



Studies on mobilization of chromium with reference to its plant availability – Role of organic acids

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Received 3 June 1998; accepted 17 November 1998

Key words: organic acids, complexation, CrIII, solubilization, maize plant, chromium enrichment

Abstract

Metal-organic acid interactions with special reference to their plant availability have been studied. The role of organic acids in the mobilization of Cr by converting it into labile organically bound form, enhancing its availability, is highlighted. Pot experiments are conducted to investigate the effect of various organic acids on the uptake and translocation of root absorbed trivalent and hexavalent chromium by maize (*z. mays*) plants grown in sand and soil culture. Statistically significant increases in chromium accumulation from CrIII-treated plants in the presence of increasing concentrations of organic acid suggest the existence of CrIII – organic acid interactions in the soil plant system. In order to support the above mentioned hypothesis of formation of organically bound CrIII in the presence of various organic acids (carboxylic and amino acids), separate experiments have been performed to synthesize and estimate its respective organically bound forms. Amendments with organic acids, however, do not appear to markedly affect chromium accumulation from CrVI treatment. The results are discussed on the basis of the potential of organic acids to form complexes with CrIII.

Introduction

Elevated concentrations of metals in human diet constitute a potential health hazard in the long term. Agricultural plants represent an important pathway for movement of potentially toxic trace elements from soil to human beings. Contaminant metals can often accumulate in considerable amounts in plant tissues and exceed the levels that are toxic to the human and animal system. Chromium is unique among the heavy metals because of its existence in two environmentally important oxidation states: trivalent (CrIII) and hexavalent (CrVI) in the pH range of agricultural soil. Industrial waste waters from metallurgical processes, refractories, electroplating units and tanneries contain various amount of CrIII mainly as $\text{Cr}(\text{OH})_3$ (NRC 1974). It was believed that disposal of this metal in soil would not cause problems because of the extremely low solubility of $\text{Cr}(\text{OH})_3$, preventing its leaching into ground water or its uptake by plants (EPA 1984). However, chromium pollution problems will arise if CrIII

becomes mobilized by any means of its solubilization. Mobilization of CrIII in soils through its oxidation into soluble CrVI species has been suggested (Bartlett & James 1979). Another possibility of organic complexation of CrIII with naturally occurring ligands for its solubilization has also been explored (Lundstrom 1993). Root exudates released in the rhizosphere have been implicated in several mechanisms for altering the level of ions and molecules within the rhizosphere (Cataldo et al. 1988; White et al. 1981). Among various root exudates, organic acids, being negatively charged species, are likely to interact with metal ions under a wide range of aqueous and soil conditions (Jones & Darrah 1994; Zhang et al. 1989; Delhaize et al. 1993). Metal-organic acid interactions in the soil plant system are important for solubilization/binding of metals from the highly insoluble soil mineral phase, and should become an area of sustained research.

The present communication highlights the role that organic acids play in the solubilization of chromium by converting it into a labile organically bound form

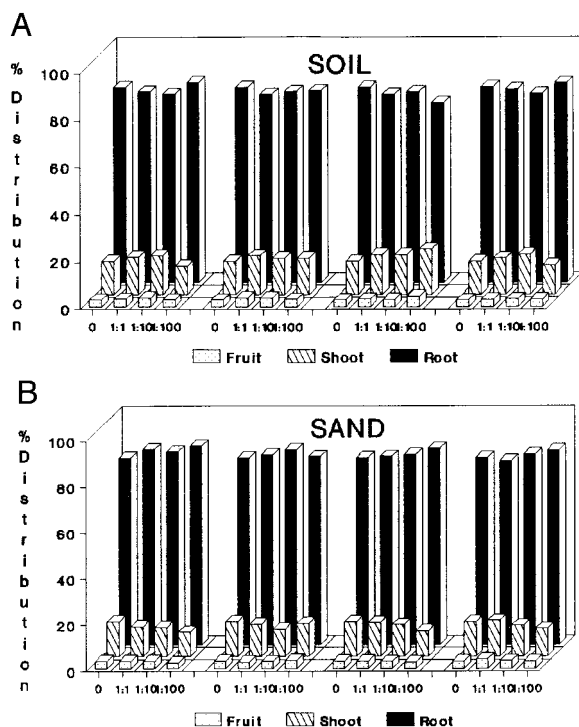


Figure 1. A) % distribution of Cr in plant (maize) grown in soil supplied with $5 \mu\text{g/ml}$ of trivalent chromium in the presence of increasing concentration of organic acids. B) % distribution of Cr in plant (maize) grown in sand supplied with $5 \mu\text{g/ml}$ of trivalent chromium in the presence of increasing concentration of organic acids.

