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Effect of Addition of Farmyard Manure and Lime on Chemical Fractionation of Chromium in Soils and its Bioavailability to Maize

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Glasshouse experiment was carried out by growing maize crop in a tubewell water irrigated soil after spiking with different levels of chromium (Cr) in the presence and absence of amendments. The experiment was laid out in a completely randomized factorial design with three replications. Maize crop was harvested at 45 days of growth. The soil samples after harvest of maize were collected. To study the change in bioavailability of Cr to maize in the presence of amendments, soil samples collected after harvest were analyzed for transformations in Cr associated with different soil fractions namely, exchangeable + water soluble, carbonate bound, easily reducible oxide bound, organic matter bound, reducible oxides and residual fractions. The results revealed that with the increase in level of applied Cr, the content of Cr in maize shoot increased from 1.85 to 28.2 mg kg^{-1}. The application of farmyard manure (FYM) decreased the Cr content by 2.6 per cent in maize shoot at 320 mg kg^{-1} of applied Cr in comparison to uncontaminated treatment. Application of lime also decreased the shoot Cr content by 11.5 per cent up to 160 mg kg^{-1} of applied Cr. It was observed that increase in levels of Cr increased the Cr content in each soil fraction with higher increase in more labile fraction and further transformation of Cr to relatively lesser available fraction. Addition of amendments significantly decreased the concentration of exchangeable + water soluble Cr, carbonate bound, easily reducible oxide bound fractions but increased the organic matter bound, reducible oxide bound and residual fraction of chromium.

Key words: Chromium, bioavailability, maize, soil fractions, transformation

Chromium (Cr) occurs naturally in the earth's crust and soils. Chromium concentration ranges from 10-150 mg Cr kg^{-1} of soil (Mcgrath 1995). Anthropogenic Cr sources such as ore refining, production of steel and alloys, pigment manufacture, leather, tanning, wood preservation, combustion of coal and oil are polluting the soils with Cr (Adriano 2001). In soils, the most stable forms of Cr are Cr(III) and Cr(VI) (Kabata-Pendias *et al.* 1992), among which latter is more mobile and toxic. Once Cr entered the soil, it interacts with various organic and inorganic constituents undergoing transformation into various forms like soluble + exchangeable, carbonate bound, easily reducible oxide bound and organic matter bound. The magnitude of these forms is not only controlled by pH, organic matter, cation exchange capacity (CEC), calcium carbonate content of the soil through dissolution, precipitation and chelation reactions but also on the rates of metal loading from inorganic or organic sources, climate and crop species. Knowledge of how contaminants are partitioned among various chemical forms gives a better idea about the mechanisms of their retention and release (Cabral and Lefebvre 1998) and hence their solubility, mobility and bioavailability in the contaminated soil (Wasay *et al.* 2001). It is, therefore, important to understand the chemical forms of heavy metals in soils, as multistep sequential extraction scheme provide a more complete picture for predicting the heavy metal distribution, mobility and bioavailability in sewage irrigated soils. The bioavailability of Cr depends upon number of factors such as pH, organic matter, clay minerals and oxides of Fe and Al. Application of amendments such as organic manures and lime has been reported to enhance their immobilization of metals and thus reducing their bioavailability in contaminated soil. Farmyard manure (FYM) is a cost effective option as it acts as pollutant ameliorant and thus expected to mitigate the toxic

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effect of Cr. Liming of contaminated soils is also a remediation treatment for reducing the mobilization and bioavailability of heavy metals (Hong *et al*. 2010; Kalsi *et al*. 2016). Keeping these two amendments, in terms of remediating the toxic effect of Cr in mind, the study was planned to investigate the effect of organic manure and lime on the transformation of Cr in soils and hence to determine its bioavailability to maize grown on tubewell irrigated soil.

