The tannery wastes consist of high concentration of salts and chromium, both of which

threats surface and subsurface land and water resources. Chromium is considered as an essential nutrient for numerous organisms but at higher level it is toxic and mutagenic. In plants high levels of chromium supply can inhibit seed germination and subsequent seedling growth. Chromium toxicity depends on its oxidation state. Trivalent forms are immobile, more stable and less toxic than the hexavalent forms. Chromium (VI) is highly toxic and it is a carcinogen.[1]

Organic manures bind the sandy soil and improve its water holding capacity. They open the clayey soil, help in aeration and better root growth. They add plant nutrients in small percentage and also add micronutrients which are essential for plant growth. The microbial activity is increased which helps in releasing plant nutrients in available form. Organic manures should be incorporated before sowing or planting because of slow release of nutrients. As the organic amendments contain significantly higher amount of N, P, K and large amounts of these nutrients might have added to the soil and increased the nutrient status.[2,3,4] The ultimate goal of fertilizer application is to maximise the productivity and economic returns. Several studies have shown that the application of organic fertilisers reduces the incidence of soil-borne pathogens.[5.6.7.8] The plant height, stem girth, number of leaves, leaf area index and yield were influenced by the application of organic manures compared to control.[9]

A micronutrient is an element that plants must have to complete their life cycles but need only in small amount. These elements have often been called trace elements or minor elements. Copper is important as a coenzyme that is needed to activate several plant enzymes. It is also involved in chlorophyll formation**.** It is necessary for the formation of chlorophyll and functions in some of the enzymes of the respiratory system. An iron deficiency results in the younger leaves being small and pale green or yellow in colour. The shortage of chlorophyll is called chlorosis. A small amount of manganese is essential but a large amount is toxic to plants. Manganese can exist in several different oxidation states, but most of the manganese in the soil solution is present as Mn^{2+}. Manganese increased plant height, dry weight/ plant, green leaves/ plant, grain yield, straw yield and protein content in grains.[10] Zinc is needed for protein metabolism and appears to be involved in the production of chlorophyll. A characteristic zinc deficiency symptom in citrus and corn is a green midrib and veins in the leaves with white areas between the veins.

The restoration of soil quality through soil organic carbon (SOC) on physical, chemical and biological properties of soil in sustaining their productivity is being realised since the dawn of human civilisation. The soil organic carbon was remarkably improved due to the application of organic amendments. As a source of energy for microbes and electron donors for the reduction of toxic Cr(VI) to Cr(III), it plays a major role in the successful implementation of bioremediation technology. The high amount of organic carbon added through organic amendments also might have stimulated microbial and enzyme activities in soil which may be important for various biochemical processes involving soil fertility.

These organic amendments are also helpful in remediating the heavy metal contaminated soil. Since chromium exists in many forms, its toxicity to plants depends on its valence state, with Cr(VI) found to be highly toxic and mobile than Cr(III). Symptoms of chromium phytoyoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass. There are many studies on chromium toxicity in crop plants. Chromium significantly affects the metabolism of plants such as barley, citrullus, cauliflower, vegetable crops, wheat and maize. The incorporation of organic amendments had remarkable impact on the biotransformation of Cr in the soil. During the decomposition of organic matter, compounds such as citric acid and gallic acid are formed that have been the potential for chelating chromium (III) and reducing chromium (VI) thereby reducing the toxicity of chromium.

Humic substances present in the organic manures play a major role in the reduction of chromium (VI). The amount of Cr(VI) reduced was estimated from the decrease in the concentration of Cr(VI) in soil and the decrease was attributed mainly to the reduction of Cr(VI) to Cr(III) (8). 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 donors . The organic amendments also immobilize chromium mainly by forming organometallic complexes.[11,12,13] Therefore, in the current study, the potential of selective organic amendments on soil fertility and bioavailability of chromium and its uptake by maize was examined by conducting a pot experiment. The transformation of chromium was examined by determining the different fractions of chromium in soil following the sequential fractionation procedure.