and thus improving its plant availability. Pot experiments have been conducted to study the effect of some organic acids (carboxylic acids and amino acids) on the uptake and translocation of root-absorbed trivalent and hexavalent chromium in various parts of maize plants grown in soil and sand culture. Organically bound forms of chromium, formed in the presence of different organic acids, have been estimated in separate experiments. Attempts have been made to relate the potentiality of organic acids to form labile organically bound Cr(III) to that of chromium accumulation in the plants. Comparative studies in soil and quartz sand (inert matrix) are expected to point out the existence of metal-organic acid interactions, modifying the chemical nature of chromium supplied and its subsequent uptake by plants.

Materials and methods

Pot experiments under laboratory conditions were performed using maize (*Z. mays*) plants grown for 60

days in sand and soil (2.5 kg) using plastic containers. Quartz sand was used after prescribed washings (Hewitt 1966). Plants grown in sand were irrigated with a complete nutrient solution (Hoagland & Arnon 1950). The soil used in the experiment has the following characteristics: Sandy loamincepistol, pH 7.4, EC 0.23 ds/m, organic carbon 0.08%. Total Cr 0.05 ppm. Total Mn 80 ppm, DTPA extratable Mn 2.6 ppm, bulk density 1.25 gm/cm^3 , CEC (Cation Exchange Capacity) $25.7 \text{ c mol (p}^+)/\text{kg}$, soluble ions Ca 0.54, Mg 1.80, Na 6.10, K 0.09, Cl 5.7, SO_4 $1.3 \text{ c mol (p}^+)/\text{kg}$. A basal dose of N:P:K (60:20:18) mg kg^{-1} of soil was initially supplied. The plants were irrigated with distilled water as and when required.

Carrier solutions of two chromium sources i.e., potassium dichromate (CrVI) and chromic chloride (CrIII), were tagged with trivalent and hexavalent chromium radiotracers, prepared from supplied ^{51}Cr isotope using its redox reactions. Treatments comprised a single pulse addition of radio-labelled CrVI and CrIII solutions at $5 \mu\text{g/ml}$ level in combination with different levels of (1:1, 1:10, 1:100 w/w) organic acids, i.e., citric acid, malic acid, aspartic acid and glycine. The predominant acids released by maize plants as root exudates were considered for the present study (Mench & Martin 1991). The pH of the solution was finally adjusted to 5.5 with 0.1 N HCl. ^{51}Cr was obtained from the Board of Radiation and Isotope Technology (BRIT) BARC, Bombay. Plants were incubated with these treatments for 10 days. Natural light (diurnal cycle of 14 h) was supplemented with Phillips Fluorescent tubes 40 W and Toshiba lamps 15W providing an irradiance of approximately 600 w/m^2 at the plant tops with a slow ventilation system. Plants were harvested and washed thoroughly with tap water, pH 4 water and with distilled water and were cut into root, shoot and edible parts, then packed into plastic vials for oven drying (50°C) to obtain dry matter yields. The pH of the final washings were tested to ensure that no detectable acidity was left and that there was no detectable external contamination on the plant parts.

Accurately weighed amounts of plant material were counted over a planar NaI(Tl) detector coupled to a 4K MCA (Canberra Accuspec Card with PC-AT386). The counting geometry was pre-calibrated for efficiency with the known amount of ^{51}Cr activity from 0.320 Me V photopeak area. The activity of ^{51}Cr was calculated and reported as chromium in different parts of plant per gram of dry weight.

Source-to-plant transfer coefficients (SPT) for chromium with increasing organic acids supplementation in both sand and soil medium were calculated by dividing chromium concentration in the plants (DW) by chromium concentration in the feeding solution.

Experiments were also conducted for estimating organically bound CrIII using Dowex-50 (cationic exchange resin). A definite amount of CrIII (50 μg) was taken in an Erlenmeyer flask and radiolabelled with ^{51}Cr III oxidation state. The solution was kept shaking for 72 h for complexation with increasing concentration of citric acid, malic acid, aspartic acid and glycine separately (1:1, 1:10, 1:100 w/v). After adjusting the total volume (25 ml) with pH 5 water, Dowex-50 resin (0.5 gm) was added and the solution was equilibrated by shaking for 1 h. The Resin was allowed to settle and the supernatant was taken in a vial of standard geometry and counted. The percentage of CrIII complexed by different organic acids was calculated by difference, using the procedure of Deb *et al.* (1976).