Materials and Methods

A glasshouse experiment was conducted on tubewell irrigated soil. The physicochemical characteristics of the experimental soil and nutrient composition of FYM used are given in tables 1 and 2. The pots were filled with 6 kg dried soil. The application of Cr consisted of 0, 40, 80, 160 and 320 mg Cr kg^{-1} soil and was added in solution form using potassium dichromate. The amendments (FYM and lime) were added @1% each *i.e.* 60 g on dry weight basis to each pot. Basal dose of nitrogen (N) was applied through urea. Phosphorus (P) and potassium (K) were applied through potassium dihydrogen orthophosphate and potassium chloride, respectively. The soil in the pots was subjected to alternative wetting and drying for three weeks to maintain equilibrium. Twelve seeds of maize (*Zea mays*) Var PMH 1 were sown in pots under optimum moisture conditions. Thinning was done after germination to maintain six plants per pot. The crop was irrigated regularly with water and it was harvested after 45 days of growth. The plant samples were weighed and ground in a willey grinding mill and were digested in di-acid mixture of HNO_3 and $HClO_4$ in the ratio of 3: 1 and the digests were analyzed for Cr content using ICAP-AES (Jones 1977). The representative soil samples from each pot were taken with the help of steel tube augar. The soil samples were air-dried, ground, sieved and stored in polythene bags for chemical analysis. A six step sequential extraction procedure was followed to evaluate the different forms of Cr such as EX + WS (exchangeable + water soluble), CARB (carbonate bound), ERO (easily reducible oxide bound), OM (organic matter bound), RO (reducible oxides) and RES (residual fraction). Sequential extraction scheme used by Han and Banin (1997) was followed to fractionate Cr (Table 3).

Table 1. Physicochemical properties of the soil used for experiment

Properties	Tubewell water irrigated soil (TWI)
pH	8.03
EC (dS m^{-1})	0.16
OC (%)	0.52
$CaCO_3$ (%)	3.68
Sand (%)	76.2
Silt (%)	14.0
Clay (%)	9.8
Texture	Sandy loam
CEC [cmol(p^+)kg^{-1}]	8.31
Available N (kg ha^{-1})	198.6
Available P (kg ha^{-1})	27.8
Available K (kg ha^{-1})	174.0
DTPA-Fe (mg kg^{-1})	9.55
DTPA-Mn (mg kg^{-1})	14.4
DTPA-Zn (mg kg^{-1})	2.89
DTPA-Pb (mg kg^{-1})	1.22
DTPA-Ni (mg kg^{-1})	0.30
DTPA-Cr (mg kg^{-1})	0.02
DTPA-Cd (mg kg^{-1})	0.05
Total Cr (mg kg^{-1})	50.1

Table 2. Elemental composition of farm yard manure used for study

Element	Concentration
Cd content (mg kg^{-1})	1.23
Cr content (mg kg^{-1})	15.4
Fe content (%)	0.49
Mg content (%)	0.48
Mn content (mg kg^{-1})	662
Ni content (mg kg^{-1})	6.83
P content (%)	0.53
Pb content (mg kg^{-1})	12.3
Zn content (mg kg^{-1})	186

The steps used for fractionation are as follows.

1) *Water soluble + exchangeable fraction:* Twenty five mL of 1 *M* NH_4NO_3 was added to 1 g of dry soil in a 50 mL Teflon tube and the mixture was shaken for 30 min at 25 °C. The mixture was then centrifuged at 12,000 rpm for 10 min (Table 3).
2) *Carbonate bound fraction*: Twenty five mL of 1 *M* NaOAC-HOAC was added to the soil residue from the previous step and the mixture was shaken for 6 h. Excess CO_2 was released by opening the tube cap during the first two h.
3) *Easily reducible oxide-bound fraction*: Twenty five mL of 0.04 *M* $NH_2OH.HCl$ in 25% HOAc solution was added to the soil residue and shaken for 30 min (Han and Banin 1996, 1997).
4) *Organic matter-bound fraction*: Three mL of a 0.01 *M* HNO_3 and 5 mL of 30% H_2O_2 were added to the soil residue. The mixture was digested on a water-bath at 80 °C for 2 h. An additional 2 mL of H_2O_2 was added, and the mixture was heated for 1 h. Fifteen mL of a 1 *M* NH_4NO_3 solution

