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Mobility and Bioavailability of Chromium in the Environment: Physico-Chemical and Microbial Oxidation of Cr (III) to Cr (VI)

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ABSTRACT: The physico-chemical and microbial characteristics in soil was investigated in order to study the mobility and bioavailability of Chromium in the environment. In the present investigation the role of soil microbes along with some physico-chemical agents (UV ray, Mn and Fe) were studied for possible oxidation of Cr(III) to Cr(VI) in laboratory conditions. Photochemical oxidation of Cr(III) to Cr(VI) was found to be significantly high at the surface level of the soil studied (conversion of Cr^{6+} in control soil was calculated as 0.15 mg kg^{-1} dry weight). Metal catalyst also contributed to oxidation of chromium in soil. Conversion of Cr^{6+} by metal catalysis ranged between 0.00 and 0.08 mg kg^{-1} dry weight (mean 0.05 mg kg^{-1} dry weight). However, metal catalysts such as, Fe and Mn together or these metals alone were not as efficient as the microbes or the UV mediated conversion during the given period of exposure (two weeks). The data indicates that microbial conversion of Cr(III) to Cr(VI) varied from 0.12 – 0.18 mg kg^{-1} dry weight, while mean was 0.14 mg kg^{-1} dry weight. Since, at the plant root zone, where the UV penetration is negligible, metal catalysts could be inferred to be not much efficient in conversion of Cr(III) to Cr(VI) as compared to microbes. Therefore, the present findings suggested that microbial mediated conversion is the preferred pathway and consequently responsible for root uptake of chromium by plants. @ JASEM

Heavy metals, which are recognised as toxic, placed in the second rank, next to pesticides in environmental importance. Cr, a much debated metal with significant toxic effects has multifarious industrial uses (Nriagu, 1988). Leather industry has been held responsible for the major influx of Cr to the biosphere, accounting for 40% of the total industrial use (Barnhart, 1997). Out of the different variable valance states of Chromium, Cr(VI) and Cr(III) are most stable; Cr(VI) owing to filled and Cr(III) due to half filled orbital stability. Cr(VI) is extremely labile in the biological system and it can easily pass through cell membranes, often via sulphate transport system (Costa, 2003). Hexavalent Cr is the predominant species involved in mutagenicity, carcinogenicity, and teratogenicity and is reported to be 100-fold more toxic than the trivalent form (Petrilli and DeFlora, 1977). On the other hand, Cr(III) is generally impermeable through cell membrane, hence considered to be less toxic (Kumaresan and Riyazuddin, 1999; Murray *et al*., 2005). Differential accumulation pattern for Cr(III) and Cr(VI) in plants are on record. Zayed *et al*. (1998), reported that Cr accumulation were higher when plants were grown with Cr(VI) than with Cr(III). The present investigation was carried out to find out the possible mechanism available at the plant root zone by which impermeable trivalent Cr is converted in the permeable hexavalent form in order to be transported into the plant parts.

MATERIALS AND METHODS

Native agro-soil was collected from Bandipur, West Bengal (about 25 km N of East Calcutta Wetlands, a Ramsar Site) that served as culture medium and solid support in the present investigation. For physico-chemical parameter analysis 10.0 ± 0.005 g. of native soil was diluted in 100 ml of distilled water in a stoppered conical flask and shaken in a mechanical shaker for 1.5 h. The suspension was allowed to settle and the supernatant was decanted. To it was added 3.0 g. of activated charcoal (E. Merck India) swirled and filtered using Whatman no. 40 filter paper. pH, Conductivity, Total Dissolved Solids (TDS), and Dissolved O_2 (DO) were measured potentiometrically using Mettler Checkmate 90 Toledo. NO_3^-, PO_4^{3-}, SiO_2, Cl^-, total hardness, CO_3 hardness, Alkalinity and Acidity were analysed calorimetrically and titrimetrically using E. Merck, Germany, Field Testing Aquamerck reagent kits. Total Suspended Solids (TSS) was analysed gravimetrically.

