Bull. Environ. Contam. Toxicol. (1999) 63:524-530

Bulletin of Environmental Contamination and Toxicology

Mobilization of Trivalent Chromium in Presence of Organic Acids: A Hydroponic Study of Wheat Plant *(Triticum vulgare)*

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Received: 15 January 1999/Accepted: 15 July 1999

World Health Organization (WHO) has recognized health hazards of metals in food chain even at low concentration (WHO 1984). The deposition of metals in soil and water find their way to human beings via plant uptake process. Uptake and translocation of metals cannot be considered as an isolated action but seems to be affected by several synergistic and antagonistic reactions with other naturally occurring species (Delhaize etal 1993). Toxicity and mobility of metals, relates to the total metal concentration but more realistically on the forms resulting from various physical and chemical interactions which they undergo in natural system (Cataldo et al 1988). Among various toxic metals, chromium, is unique giving its paradoxical role in both nutrition and carcinogenesis. It exists in the environment as trivalent and hexavalent oxidation states. Trivalent cation (CrIII) is less mobile and considered essential for carbohydrate metabolism (Anderson 1981), while hexavalent form (CrVI) is soluble anion and has been placed in first quartile of 53 compounds, evaluated by Cancer Assessment Group (NIOSH 1979). Industrial waste waters from metallurgical processes, refractories, electroplating units, tanneries contain various amount of CrIII mainly as $Cr(OH)_3$ (NRC 1974). It was believed that disposal of this metal in soil would not impose problems because of extremely low solubility of $Cr(OH)_3$, preventing its leaching into ground water or its uptake by plants (EPA 1984). However, chromium pollution problems would 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 and James 1979). Another possibility of organic complexation of CrIII with naturally occurring ligands for its solubilization has also been explored (Lundstrom 1993). Among root exudates, low molecular weight organic acids (carboxylic and amino acids) have been indicated to effect solubilization/ mobilization and redox behavior of chromium (James and Bartlett 1984). It is suggested that besides, CrIII and CrVI species, organically bound CrIII complexes also prevail in agricultural soil and water (Florence 1982).

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In order to highlight the role of organic acids in solubilization of trivalent chromium, hydroponic experiments have been conducted to study the effect of some organic acids on the chromium enrichment from trivalent chromium in wheat *(Triticum vulgare)* plant.

MATERIALS AND METHODS

Hydroponic experiments on the plant wheat *(Triticum vulgare)* were carried out using Hoagland nutrient solution (Hoagland and Amon 1950) at a temp 24 ± 0.2°C. Natural light (diurnal cycle of 15 hrs.) was supplemented by the combination of Philips Fluorescent tubes 40 W and Toshiba lamps 15 W giving an irradiance of approximately 600 W/m^2 at the plant tops with a slow ventilation system. The seedlings of the plant were carefully transplanted to the beakers with the support of thermocol. Plants were supplied nutrient solution (half strength) every alternate days and aerated with filtered compressed air. After establishment period of four weeks, plants were shifted to fumehood of radiochemical laboratory. Treatments comprised trivalent chromium ($CrCl_3$), labelled with ^{51}Cr (0.1 µci) at 2.0 µg/ml level in combination with different levels (1: 1, 1:5, 1: 10, 1:50, 1: 100) of organic acids viz. oxalic acid, malic acid and glycine. These are the acids predominately released by wheat plants as root exudates (Vancura 1964) and are considered for the present study. Experiments when no organic acids were supplemented served as control. The pH of the solution was maintained at 5.5-6. ^{51}Cr was obtained from Board of Radiation and Isotope Technology (BRIT) BARC, Bombay.