Table 3. Sequential extraction method used for Cr fractions

Fraction	Solution mixture	Soil (g)	Solution quantity (mL)	Condition
Water soluble + exchangeable fraction	1 *M* NH_4NO_3 (pH 7.0 with NH_4OH	1	25	Shake for 30 min at 25 °C
Carbonate bound fraction	of 1*M* NaOAC-HOAC at pH 5	1	25	Shake for 3 h at 25 °C
Easily reducible oxide bound fraction	0.04*M* hydroxyl amine hydrochloride in 25% HOAc	1	25	Shake for 30 min
Organic matter-bound fraction	0.01*M* HNO_3 and 30%H_2O_2.H_2O_2 1*M* NH_4NO_3	1	3 mL HNO_3 + 5 mL H_2O_2 2 mL H_2O_2 15 mL NH_4NO_3	Digestion at 80 °C for 2 h Digested for 1 h Heated for 1 h Shaken for 15 min, centrifuged and decanted
Reducible oxide-bound fraction	0.04 *M* NH_2OH.HCl in 25% HOAc	1	25	Digested for 3 h at 90 °C in water bath
Residual fraction	Aqua Regia (3:1) HCl: HNO_3)	1	25	Digested for 30 min

was then added and the sample was shaken for 10 mine (Tessier *et al.* 1979; Han and Banin 1996, 1997).

5) *Reducible oxide-bound fraction*: Twenty five mL of 0.04 M NH_2OH.HCl in 25% HOAc solution was added to soil residue and the sample digested in a water bath at 90 °C for 3 h (Gupta and Chen 1975; Banin *et al.* 1990).
6) *Residual fraction and total metal concentration:* Twenty five mL of aqua regia was added to the soil residues (Sposito *et al.* 1982), and the sample was transferred to a Teflon microwave digestion vessel. Digestion was conducted at 100% of power (1600 watts) for 30 min. The temperature of the mixture reaches 140 to 160 °C (Han and Banin 1996, 1997).

It is pertinent to mention here that essential conditions like boiling, digestion and shaking were fulfilled during sequential extraction procedure. The amount of Cr present in different soil fractions was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The detection limits for Cr on ICP-AES was 7 µg L^{-1}. Calibration was done before measurement of samples using a series of standard Cr solutions. The data generated were analyzed using CPCS 1 statistical program by Cheema and Singh (1991).

Organic carbon (OC), pH, electrical conductivity (EC), calcium carbonate, available N, P, K and total contents of Cr in soil were determined by standard methods (Page *et al*. 1982). Soil texture was determined by International Pippet' method (Piper 1966). DTPA extractable Cr was determined by employing the method of Lindsay and Norvell (1978).

Result and Discussion

Significant reduction in dry matter yield (DMY) of maize was observed with increasing the rates of Cr in soil both in the presence and absence of amendments (Table 4). The effect of added Cr was more pronounced at highest rate of its application. Mean dry matter yield of maize (at 45 days of growth) decreased significantly with the application of Cr and recorded 16.7, 14.5, 10.9, 0.11 and 0.06 g pot^{-1} with the application of Cr levels of 0, 40, 80, 160 and 320 mg Cr kg^{-1} soil, respectively (Table 4). Addition of both amendments significantly reduced the toxic effect of Cr on dry matter yield of maize shoots at each level of applied Cr. The application of 40 mg Cr kg^{-1} soil, decreased the maize yield by 13.45 per cent in comparison to uncontaminated soil. Addition of FYM, reduced the yield decline to 7 per cent. This could be due to increased organically bound, reducible oxide bound and residual Cr fractions. Furthermore, it decreased the exchangeable + water soluble and carbonate bound Cr probably due to increase in SOC, EC and available P in soil treated with FYM. Rangasamy *et al*. (2013) found that biomass of maize was significantly lower when grown on uncontaminated soil but when the soil was amended with vermicompost with or without microbial strains and earthworm, recorded higher biomass. Maize plants were healthier in all the FYM treated pots throughout the growing season. The application of lime also significantly increased the mean dry matter yield from 8.44 g pot^{-1} in control to 9.29 g pot^{-1}, indicating an increase of 10.1 per cent.