MATERIALS AND METHODS

Soil testing is a key weapon in assessing the fertility of the soil. Soil testing results can be effectively used for assessing the plant nutrient requirements. The soil samples were collected at

22 cm depths, dried in shade and sieved through 2 mm sieve. They were stored in polythene bags for laboratory analysis. Effect of organic manures on fertility of tannery effluent contaminated soil was studied. Soil sample was collected at Govindapuram of Ambur. Soil was dried in shade and sieved using 2 mm sieve. 5 kg of soil was taken in 11 inches width pot. Organic composts were mixed at a rate equivalent to 12.5 t/ha. The soil-compost was moistened to about 60%. It was left for a month and moisture content was maintained.

Laboratory Experiment I

Effect of Organic Amendments on Chromium Contaminated Soil and Crop.

T_1 Control

T_2 Control + Press mud

T_3 Control + Poultry manure

T_4 Control + Vermicompost

T_5 Control + Farmyard manure

T_6 Control + Composted coir pith

Ten maize seeds were sown in each pot. Except two plants, other plants were removed. The plants were allowed to grow until cob formation. After 120 days, the plants of each treatment were harvested, separated into roots, shoots, cobs and dried at 40 ^{o}C and weighed. Soil samples were analyzed for soil parameters such as EC, pH, N, P, K, micronutrients copper, zinc, manganese, iron and chromium.

The pH was measured using pH meter. Electrical Conductivity (EC) was measured with the help of a conductivity meter. Nitrogen was estimated by permanganate method using (BCG) Bromocresol Green as indicator. Phosphorus was determined by colourimetry using spectrophotometer. Potassium was determined using flame photometer. Organic carbon content of the soil was estimated by the wet digestion method (Walkley and Black, 1934). One gram of soil sample was weighed in a conical flask and added 15 mL of aqua- regia and digested. The chromium concentration was measured using an AAS with air- acetylene flame at 357.9 nm.

Speciation Studies of Chromium

The soil was mixed with different organic manures. The soil compost mixture was moistened to about 60%. The moisture content was maintained throughout the experiment. After six weeks, a portion of the soil sample was collected and the transformation of chromium was examined by determining the different fractions of chromium in soil following a sequential fractionation procedure. About 1g soil was taken in a centrifuge tube and extracted at room temperature with 25 mL of distilled water with continuous shaking for 2 h. It was centrifuged and filtered through Whatmann- 40 filter paper. The filtrate was analysed for water soluble chromium content using AAS. The soil was extracted with 25 mL of 0.5 M KNO_3 and shaken for 16 h. It was centrifuged and filtered through Whatmann- 40 filter paper. The filtrate was analysed for exchangeable chromium content using AAS.

The soil was extracted with 25 mL of 0.5 M NaOH and shaken for 16 h. It was centrifuged and filtered through Whatmann-40 filter paper. The filtrate was analysed for organic chromium content using AAS. The soil was extracted with 0.05 M Na_2EDTA and shaken for 6 h. It was centrifuged and then filtered through Whatmann-40 filter paper. The filtrate was analysed for iron/ aluminium oxide bound chromium content using AAS. The soil in the centrifuge tube was transferred to a dry conical flask using 1M HNO_3 and digested on a hot plate at 110^{o} C for 2 h. The residue was transferred to 100 mL standard flask using distilled water and shaken well. It was analysed for residual chromium content using AAS.

RESULTS AND DISCUSSION

Effect of Organic Amendments on Maize growth

The values of all growth parameters shoot height, root length, fresh shoot weight, root weight, cob weight and straw yield increased in all the organic amendments treated soil compared to control. However the rate of increase differed among the amendments (Table 1). Except poultry manure treatment, other treatments did not differ significantly (Plate-1). Poultry manure amendment showed the maximum straw yield (97.67 g) and the composted coconut coir pith, the lowest (18.67 g).[14] The composted coir pith was found to be the best amendment in increasing the organic carbon content. The growth was slow but the stems and roots were strong. The percentage of seed germination was found to be high in press mud amendment. The tassel came first for poultry manure and farmyard manure amended soil.

Poultry manure was found to be the best nitrogen additive. Poultry manure has long been recognized as the most desirable organic fertilizer, as it improves soil fertility by adding both major and essential plant nutrients as well as soil organic matter which improves moisture and nutrient retention. The corn came first in poultry manure amended soil. The results are corroborated with the findings of other researchers.[3,15,16,17,18,19,20]

Chromium was found to be more toxic affecting root and shoot length. The reduction in the plant height might be due to the reduced root growth and consequent lesser nutrient and water transport to the above parts of the plant. In addition to this chromium transport to the aerial parts of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction of plant height.[21]

Table 1: Effect of Organic Amendments on Maize Growth.

