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Effect of Selenium Supplementation on the Uptake and Translocation of Chromium by Spinach (*Spinacea oleracea)*

So. Srivastava,[1] K. Shanker,[2] Sh. Srivastava,[1] R. Shrivastav,[1] S. Das,[1] S. Prakash,[1] M. M. Srivastava[1]

[1] Department of Chemistry, Faculty of Science, Dayalbagh Educational Institute, Dayalbagh, Agra 282 005, India
[2] Central Board of Excise and Customs Laboratories, Neemach, India

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Agricultural plants represent an important pathway for the movement of potentially toxic trace elements from soil to human beings. Contaminant metals can often accumulate in considerable amount in plant tissues and exceed the levels that are toxic to human and animal system, before they produce visible phytotoxic effects. Chromium is encountered as a metal pollutant in the effluent from tanneries and electroplating industries and accumulates in the top layer of the soil. Chromium in its hexavalent form is highly carcinogenic (NIOSH 1979) and, being highly soluble, readily available to the plants (Bartlett and James 1988). The discovery that an element like selenium counteracts the toxicity, chemical carcinogenesis and may reduce the plant uptake of other toxic metals, has resulted into extensive research on relationship of selenium to other metals in terms of bioavailability (Cary1981) However, studies are mainly confined to zooplankton (Whanger1981, Zakaria et. al. 1993) and, little attention has been paid on selenium-metal interactions in soil plant system. Studies on cadmium-selenium and mercury-selenium interactions in various plants have recently been reported by us. (*Shanker, 1995*a,b and 1996a, b). Apparently, no study was accessable to us on the possibility of chromium - selenium interactions in soil plant system, particularly those with different oxidation states of chromium and selenium.

The present communication describes our preliminary work on the antagonistic effect of selenium (selenite and selenate) species on the uptake of chromium from its two environmentally important oxidation states (trivalent and hexavalent chromium) in spinach (Spinacea oleracea) plant, grown in sand and soil growth medium. Comparative study in sand (inert matrix) and soil is expected to highlight the role of various naturally occurring species present in soil, in modifying the chromium-selenium interactions and their subsequent uptake by plants.

Correspondence to: M. M. Srivastava

MATERIALS AND METHODS

Pot culture experiments under laboratory conditions were performed on the spinach (Spinacea oleracea) plant for a growth period of 60 days in sand and soil (2.5 kg) using plastic containers. The quartz sand was used after prescribed washings.Plants grown on sand culture were irrigated with complete nutrient solution (Hogland and Arnon1950). The Soil used in the experiment has the following characteristics:pH 7.4, EC-0.23 mmho cm^{-1}; organic carbon 0.08%, texture -sandy loam. 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.

Sodium selenite ($Na^{2}SeO_3$), sodium selenate (Na_2SeO_4), potassium dichromate ($K_2Cr_2O_7$) AR grade were used for treatments. Selenite and selenate (inactive) at concentrations (00,0.5,1.0, 2.0, 4.0, 6.0 µg/ml and hexavalent and trivalent chromium (reduced form of $K_2Cr_2O_7$) at 2 and 5 µg/ ml labelled with ^{51}Cr tracer were applied to the plants separately. The pH of the stock solution was maintained between 6 and 7 while trivalent chromium was prepared at pH 5.2. ^{51}Cr was obtained from the Board of Radiation and Isotope Technology (BRIT) BARC, Bombay (India).Plants were kept in the fumehood of the radio chemical laboratory for 10 days. Natural light (diurnal cycle of 15 hrs.) was supplemented with Philips Fluorescent Tubes 40 W and Toshiba lamps 15 W, 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 pH 2 water, tap water and finally with distilled water. Plant tissues were cut into root and aerial part and then packed into plastic vials of standard geometry for drying in an oven at 50°C. Dry mattter yield of the plants in each case were obtained. 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. Control experiments for each substrate type and chromium concentration served for both oxidation forms of selenium (selenite and selenate).

Accurately weighed amounts of plant-material were counted over a planar Nal (TI) detector coupled to a 4k MCA (Canberra Accuspec Card with PC-AT 386). The counting geometry was pre-calibrated for efficiency with known amount of ^{51}Cr activity from 0.320 MeV photopeak area. From photopeak area, activity was calculated and converted to total amount of element in different plant tissue per gram 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 mts to 120 mts, so as to accumulate at least 8 to 10 thousand counts under photopeak area to keep statistical errors in counting below a few percent.

Values represent the mean of four plants per pot. Data were analysed statistically using SPSS/PC$_{+TM}$ statistical packages. Tests for non-normal data were computed for the Mann-Whitney (independent) U test to compare individual means significant at $p \leq 0.05$. Correlation coefficients were used to relate chromium concentration in different parts of the plant and different forms of selenium supplied to the plants.

RESULTS AND DISCUSSION

Summaries of the effects of various concentrations of selenite and selenate (0.5-6.0 µg/ml) on the uptake and translocation of chromium at two different levels (2 and 5 µg/ml) from its trivalent and hexavalent states, are depicted in table 1 and 2.