After 10 days of exposure to different treatments plants were harvested and sized in root and aerial parts. Plant tissues were washed thoroughly with tap water, acidic water (pH 4), finally with distilled water and dried in oven at 50°C. Dry matter yield of the plants in each case was obtained. The pH of washings were tested to ensure that no detectable acidity left and there was no detectable external contamination of the plant. Accurately weighed amount of dried material was counted over a planar NaI (T1) detector coupled to a 4K MCA (Canberra Accuspec Card controlled by a PC- AT 386). The counting geometry was precaliberated for efficiency with known amount of ^{51}Cr activity using 0.320 MeV photopeak area. The activity of ^{51}Cr was calculated and converted to total amount of chromium in different parts per gram of dry weight. All the activity was corrected for its decay to arrive at an activity on a common time and date also for different sample matrices. Samples were counted for varying duration

30 min. to 120 min., so as to accumulate at least 8 to 10 thousand counts under photo peak area to keep statistical errors in counting below a few percent.

Experiments were also conducted to estimate organically bound CrIII using Dowex-50 (cationic exchange resin). A definite amount of CrIII (50 μg) was taken in Erlenmeyer flask and radiolabelled with ^{51}CrIll oxidation state. The solution was kept for 72 hrs shaking for complexation with increasing concentrations of oxalic and malic acid, separately (1:1, 1:10, 1: 100 w/w). After adjusting the total volume (25 ml) with pH 5 water, Dowex -50 resin (0.5gm) was added and the solution was equilibrated by shaking for 1 hr. Resin was allowed to settle and supernatant was taken in 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).

Source to plant transfer coefficients (SPT) for chromium with increasing organic acids supplementation have been calculated by dividing chromium concentration in the plants (DW) by chromium concentration in feeding solution.

Data represent the mean of four plants per pot after doing experiments in triplicate. Statistical analysis was performed using SPSS/Pc$^+$M software

package. Test for non normal data were computed by Mann Whitney (independent) U test to compare individual mean. Correlation coefficients were used to relate chromium concentration in root and aerial parts to various treatments of organic acids.

RESULTS AND DISCUSSION

Table 1 includes data on the distribution of chromium in root and aerial parts of the plant, supplied with 2 µg/ml of CrIII, in the presence of varying concentrations of different organic acids. The distribution of chromium in the plant tissues viz. root and aerial parts indicate that (70-80%) of chromium is retained in roots and only a small part of it is translocated to aerial parts. Huffman and 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. It is interesting to note that increasing concentration of organic acids, increases plant uptake of chromium without affecting its distribution between the root and aerial parts of the plant.

Table 1. Plant tissue concentration of chromium (µg/g dry weight) in wheat plant supplied with chromium III at 2µg/ml.

Conc.	Carboxylic acids				Amino acid	
Ratio	Oxalic acid		Malic acid		Glycine	
Cr III: acids	Root*	Shoot*	Root*	Shoot*	Root*	Shoot*
1:0	94.8 ± 3.8	24.2 ± 1.1	94.8 ± 3.8	24.2 ± 1.1	94.8 ± 3.8	24.2 ± 1.1
1:1	382.4 ± 4.2	90.1 ± 1.8	296.2 +5.4	60.4 ± 1.8	162.3 ± 4.2	39.4 ± 2.1
1:5	403.2 ± 2.2	108.1 ± 2.1	323.4 ± 5.2	80.4 ± 2.2	218.4 ± 2.9	50.4 ± 3.6
1:10	425.1 ± 2.5	120.4 ± 1.5	340.5 ± 4.6	90.2 ± 1.9	256.4 ± 3.2	62.3 ± 4.3
1:50	479.1 ± 3.2	149.2 ± 2.2	393.7 ± 4.3	112.1 ± 2.5	301.3 ± 3.9	77.4 ± 2.2
1:100	578.4 ± 4.8	163.1 ± 2.5	477.3 ± 3.7	128.3 ± 2.8	360.1 ± 4.8	99.2 ± 4.1
Corr. Coef.	0.7*	0.7*	0.7*	0.8*	0.8*	0.9*

± = SD, *$p < 0.05$

An increase in chromium uptake from CrIII treatments with increasing supplementation of organic acids may be ascribed to the interaction of CrIII with organic ligands leading to the formationof mobile organically bound CrIII. Organic compounds such as oxalic, malic and glycine have been reported potential chelators for CrIII (Nakayama, 1981).