Mean Cr concentration in maize shoots increased significantly from 5.50 to 29.0 mg kg^{-1}, with the

Table 4. Effect of chromium and amendments (FYM and lime) on dry matter yield, chromium content and chromium uptake of maize

Amendments	Rates of chromium application (mg kg^{-1} soil)					
	0	40	80	160	320	Mean
Dry matter yield (g pot^{-1})						
Control	16.7	14.4	10.8	0.11	0.06	8.44
FYM	17.4	16.1	12.6	3.21	0.13	9.92
Lime	17.1	15.8	11.1	2.37	0.03	9.29
Mean	17.5	15.4	12.5	1.90	0.08	
CD (P=0.05)	Soil = 0.22, Amendments = 0.27, Cr = 0.35, Soil × Amendments = 0.39, Amendments × Cr = 0.61, Soil × Amendments × Cr = NS					
Chromium concentration (mg kg^{-1})						
Control	5.50	11.5	18.7	23.2	29.0	17.6
FYM	1.85	7.57	8.16	17.5	28.2	12.6
Lime	2.36	8.14	15.5	20.6	29.8	15.3
Mean	3.24	9.07	14.1	20.4	29.0	
CD (P=0.05)	Soil = 0.21, Amendments = 0.25, Cr levels = 0.33, Soil × Amendments = 0.36, Soil × Cr = 0.46, Amendment × Cr = 0.57, Soil × Amendments × Cr = 0.81					
Chromium uptake (µg pot^{-1})						
Control	91.9	166.5	203.7	2.6	1.85	93.3
FYM	32.1	121.9	103.4	56.2	3.76	63.5
Lime	40.4	129.0	172.2	48.9	0.99	78.3
Mean	54.8	139.1	159.8	35.9	2.20	
CD (P=0.05)	Soil = 3.86, Amendments = 4.73, Cr levels = 6.11, Soil × Amendments = NS, Soil × Cr = 8.63, Amendment × Cr = 10.57, Soil × Amendments × Cr = 14.96					

application of 0, 40, 80, 160 and 320 mg Cr kg^{-1} levels (Table 4). This was due to significant increase in exchangeable + water soluble Cr fraction which results an increased amount of metal being absorbed by the plants. The concentration of Cr in maize shoots grown in FYM treated soil decreased from 17.6 mg kg^{-1} in uncontaminated soil to 12.6 mg kg^{-1}. In lime treated pots, it decreased from 17.6 mg kg^{-1} in control to 15.3 mg kg^{-1}. Application of FYM increased the dry matter yield, hence reducing the Cr concentration in maize plants. Moreover, reaction of organic matter with Cr provide fixation sink due to chelation reactions leaving lesser Cr immediately available for plant. Gheju *et al.* (2009) found that *Zea mays* roots have the greatest tendency to concentrate Cr(VI), the concentration in these plant parts being 11.7 times greater than in the surrounding soil.