Total Cr was detected by Atomic Absorption Spectrophotometer (Perkin-Elmer AAnalyst-100 with interfacing AAWinlab Software), using element-specific hollow cathode lamps in default condition, by flame absorption mode following the method described by Chatterjee *et al*. (2007). Hexavalent Cr determination was made by the 1,5-diphenylcarbazide method (Bassett *et al*., 1978) by employing a UV-VIS Spectrophotometer (Perkin Elmer Lambda 25 with interfacing UV WinLab software) having reproducibility ±0.002 A at 1A.

Twelve Borosil petridishes were marked as A_m, B_m, C_m, D_m, E_m, F_m and A_c, B_c, C_c, D_c, E_c, F_c, around 10 ± 0.86g agro soil was added in them. The former set was conserved for microbial seeding, while the latter was taken for chemical dosing. The petridishes containing known amount of soil marked, A_m, B_m, C_m, D_m, E_m and F_m were kept inside an incubator at 37°C while the A_C, B_C, C_C, D_C, E_C and F_C marked

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petridishes were sterilised in an autoclave at 20 psi pressure, at 120°C for 45 minutes carefully in order to kill even the microbial spores. They ware sealed and taken to a laminar flow to prevent further contamination of microbes. Basic chromic sulphate (BCS) solution of known total Cr content (determined by AAS) and Cr(VI) content (determined by UV-Vis Spectrophotometer) were added daily inside the incubator for seven consecutive days, in progressively increasing order, from A_m to E_m, while F_m was made control. Similarly, known concentration of BCS solution was added for A_c to E_c while F_c was made control inside the laminar flow in presence of UV light. Every day all the dosing chemical solutions to be added in the C-series petridishes and the glassware needed for dosing, were kept exposed to UV light for 45 minutes prior to addition. In addition in C_c known amount of both Fe^{2+} and Mn^{2+} were added. On the other hand, in D_C known amount of Mn^{2+} and in E_c known amount of Fe^{2+} were added, besides Cr dosing as scheduled. After one-week addition of all the solutions, both the series of petridishes were kept in their respective places for a further period of two weeks with no further dosing of chemicals. They were then taken out and analysed to determine both total Cr (by AAS, as described) and Cr(VI) (by UV-Vis Spectrophotometer as described).

RESULTS AND DISCUSSION

Bandipur soil was supposed to be away from the influence of any sort of Cr contamination and was observed to contain mean Cr concentration of 48.15 mg kg^{-1} which included 0.03 mg kg^{-1} dw of Cr(VI). Mention may be made that on an average total Cr in earth crust is 200 mg kg^{-1} (Shanker *et al*., 2005). Physico-chemical parameters for the initial chemical environment of the Bandipur soil are presented in Table 1 where moisture content was recorded as 28.93% w/w and pH was near neutral (7.3). Commercial BCS powder that was used in this experiment was found to contain a total Cr of 15.06% w/w and Cr(VI) of 4.14 mg kg^{-1}. Chemical dosing with BCS, Fe and Mn solutions in the experimental set up are given in Table 2. Both the controls (*i.e.*, F_M and F_C) did not show any perceptible change in total Cr concentration as compared to that of the native soil during the period of incubation. At the end of the experiment total Cr of F_M and F_C were 47.86 and 48.36 mg kg^{-1} dw respectively. Cr(VI), on the other hand, reacted differently. Though in F_M it was unchanged (0.03 mg kg^{-1} dw), in F_C, however, it increased to 0.18 mg kg^{-1} dw after the incubation period. It may be recalled, that the set X_Cs (X = A, B, C, ….,F) were kept in laminar flow in order to discourage the microbial growth. The UV light might had contributed to the photochemical conversion of a part of Cr(III) to Cr(VI), while the Mn and Fe, which were present in the control soil, might also helped for chemical oxidation. In the five replicates of microbe induced conversion study, net total Cr input varied between 60.75 and 249.40 mg kg^{-1} dw (mean 169.13 mg kg^{-1} dw) for five days dosing. The final total Cr content was however, between 70.08 and 318.00 mg kg^{-1} dw (mean 181.22 mg kg^{-1} dw). Microbial conversion of Cr(III) to Cr(VI) varied from 0.12 – 0.18 mg kg^{-1} dw, while mean was 0.14 mg kg^{-1} dw . Oxidation by chemical catalysis, on the other hand, to convert Cr(III) to Cr(VI) appeared to be much inefficient as compared to microbial assisted conversion. Conversion by chemical catalysis ranged between 0.00 and 0.08 mg kg^{-1} dw (mean 0.05 mg kg^{-1} dw) indicating nearly 3 times greater efficiencies in microbial conversion.