The oxidation of organically bound CrIII by naturally occurring oxidizing species appears to be kinetically feasible only because of several complex side reactions which tend to make the oxidation of organically bound CrIII very slow (Bartlett, 1991) minimizing the scope of formation of mobile CrVI. Chromium enrichment from the metal organic acid amendments in the present hydroponic system, which does not contain any oxidizing species, eliminating the possibility of Cr III oxidation and support the school of thoughts highlighting organic complexation of Cr III as a major contributor for Cr accumulation in the plants

This finds further support by the increasing trend in SPT values of chromium with increasing concentration of organic acids (Table 2) 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 affinity of organic acids for complexation with CrIll was found to be as follows (Man Whitney U test):

Oxalic > Malic > Glycine

Table 2. Source to Plant Transfer Coefftcients (SPT) for chromium in wheat plant supplied with trivalent chromium at 2 µg/ml in presence of increasing organic acids supplementation.

Conc. Ratio CrIII: acids	Oxalic acid*	Malic acid*	Glycine*
1:0	59.5	59.6	59.5
1:1	236.3	178.3	100.9
1:5	255.7	201.9	134.4
1:10	272.7	215.3	159.6
1:50	314.2	507.8	189.4
1:100	370.8	302.8	229.7
Corr. Coef.	0.7*	0.7*	0.8*

*P < 0.05

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 are reported to be parallel to their metal binding ability (Mench and Martin 1991) which in turn is correlated with their dissociation constants. The dissociation constants (Ka$_1$, Ka$_2$) for oxalic acid (5.9x 10^{-2}, 6.4 x 10^{-2}), malic acid (3.9x10^{-4}, 7.8x10^{-6}), glycine (1.6x10^{-10}) are in conformity with the order obtained in our experiments.

Our findings also indicate that chromium complexation and their resultant uptake are low for amino acids as compared to carboxylic acids (Table 1). It is suggested that proteinaceous amino acids released into the rhizosphere do not play a major role in mobilizing metals from the soil. Jones et al. (1994) have indicated that amino acids effect complexation to lesser extent than carboxylic acids having limited nutrient mobilization capacity.

In order to support the hypothesis of the formation of organically bound CrIII, separate experiments were performed to synthesize organically bound CrIII,

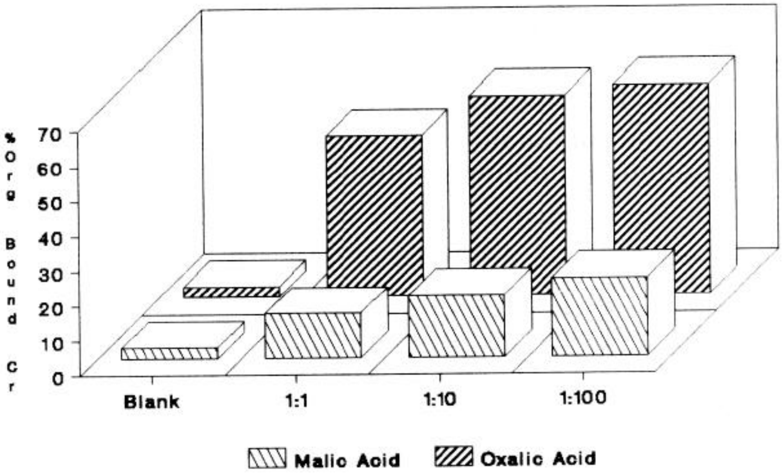

Figure 1. % formation of organically bound chromium with increasing concentration of malic and oxalic acid

(Crlll -Oxalic acid, Crlll - Malic acid). Organically bound Crlll was estimated on Dowex-50 (Cationic exchange resin). Results (Fig. 1) show that with increasing concentrations of oxalic acid and malic acid (1:1, 1:10, 1:100 w/w), 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.

Acknowledgments We thank Prof. P.S. Satsangi, Director, Dayalbagh Educational Institute, Agra for providing necessary facilities. Financial support given by Board of Studies in Nuclear Science (BRNS), Department of Atomic Energy (DAE) is gratefully acknowledged.

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