Treatment	Shoot height (cm)	Root length (cm)	Fresh shoot Weight (g)	Dry shoot Weight (g)	Root weight (g pot^{-1})	Cob weight (g pot^{-1})	Straw yield (g pot^{-1})
T_1	100.3	20.6	29.4	9.17	1.019	0.521	18.33
T_2	102.8	21.75	34.4	11.5	1.635	0.738	23.0
T_3	**145.3**	**28.0**	**74.0**	**48.9**	**5.864**	**20.230**	**97.67**
T_4	103.4	20.84	30.5	15.0	2.139	3.836	30.0
T_5	107.2	21.2	30.7	14.5	2.204	2.042	29.0
T_6	101.5	21.0	30.0	9.4	1.270	2.684	18.67

Effect of Organic Amendments on the Characteristics of Tannery Effluents Contaminated Soil

The pH ranged from 8.7 to 8.22. The highest pH was recorded in the control soil (T_1); whereas, the lowest pH was observed in soil with the application of poultry manure. The reduction in pH was mainly due to organic acids produced during the decomposition of poultry manure[2] and also due to protons generated during the process of nitrification. The organic amendments are usually rich in nitrogen, part of which was in ammoniacal form. Oxidation of NH_4^{+} - N to NO_3^{-}- N resulted in the release of protons. This may be one of the reasons for the decrease in soil pH with the incorporation of organic amendments. The reduction of Cr (VI) to Cr (III), being a proton consumption (or hydroxyl release) reaction, was found to increase with a decrease in soil pH.[22,23] The increase in pH could be attributed to the production of NH_4^{+} and addition of base materials from poultry manure and composted coir pith. Initially, the Soil Organic Carbon (SOC) ranged from 5.2 to 7.24%. The soil with the application of composted coir pith (T_6) had the highest amount of SOC. Irrespective of treatments, the SOC was found decreased gradually during the incubation. At the end of 120 days of incubation, after harvesting the SOC ranged between 3.28 and 4.71% (Table 2). The contaminated soils had EC values between 0.21 d Sm^{-1} to 0.37 d Sm^{-1}.

Table 2: Effect of Organic Amendments on Soil Characteristics when Maize Crop Grown in Tannery Effluents Contaminated Soil.

Treatment	Before Sowing						After Harvesting					
	EC (dSm^{-1})	pH	N ($kgha^{-1}$)	P ($kgha^{1}$)	K ($kg\ ha^{1}$)	SOC (%)	EC (dSm^{-1})	pH	N ($kgha^{-1}$)	P ($kgha^{-1}$)	K ($kgha^{-1}$)	SOC (%)
T_1	0.32	8.70	220	3.4	368	5.2	0.23	8.5	182	2.41	274	3.28
T_2	0.36	8.40	295	11.1	416	6.92	0.33	8.4	138	8.4	273	4.71
T_3	0.37	8.22	282	18.4	435	6.06	0.30	8.31	114	12.5	294	4.11
T_4	0.21	8.43	298	18.2	466	6.91	0.30	8.34	110	10.5	242	4.04
T_5	0.27	8.50	302	11.9	489	7.06	0.31	8.15	160	4.9	262	3.56
T_6	0.29	8.23	265	19.8	392	7.24	0.30	8.3	140	14.5	210	4.13

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Table 3: Effect of Organic Amendments on Micronutrient Content of Soils.

Treatment	Before Sowing				After Harvesting			
	Cu ($mgkg^{1}$)	Mn ($mgkg^{-1}$)	Zn ($mgkg^{-1}$)	Fe ($mgkg^{-1}$)	Cu ($mgkg^{-1}$)	Mn ($mgkg^{-1}$)	Zn ($mgkg^{-1}$)	Fe ($mgkg^{-1}$)
T_1	2.5	7.4	0.45	2.4	1.03	7.02	0.39	1.03
T_2	2.89	14.21	1.11	2.73	1.59	12.65	0.98	1.63
T_3	3.91	11.62	3.04	2.48	1.56	11.39	0.5	1.22
T_4	2.92	16.46	0.72	2.59	2.03	13.7	0.68	1.41
T_5	2.81	14.79	0.46	2.56	1.74	13.5	0.44	1.36
T_6	2.69	14.46	0.87	2.5	1.99	13.18	0.64	1.39