Mean Cr uptake also increased significantly with increasing levels of applied Cr to soil irrespective of FYM levels. The mean Cr uptake increased from 91.9 µg pot^{-1} in control treatment to 203.7 µg pot^{-1}, when 80 mg Cr kg^{-1} soil was applied (Table 4). Raising the Cr levels beyond 80 mg Cr kg^{-1} did not increase Cr uptake by maize to an extent similar to that observed with 80 mg Cr kg^{-1}. This kind of Cr uptake pattern was consequence of both reduction in yield due to Cr toxicity and increased concentration of Cr at higher application rate as a reduction in yield was compensated by higher Cr absorption. Rangasamy *et al*. (2014) observed that the addition of poultry manure or vermicompost with or without microbial strains significantly (60%) reduced the Cr content and uptake by maize due to manure-induced Cr immobilization in soil.

Exchangeable and water soluble fraction increased significantly with each increasing levels of Cr (Table 5). It increased from 0.24 to 79.7 mg kg^{-1} when Cr application was increased from 0 to 320 mg kg^{-1}. Since coarse textured soil offered little resistance, due to low CEC, thereby the extractability of this fraction was high in these soils. Application of FYM decreased the amount of the above fraction at each level of applied Cr. Interaction of Cr levels and amendments were found to be significant (Table 5). Rendina *et al*. (2011) found that the concentration of water soluble and exchangeable Cr decreased with the addition of organic amendments and Cr concentration increased in the organic fraction. This indicated that a decrease in phytotoxicity which was attributed to redistribution of Cr from the water soluble and exchangeable fractions to the organic fraction, which decreased the plant availability and uptake of Cr. Chromium associated with carbonate bound fraction increased from 1.81 to 29.9 mg kg^{-1}

Table 5. Effect of chromium and amendments (FYM and lime) on various fractions (mg kg^{-1} soil)

Amendment	Levels of applied Cr (mg kg^{-1})					
	0	40	80	160	320	Mean
	Exchangeable + water soluble chromium					
No amendment	0.30	5.62	11.3	30.4	85.1	26.5
FYM @1%	0.24	3.76	8.34	26.2	59.1	19.5
Lime @1%	0.19	5.75	12.3	33.5	94.8	29.3
Mean	0.24	5.04	10.6	30.1	79.7	
CD (P=0.05)	Amendments = 0.11; Cr = 0.14; Cr × Amendments = 0.24					
	Carbonate bound Cr					
No amendment	1.81	6.23	9.74	15.2	29.8	12.5
FYM @1%	1.72	4.80	9.59	14.6	28.9	11.9
Lime @1%	1.73	7.36	9.96	19.0	33.2	14.2
Mean	1.75	6.13	9.76	16.3	30.7	
CD (P=0.05)	Amendments = 0.62; Cr = 0.81; Cr × Amendments = 1.39					
	Easily reducible oxide bound fraction					
No amendment	1.31	6.59	12.1	23.2	40.2	16.7
FYM @1%	1.13	5.77	10.5	19.8	23.4	12.1
Lime @1%	1.77	5.83	11.5	18.7	25.1	12.6
Mean	1.40	6.06	11.4	20.6	29.6	
CD (P=0.05)	Amendments = 0.59; Cr = 0.76; Cr × Amendments = 1.32					
	Organic matter bound					
No amendment	3.90	21.6	32.6	68.0	115	48.3
FYM @1%	4.37	22.8	37.1	82.4	152	59.9
Lime @1%	3.93	22.6	33.9	69.3	118	49.6
Mean	4.07	22.3	34.5	73.2	128	
CD (P=0.05)	Amendments = 0.84; Cr = 1.08; Cr × Amendments = 1.88					
	Reducible oxide bound fraction					
No amendment	10.0	11.8	12.5	14.8	24.3	14.7
FYM @1%	11.3	12.7	14.2	17.8	24.4	16.1
Lime @1%	8.49	8.3	10.0	12.1	20.6	10.9
Mean	8.98	10.2	12.3	14.9	23.1	
CD (P=0.05)	Amendments = 0.45; Cr = 0.58; Cr × Amendments = 1.01					
	Residual fraction					
No amendment	28.2	28.3	35.6	35.9	50.0	35.6
FYM @1%	27.3	28.3	35.9	35.5	51.2	35.6
Lime @1%	27.7	28.4	35.9	36.3	53.3	36.3
Mean	27.7	28.3	35.8	35.9	51.5	
CD (P=0.05)	Amendments = NS; Cr = 1.26; Cr × Amendments = NS					