The data represent the mean of four plants per pot after performing experiments in triplicate. Statistical analysis were performed using SPSS/PC+M software package. Tests for nonnormal data were computed by Mann Whitney (independent) U test comparing individual means. Correlation coefficients were used to relate concentration in root and aerial parts to various treatments of organic acids.

Results

Tables 1 and 2 show the accumulation of chromium in various parts of the plants, supplied with 5 $\mu\text{g}/\text{ml}$ of trivalent and hexavalent chromium in the presence of increasing concentrations of various organic acids.

Addition of organic acids to CrIII (1:1, 1:10, 1:100 w/w) resulted in a statistically significant increase in chromium accumulation in root and aerial parts of the plant in both the soil and sand cultures ($p < 0.05$). Relatively higher increases in chromium accumulation were observed in plants grown in sand (Mann Whitney-U test $p < 0.05$). In contrast, no well-defined order in the uptake pattern of chromium from CrVI-treated plants was observed in the presence of organic acids in either sand or soil culture ($p > 0.05$).

The uptake of chromium with increasing supplementation of organic acids was calculated in terms of percentage distribution (Figure 1) and percentage increase (Figure 2a). The effects of organic acid amendments on the chromium enrichment from CrIII

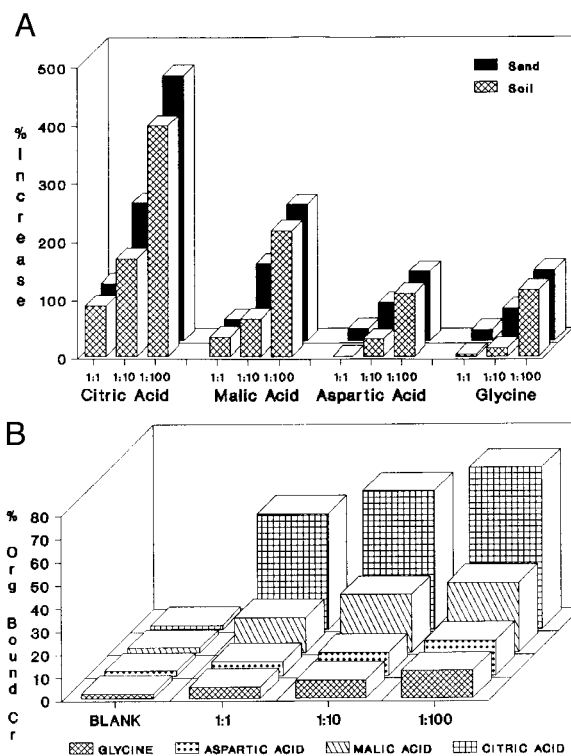


Figure 2. A) % increase in Cr uptake of whole plant (maize) supplied with 5 $\mu\text{g}/\text{ml}$ of trivalent Cr in the presence of increasing concentration of organic acids. B) % formation of organically bound chromium with increasing concentration of Citric, Malic, Aspartic Acid & Glycine.

treatment were also calculated in terms of source-to-plant transfer (SPT) coefficient (Table 3). Experiments showed that the affinity of organic acids for complexation with CrIII, was found to be as follows (Mann Whitney U test):

Citric acid > Malic acid > Aspartic acid \approx Glycine

Discussion

The distribution of chromium in plant tissues (root, shoot, fruit) indicate that (80–85%) of chromium is retained in the roots and only a small part of it is translocated to the aerial parts. It is interesting to note that increasing concentrations of organic acids increases the plant's uptake of chromium without affecting its distribution between the root and aerial parts of the plant. Huffman & Allaway (1973) reported that over 90% of supplied chromium gets incorporated in roots of barley and wheat plants with poor translocation to the aerial parts.

Table 1. Plant tissue concentration of chromium ($\mu\text{g/g}$ dry weight) in maize plants supplied with chromium III ($5 \mu\text{g/ml}$) in the presence of organic acids