Effect of Organic Amendments on Micronutrients Content of Soil

The organic amendments treated soil samples were analysed for the micronutrients Copper (Cu), Manganese (Mn), Zinc (Zn) and iron (Fe) before sowing and after maize harvesting. It was found that the concentration of micronutrients Copper, Manganese, Zinc and iron present in the soil increased compared to the control irrespective of the organic amendments (Table 3). After harvesting the amount of micronutrients present decreased. It indicated that the micronutrients also contributed for the growth of maize. The results are corroborated with the findings of other researchers.[10]

Speciation of Chromium

Depending upon the chemical valence state of chromium and soil environmental conditions, the added chromium 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 chromium (H_2O-Cr), exchangeable and adsorbed chromium (KNO_3-Cr), organic chromium (NaOH-Cr), iron and aluminium oxide bound – chromium (EDTA-Cr) and the residual chromium or acid soluble – chromium (HNO_3-Cr) during the incubation are presented in Table 4. In the control soil, initially, the relative distribution of chromium species followed:

HNO_3-Cr >> NaOH-Cr > EDTA-Cr > KNO_3-Cr > H_2O-Cr

Only a small amount of (5.6 µgkg-1) water soluble chromium was present initially, and it was markedly increased (12 µg kg-1). There was a significant reduction in the concentration of exchangeable and adsorbed – chromium (KNO_3-Cr) ie. 24.5 to 12.7 µg g^{-1}.

Only a small increase in the concentration of organic- chromium and iron and aluminium oxide bound- chromium was observed (220 to 306 µg g^{-1} and 126 to 180 µg g^{-1}). The acid soluble fraction (HNO_3-Cr) was found decreased (768 to 620 µg g^{-1}). The result showed that the added Cr(III) was oxidised to Cr(VI) which is soluble in water and increased the soluble chromium in soil. The reduction in exchangeable and adsorbed chromium and the increase in Cr(VI) also provided evidence for the oxidation of Cr(III).

When organic amendments were added, the water soluble chromium decreased irrespective of the amendments (Table 4). The rate of decrease in the concentration of water soluble-chromium was more in farmyard manure and vermicompost treated soil.

The concentration of organically bound- chromium (NaOH-Cr) and Fe/Al oxide bound chromium (Na_2EDTA-Cr) was found significantly increased, indicating large amount of chromium could have adsorbed and also converted into organic form. The rate of increase in organic bound- chromium (NaOH-Cr) was more in vermicompost treated soil. The greater concentration of NaOH-Cr and Na_2EDTA-Cr provided evidence for the occurrence of complexation reaction resulting in the formation of large amount of organochromic complexes and chelates in the soil due to addition of organic amendments.[19]

Table 4: Effect of Organic Amendments on Different Species of Chromium in Soil.

Treatment	Water-Cr (µg g^{-1})		KNO_3-Cr (µg g^{-1})		NaOH-Cr (µg g^{-1})		Na_2EDTA-Cr (µg g^{-1})		HNO_3-Cr (µg g^{-1})	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	5.6	12	24.5	12.7	220	306	126	180	768	620
T_2	4.8	2.1	18.2	6.4	180	362	112	186	727	527
T_3	4.0	1.8	16.8	7.3	176	380	108	170	716	498
T_4	4.8	1.0	15.3	6.8	188	406	98	165	732	460
T_5	4.8	0.9	19.5	8.2	170	222	117	212	740	430
T_6	4.6	1.4	16.2	8.2	188	260	125	230	732	422

Growth of Maize and Chromium Uptake

The results of the pot experiment showed that the maize growth and chromium uptake was significantly affected due to application of organic amendments. The plants grown in soil without any amendment had lesser biomass. Application of organic amendments resulted in a significant greater biomass of maize. Addition of organic amendments reduced the bioavailability of chromium. The reduction in the bioavailability of chromium would also be due to either formation of organochromic complexes or reduction of toxic, soluble chromium (VI) to non- toxic, less soluble chromium (III) and subsequent precipitation as chromic hydroxide in soil.[24,25] Significant variation in chromium content and uptake by maize was observed. The plants grown on the control soil had higher concentration of chromium. The biomass yield was found decreased with increasing concentration of chromium in plants as well as chromium uptake (Table 5). There exists a negative correlation between the biomass yield and water soluble fraction of chromium in soil.