soil, with the increase in Cr application from 0 to 320 mg kg^{-1} soil. Application of FYM, irrespective of Cr levels decreased significantly the amount of Cr in this fraction from 12.6 mg kg^{-1} to 11.9 mg kg^{-1} soil . The results indicated the usefulness of organic matter in retarding the release of Cr from carbonate bound pool which is considered to be slowly available to plants. Saffari *et al.* (2014) reported that municipal solid waste compost and rice husk biochar significantly decreased carbonate bound-Cr fraction. The carbonate bound fraction characterize the most labile and bioavailable form, hence, decreasing this fraction in treated soil showed that these amendments are able to increase Cr stabilization. Mean Cr associated with easily oxide bound fraction increased significantly from 1.40 to 29.6 mg kg^{-1} soil when Cr was applied at 0, 40, 80, 160 and 320 mg kg^{-1} soil. Application of FYM and lime significantly decreased its contents from 16.7 to 12.1 mg kg^{-1} and 16.7 to 12.6 mg kg^{-1} soil, respectively. Kalembasa *et al.* (2009) reported that the percentage of Cr in the fractions bound to manganese oxides and hydroxides (F3), amorphous (F5) and crystalline (F6) iron oxides and hydroxides varied between within the analyzed profiles. Higher Cr amounts were found in the form occluded on crystalline iron oxides (14.9-17.6% on average for profiles) than on amorphous ones (8.67-2.80%). Amount of mean Cr associated with organic matter bound fraction, residual fraction increased significantly from 4.07 to 128.9 mg kg^{-1} soil. Addition of FYM and lime further increased their content from 48.3 to 59.9 mg kg^{-1} and 48.3 to 49.6 mg kg^{-1}, respectively. Amount of mean Cr associated with reducible oxide bound increased significantly from 8.98 to 23.1 mg kg^{-1} soil. Application of FYM further increase their content to 16.1 from 14.7 mg kg^{-1} and Amount of mean Cr associated with residual fraction increased significantly from 27.8 to 51.5 mg kg^{-1} soil with the application of respective levels of Cr. Application of amendment like FYM and lime further increase their contents. Osakwe (2012) found that Cr with organic fraction has been observed in sandy soils where Cr had a strong affinity for organic matter. It has been suggested that the existence of Cr in the organic bound fraction results from the existing physicochemical conditions. Erzen and Stupar (2003) found that among different soils (clay, peat, sand and luvichromic cambisols), the concentration of Cr in Fe- and Mn-oxides and hydroxides fractions were higher in comparison to sparingly soluble fractions of soil. Han *et al.* (2004) observed that residual fraction was constant in both Cr(III) and Cr(VI) contaminated soil, therefore its relative percentage decreased with Cr loading levels.

Results emanating from present investigation suggest that greater amount of Cr was associated with residual fraction followed by reducible oxide and organic matter fractions with by far the lowest concentration of Cr in exchangeable fraction. Addition of different concentration of Cr application increased the Cr content in each fraction, with higher increase in more labile fraction and further transformation of Cr into relatively less mobile fractions. Fractionation result (Table 5) revealed that all the fractions exhibited an increase with Cr rates but addition of FYM increased organically bound Cr, reducible oxide bound fractions and residual fractions but decreased the exchangeable + water soluble and carbonate bound fraction. The addition of lime also decreased the exchangeable + water soluble Cr and reducible oxide bound fractions and while all other fractions increased. So, both the amendments were equally effective in reducing the highly mobile fractions of Cr but FYM was effective in reducing the other fractions and toxic effect of Cr in maize shoots even at 320 mg kg^{-1}. So, out of both the amendments, FYM was considered as the best amendment

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