Conc. ratio CrIII: Org. acids	Sand culture			Soil culture		
	Root*	Shoot*	Fruit*	Root*	Shoot*	Fruit*
Citric acid						
1:1	93 (5.2)	13 (1.2)	3 (0.8)	45 (1.5)	9 (1.0)	2 (0.7)
1:10	155 (3.2)	23 (1.8)	6 (0.9)	63 (1.9)	13 (1.2)	3 (0.8)
1:100	263 (2.9)	32 (1.9)	8 (1.1)	123 (3.0)	18 (1.3)	4 (0.8)
Corr. Coeff.	0.91	0.86	0.82	0.95	0.83	0.86
Malic acid						
1:1	62 (5.1)	11 (1.2)	2 (0.6)	31 (3.2)	6 (0.2)	1 (0.1)
1:10	108 (4.6)	15 (1.9)	4 (0.8)	39 (3.8)	7 (0.2)	2 (0.1)
1:100	150 (4.8)	26 (2.1)	7 (0.9)	75 (4.5)	14 (0.2)	3 (0.8)
Corr. coeff.	0.87	0.96	0.94	0.98	0.96	0.93
Aspartic acid						
1:1	54 (5.0)	10 (1.3)	2 (0.6)	23 (1.4)	5 (0.6)	09 (0.1)
1:10	75 (4.6)	13 (1.1)	3 (0.8)	31 (1.6)	6 (0.5)	2 (0.1)
1:100	103 (3.8)	13 (1.9)	3 (0.8)	46 (1.4)	12 (0.8)	3 (0.9)
Corr. coeff.	0.91	0.72	0.86	0.97	0.98	0.99
Glycine						
1:1	52 (4.6)	10 (1.1)	3 (0.8)	24 (1.3)	5 (0.9)	0.9 (0.1)
1:10	71 (5.4)	12 (0.9)	3 (0.6)	27 (1.4)	6 (0.6)	1 (0.1)
1:100	100 (5.8)	15 (0.8)	4 (0.2)	43 (1.8)	8 (0.3)	2 (0.1)
Corr. coeff.	0.93	0.89	0.86	0.99	0.95	0.95
Blank without org. acid supplementation	45 (4.3)	8 (1.1)	2 (0.9)	24 (4.8)	4 (1.2)	0.8 (0.8)

$p^* < 0.05$

Values in parenthesis (SD)

Table 2. Plant tissue concentration of chromium ($\mu\text{g/g}$ dry weight) in maize plants supplied with chromium VI ($5 \mu\text{g/ml}$) in the presence of organic acids

Conc. ratio CrVI: Org. acids	Sand culture			Soil culture		
	Root*	Shoot*	Fruit*	Root*	Shoot*	Fruit*
Citric acid						
1:1	296 (5.2)	38 (1.4)	6 (0.3)	210 (5.5)	20 (1.0)	3 (0.3)
1:10	286 (5.6)	30 (1.3)	5 (0.2)	180 (5.8)	17 (0.3)	4 (0.9)
1:100	290 (5.8)	36 (1.1)	5 (0.1)	198 (5.2)	18 (0.4)	4 (0.4)
Corr. coeff.	-0.43	-0.09	-0.54	-0.19	-0.48	-0.42
Malic acid						
1:1	300 (5.1)	36 (1.5)	6 (0.6)	213 (5.3)	15 (1.0)	4 (0.2)
1:10	293 (4.8)	28 (1.3)	5 (0.4)	192 (5.2)	18 (1.1)	5 (0.3)
1:100	299 (4.6)	33 (1.1)	6 (0.3)	204 (4.8)	15 (1.2)	4 (0.2)
Corr. coeff.	-0.28	-0.27	-0.20	-0.22	-0.59	-0.12
Aspartic acid						
1:1	311 (5.2)	38 (1.6)	8 (0.5)	211 (5.2)	21 (0.9)	4 (0.8)
1:10	297 (5.6)	32 (1.2)	7 (0.4)	205 (4.8)	22 (0.3)	3 (0.2)
1:100	301 (5.4)	36 (1.3)	8 (0.3)	209 (5.6)	21 (0.2)	3 (0.1)
Corr. coeff.	-0.47	-0.15	-0.14	-0.21	-0.59	-0.49
Glycine						
1:1	312 (5.8)	39 (1.4)	8 (0.8)	214 (4.6)	20 (1.0)	4 (0.9)
1:10	296 (5.0)	30 (1.3)	7 (0.3)	205 (4.2)	22 (0.4)	3 (0.8)
1:100	309 (5.2)	32 (1.9)	8 (0.4)	207 (4.7)	20 (0.2)	4 (0.4)
Corr. coeff.	-0.01	-0.49	-0.19	-0.58	-0.27	-0.06
Blank without org. acid supplementation	316 (5.3)	40 (3.2)	8 (2.1)	214 (5.8)	21 (2.8)	4 (1.1)

$p^* < 0.05$

Values in parenthesis (SD)