Chromium Content and Chromium Uptake by Crops

In general, 60% reduction of chromium was recorded due to the application of organic amendments. Relatively large amount of chromium was found accumulated in roots, than in shoot and cob of maize. The distribution of chromium in different parts of maize is depicted in Table 5. Large accumulation of chromium in the roots of maize could be due to the fact that they were affected earlier and subjected to accumulation of more chromium than any of the other organs.[26,27] Root was found to be affected than shoot. This is due to the fact that heavy metals accumulated on root due to binding of metals on the cell wall of root and retard cell division and cell elongation. The maize grown on control soil has shown greater accumulation of chromium (416 $\mu g\ g^{-1}$). Whereas, maize when grown on soil amended with organic amendments appeared to have significantly lesser amount of chromium in roots, leaves and shoot. The addition of organic amendments resulted in low concentration of chromium in grains and seeds.

Table 5: Effect of Organic Amendments on Chromium Content and Chromium Uptake.

Treatment	Total Chromium content			Chromium uptake		
	Root ($\mu g\ g^{-1}$)	Shoot ($\mu g\ g^{-1}$)	Cob ($\mu g\ g^{-1}$)	Root ($\mu g\ pot^{-1}$)	Shoot ($\mu g\ pot^{-1}$)	Cob ($\mu g\ pot^{-1}$)
T_1	416	116	42	424.00	2126	21.882
T_2	340	89	23	555.9	2047	16.974
T_3	162	46	12	949.91	4493	242.76
T_4	320	78	28	684.48	2340	107.408
T_5	340	60	22	749.36	1740	44.924
T_6	280	76	20	355.6	1419	53.68

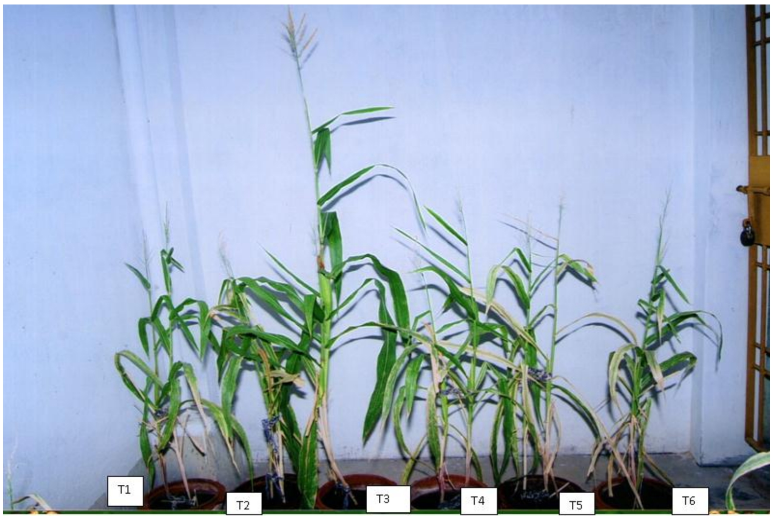

Plate 1: Effect of Different Organic Amendments on Maize growth.

Plate 2: Effect of Organic Amendments on Root Length.

Plate 3: Plants Collected After Maize Harvesting.

CONCLUSIONS

Among the organic amendments, poultry manure was found to be the best in increasing the available nutrients of N, P and K. The difference in the efficiency of poultry manure and other organic amendments 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 chromium in soil, leading to increased nutrient uptake. From the chromium study, it was found that the organic amendments can be used for the bio-remediation of chromium contaminated soils and the poultry manure was the best amendment in the treatment of chromium contaminated soil. The high amount of organic carbon added through organic amendments also might have stimulated microbial and enzyme activities in soil which may be important for various biochemical processes involving soil fertility. The application of organic amendments was found to be effective for reducing the bioavailable fractions of chromium, mainly through the formation of organic complexes demonstrating their potential in the biooremediation of chromium contaminated soil.

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