Table 1. Chemical environment of the experimental soil (Bandipur soil).

Parameters	concentrations
Moisture (%)	28.93
Total Cr (mg kg^{-1})	48.15
Cr^{6+} (mg kg^{-1})	0.03
Conductivity (µS)	0.40
pH	7.28
NO_3^{1-} (mg kg^{-1})	2498.75
NO_2^{1-} (mg kg^{-1})	99.90
P_2O_5 (mg kg^{-1})	999.50
SiO_2 (mg kg^{-1})	299.85
Cl^{1-} (mg kg^{-1})	1399.30
PO_4^{3-} (mg kg^{-1})	1337.30
Total hardness (mmol kg^{-1} $CaCO_3$)	39.90
Carbonate hardness (mmol kg^{-1} $CaCO_3$)	29.48
Total acidity (mmol kg^{-1})	19.99
Total alkalinity (mmol kg^{-1} $CaCO_3$)	29.98

Bartlett and James (1988) mentioned that almost any soil with a pH above 5.0 were able to oxidise a portion of Cr(III) provided the soil was moist and fresh. They also reported that, the amount of Cr(III) converted to Cr(VI) depends directly on the content of reducible Mn present in the soil. Amacher and Baker (1982) have studied the oxidizing abilities of Cr in an incubated condition where they found that, in a low concentration of Cr(III) in the soil, the Cr(VI) conversion is higher but after a threshold limit the conversion efficiencies decreases. They also concluded that, the organic matters present in the soil could also possibly reduce the Cr(VI) back to Cr(III) when a higher level of Cr dosing in the soil was made. From Table 2 it is quite obvious that the oxidation of Cr is more pronounced at a lower concentration of Cr in the soil (A_C amounting 0.04 mg kg^{-1} dw and B_C amounting 0.07 mg kg^{-1} dw), while the same was reduced considerably when higher Cr dosing were accomplished (D_C amounting

0.04 mg kg^{-1} dw but, E_C converting nothing). The efficiencies of the oxidising ability of Mn added in D_C got submerged for the dosing rate was higher as compared to A_C, B_C and C_C. So far as the metal catalysts were concerned, Mn showed to be more efficient in oxidising Cr(VI) as compared to Fe (Table 2). In C_C, Fe and Mn were dosed together where Fe content was higher (26.77 mg kg^{-1} dw) than Mn content (6.85 mg kg^{-1} dw). This combined dosing efficiently increased Cr(VI) output (0.18 mg kg^{-1} dw). In E_C, on the other hand, Fe dosing was even higher (57.16 mg kg^{-1} dw) but Mn was totally absent. Such situation could not increase Cr(VI) output proportionately. In another case, when only 14.65 mg kg^{-1} dw of Mn (c.a. 25% of Fe input in E_C) was added alone in D_C, it almost brought about the same result in Cr(VI) output amounting 0.15 mg kg^{-1} dw. It seemed to be relevant that Mn actually played the role of a promoter in C_C.