Table 3. Source to plant transfer coefficients for chromium in maize plants treated with trivalent chromium in the presence of organic acids supplementation

Conc. ratio CrIII: Org. acids	CrIII sand				CrIII soil			
	Citric	Malic	Aspartic	Glycine	Citric	Malic	Aspartic	Glycine
	Acid	Acid	Acid		Acid	Acid	Acid	
1:0	11.0	11.0	11.0	11.0	5.7	5.7	5.7	5.7
1:1	21.8	14.8	13.2	13.0	11.0	7.6	5.8	6.0
1:10	36.7	25.4	18.0	17.1	15.6	9.6	7.8	6.6
1:100	60.8	36.4	24.4	22.8	29.0	18.4	12.2	10.6

An increase in chromium uptake from treatment of CrIII with increasing supplementation of organic acids may be ascribed to the interaction of CrIII with organic ligands leading to the formation of mobile organically bound CrIII. Organic compounds such as citric, malic and aspartic acids and glycine have been reported to be potential chelators for CrIII (Nakayama 1981). James & Bartlett (1984) observed that addition of citric acid increases the chromium accumulation in different plant parts. They proposed that chromium uptake with citric acid was a CrVI effect that resulted from enhanced solubility of $\text{Cr}(\text{OH})_3$ by citrate in soil, which in turn led to an increased oxidation of CrIII by soil manganese oxides. However, their further experiments minimized the possibility of chromium oxidation by soil manganese oxide. Oxidation of CrIII held by low-molecular-weight organic acids in soil conditions appears to be kinetically feasible only. Some important complex side reactions tend to make the oxidation of CrIII very slow (Bartlett 1991).

A higher uptake of chromium from treatment of CrIII with organic acids in the plants grown in sand as compared to soil is observed (Table 1). Quartz sand, unlike soil, does not contain any oxidizable MnO_2 , possesses the minimum possibility of oxidation, further inert in nature and provides a better site for CrIII-organic complexation and thus highlights the complexation of CrIII as a major contributor for Cr enrichment in plants.

This finds further support by the increasing trend in SPT values of chromium with increasing concentration of organic acids (Table 3), demonstrating the existence of CrIII – organic acid interaction ($p < 0.05$). SPT coefficients for chromium uptake when no organic acid was provided, have been considered as reference value. It appears that mobilization of CrIII largely depends on organic ligands.

The order of complexation obtained is according to the ease of complex formation of CrIII by different organic acids. The metal solubilizing ability of organic acids is reported to be parallel to their metal binding ability (Mench & Martin 1991), which in turn correlates with their dissociation constants. The dissociation constants (K_{a1} , K_{a2}) for citric acid (7.10×10^{-4} , 1.68×10^{-4}), malic acid (3.9×10^{-4} , 7.8×10^{-6}), aspartic acid (1.38×10^{-4}), glycine (1.67×10^{-10}) are in conformity with the order obtained in our finding.

In CrVI-treated plants an inconsistent chromium uptake pattern but with overall decrease in the presence of increasing organic acids is observed. This behaviour can be explained on the basis of the fact that organic acids can contribute towards reduction of CrVI into less mobile CrIII and then tend to complex CrIII, thus making it plant available. Thus, two processes solubilization of CrIII and reduction of CrVI are both thermodynamically favourable in soil conditions and results in the existence of some self-preserving state of soil nonequilibrium, causing inconsistency in uptake behaviour.

In order to support the hypothesis of the formation of organically bound CrIII, separate experiments were performed to synthesize organically bound CrIII, (CrIII-Citric acid CrIII-Malic acid, CrIII-Aspartic acid and CrIII-Glycine). Organically bound CrIII was estimated on Dowex-50 (Cationic exchange resin). Results (Figure 2b) show that with increasing concentrations of citric acid, malic acid, aspartic acid and glycine (1:1, 1:10, 1:100 w/w), an increase in the formation of organically bound CrIII occurs. Increasing formation of organically bound CrIII with increasing CrIII: ligand ratio, provide support to the existence of metal-organic acid interaction, leading to the overall enrichment in chromium uptake in the presence of increasing concentration of organic acids.

Acknowledgements

The authors are grateful to Prof. P.S. Satsangi, Director, Dayalbagh Educational Institute, Agra for providing the necessary facilities. Financial support given by Board of Studies in Nuclear Science (BRNS), Department of Atomic Energy (DAE) is gratefully acknowledged. Thanks are due to Prof. D.L. Deb, Project Director, Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi for the help in getting soil samples analysed.

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