Table 2. Dosing concentrations d^{-1} for five days (mg kg^{-1}dw of soil) with BCS, Fe and Mn and resultant Cr concentrations (mg kg^{-1}dw of soil) after fourteen days' storage

Sample	Total Cr dosing	Net Total Cr input	Net Cr^{6+} input	Fe dosing	Mn dosing	Final Total Cr	Final Cr^{6+}	Conversion of Cr^{6+}
A_M	12.15	60.75	1.94*10-3		0.00	70.08	0.15	0.12
B_M	27.11	135.55	3.66*10-3	0.00	0.00	124.83	0.15	0.12
C_M	41.03	205.15	5.54*10-3	0.00	0.00	171.92	0.18	0.15
D_M	38.96	194.80	5.25*10-3	0.00	0.00	221.28	0.18	0.14
E_M	49.88	249.40	6.75*10-3	0.00	0.00	318.00	0.22	0.18
F_M (Control)	0.00	0.00	0.00	0.00	0.00	47.86	0.03	0.00
A_C	13.76	68.80	1.86*10-3	0.00	0.00	72.63	0.22	0.04
B_C	26.83	134.15	3.62*10-3	0.00	0.00	147.78	0.25	0.07
C_C	39.34	196.70	5.30*10-3	26.77	6.85	186.32	0.18	0.08
D_C	56.07	280.35	7.55*10-3	0.00	14.65	257.56	0.15	0.04
E_C	69.97	349.85	9.45*10-3	57.16	0.00	309.61	0.18	0.00
F_C (Control)	0.00	0.00	0.00	0.00	0.00	48.36	0.18	0.15

Soil had total Cr content 48.95 and Cr^{6+} content = 0.03 mg kg^{-1} dw; BCS powder had total Cr content = 1506.00 and Cr^{6+} = 4.14 mg kg^{-1} dw. Where conversion of Cr^{6+} = Final Cr^{6+} conc. – {Cr^{6+} net dosing + Cr^{6+} conc. in control soil} in mg kg^{-1} dw

It was also reported (Bartlett and James, 1988) that organic acids like, citric, gallic or esters like citrate increase the solubility and mobility of Cr(III) and thus facilitates its oxidation. They found behaviour of Cr(III) in the subsoil containing organic wastes analogous to the organic NO_3^-. Soluble and mobile Cr(III) also behaved similarly to nitrate, which was released slowly by mineralisation and did not accumulate in humid region of the sub soil. Neither Cr(VI) nor nitrates was readily reduced to Cr(III) in absence of organic reducing agents in the sub soil. However, Fe^{2+} present in the sub soil can reduce Cr(VI) very slowly in the sub soil. Under optimum conditions of oxidation Cr(III) could be oxidised to Cr(VI) on prolonged exposure in the sub soil. The exposure of the sample soils over a period of two weeks as in the present experimental condition might not be an optimum duration of exposure and hence a very negligible conversion was resulted. The availability and toxicity relationships of Chromium in microbial system appeared to be superficial. There was a good indication that, soil microbes in incubated condition were able to oxidise Cr(III) to Cr(VI) as depicted in Table 2. Unlike in X_Cs, no limiting concentration was observed in them. Soil microbes have abilities both to oxidise and reduce the Chromium present in the soil. Perhaps there existed a dynamic equilibrium between these processes and

therefore unlike the control sample F_C, F_M did not show any further enhancement of Cr(VI) content in them, though owing to incubation at 37^0C, the physical condition for bacterial growth was much more conducive than that of the field. As compared to the chemical agents, microorganisms were found to be more efficient in oxidising Cr(III) to Cr(VI). Ross and Bartlett (1981) found that 100 mg kg^{-1} soil or 10 mg Cr(VI) kg^{-1} soil reduced the respiration in soil. In axenic broth culture they also found that gram-negative bacteria were more sensitive to 10 – 12 mg Cr(VI) L^{-1} than were gram-positive ones. In a general survey of the effect of heavy metals on nitrification in soils, Liang and Tabatabai (1978) have found that, 5.0 mmol $CrCl_3$ kg^{-1} of soil inhibited this process in ten days period. The autrotophs appeared to have a lower sensitivity to Cr(VI) than heterotrophs (Bartlett and James, 1988). The low sensitivity of autrotophs was found to be due to the abilities of *Nitrosomonas* and *Nitrobacter* species to produce oxyanions (NO_2^- and NO_3^-) in their normal respiratory and metabolic function. In contrast, heterotrophs that reduced the NO_3^- might be more sensitive to relatively easily reducible Cr(VI) oxyanions. Therefore, the balance between autrotophic and heterotrophic microbial activity might be shifted in favour of autotrophs in soil containing Cr(VI).

Figure 1. Relationship between Total Cr input and net Cr (VI) output.

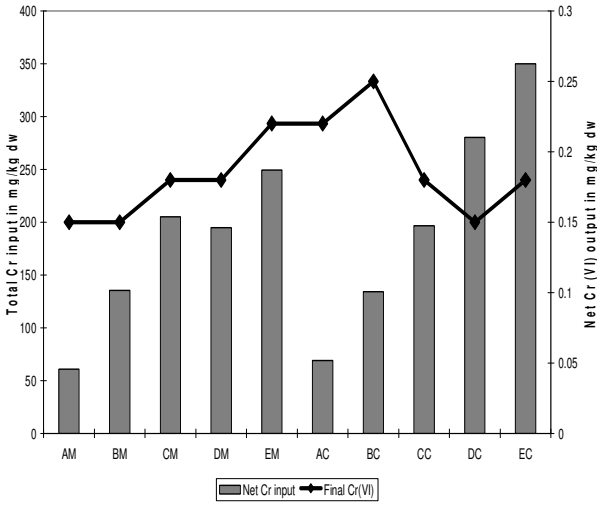
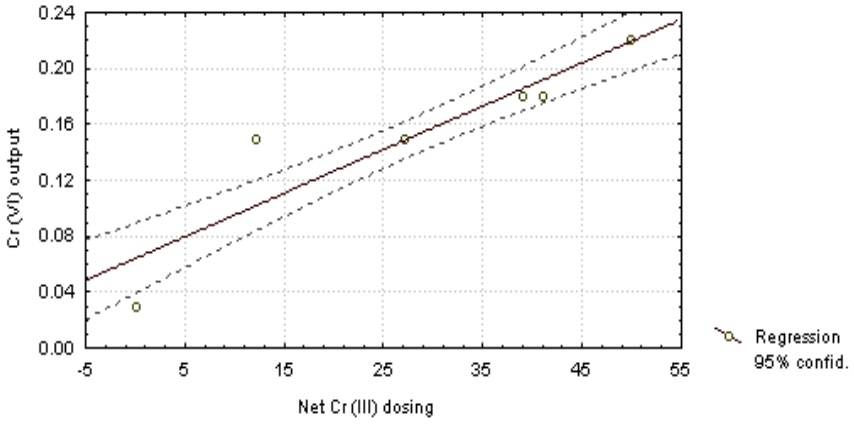


Figure 2. Relationship between net Cr (III) dosing and Cr (VI) formation in microbial induced pathway



Cr (VI) = 0.06400 + 0.00311 * Cr (III) dosing; Correlation: r = 0.90928

The relationship between different input (as regard to both metal composition and amount of dosing) and Cr(VI) conversion efficiencies in two different pathways (*i.e.,* microbial and chemical) is shown in Figure 1. It was revealed that, the microbial induced conversion was positively correlated ($r = 0.91$ at $p<0.05$) with the amount of Cr dosing (Figure 2). Higher the dosing rate d^{-1}, higher was the Cr(VI) formation in this mechanism.

Thus photochemical and microbial oxidation of Cr was found to be more efficient over chemical catalysis as far as the present investigation is concerned. To our knowledge, there are no evidences of direct oxidation of Cr(III) by microbes in literature but recent studies have shown that Mn(II) oxidizing bacteria of the genus *Bacillus* and *Pseudomonas* can actually oxidise Cr(III) indirectly (Wu *et al*., 2005; Murray and Tebo, 2007). Hexavalent Cr thus formed might be thereafter leached, reduced, absorbed, precipitated or consumed by living organisms.

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