The level of accumulation in different parts of plant-root and aerial parts indicate that a major part of the chromium is retained in roots. Greater accumulation of chromium in the underground tissues of the plant and its poor translocation to the tops have been reported (Huffman and Allaway 1973).

Significant decreases in chromium accumulation in the root and aerial parts of the spinach plant were observed with increasing concentration (0.5-6.0 µg/ml) of selenite and selenate in both culture mediums. ($p<0.05$). Both forms of selenium (selenite and selenate) were found equally effective in reducing chromium burden of the plants ($p>0.18$, Mann-Whitney U-test). However, these treatments were found more effective in reducing chromium uptake from trivalent chromium source.

This effect was more pronounced in plants grown in sand culture compared to soil culture. Source to plant transfer co-efficients (SPT) of chromium for spinach plant grown in sand and soil culture were calculated and presented in Table 3. SPT co-efficients when no selenium treatments provided, were taken as reference. SPT co-efficients for the treatments were found to follow a decreasing order with increasing concentration of selenium supplementation ($p<0.05$).

Statistically significant decrease in SPT values of chromium with increasing selenium supplementation, suggests the existence of chromium - selenium interaction causing reduction in chromium uptake. The comparison of SPT values also highlight that with increase in the chromium feed concentration (2-5 µg/ml) increasing amount of chromium was translocated to the different parts of the plant in both

Table1 Plant-tissue concentrations of chromium (µg/g, dry weight) in spinach plant supplied with chromium (III) and chromium (VI) at 2µg/ml.

Treatment Selenite/ Selenate µg/ml	Chromium concentrations (µg/g, DW) in spinach grown on: CrIII - Source: Presence of selenite Root*	Shoot*	Presence of selenate Root*	Shoot*	CrVI - Source: Presence of selenite Root*	Shoot*	Presence of selenate Root*	Shoot*
				SAND CULTURE				
0.5	26.5 *(1.00)*	11.1 *(0.75)*	26.0 *(1.05)*	11.1 *(0.72)*	08.4 *(0.72)*	03.4 *(0.29)*	07.7 *(0.63)*	03.7 *(0.30)*
1.0	26.5 (1.10)	10.1 (0.79)	23.5 (1.01)	11.1 (0.75)	06.3 (0.59)	02.6 (0.25)	07.3 (0.62)	03.2 (0.25)
2.0	20.2 (0.97)	09.3 (0.71)	20.0 (0.98)	08.4 (0.69)	06.0 (0.57)	01.6 (0.21)	06.3 (0.60)	01.9 (0.24)
4.0	18.2 (0.88)	09.1 (0.64)	19.3 (0.95)	05.9 (0.50)	05.0 (0.45)	01.4 (0.21)	06.1 (0.58)	01.6 (0.24)
6.0	07.1 (0.62)	04.6 (0.31)	08.1 (0.70)	04.0 (0.31)	04.1 (0.30)	00.9 (0.20)	02.3 (0.21)	01.0 (0.22)
Blank without Se. supplementation			Root* 26.9 *(1.12)*	Shoot* 11.8 *(0.81)*		Root* 09.0 *(0.75)*		Shoot* 03.9 *(0.31)*
Corr. Coeff.	-0.96*	-0.94*	-0.95*	-0.98*	-0.91*	-0.90*	-0.94*	-0.75*
				SOIL CULTURE				
0.5	05.0 *(0.44)*	02.6 *(0.22)*	04.2 *(0.31)*	03.4 *(0.29)*	03.4 *(0.28)*	02.6 *(0.23)*	03.4 *(0.29)*	03.0 *(0.27)*
1.0	02.6 *(0.22)*	02.4 *(0.21)*	03.5 *(0.29)*	03.0 *(0.24)*	03.1 *(0.27)*	02.2 *(0.21)*	03.2 *(0.25)*	02.7 *(0.24)*
2.0	02.4 *(0.19)*	01.9 *(0.23)*	03.3 *(0.26)*	02.4 *(0.21)*	01.9 *(0.21)*	02.0 *(0.19)*	01.9 *(0.21)*	01.7 *(0.25)*
4.0	02.4 *(0.21)*	0.20 *(0.15)*	02.7 *(0.21)*	02.3 *(0.20)*	01.8 *(0.19)*	01.9 *(0.21)*	01.8 *(0.20)*	01.5 *(0.23)*
6.0	01.2 *(0.21)*	00.1 *(0.11)*	01.2 *(0.19)*	01.4 *(0.20)*	01.7 *(0.21)*	01.8 *(0.22)*	01.7 *(0.22)*	01.4 *(0.21)*
Blank without Se. supplementation			Root* 5.3 *(0.40)*	Shoot* 4.5 *(0.32)*		Root* 3.5 *(0.30)*		Shoot* 3.3 *(0.20)*
Corr. Coeff.	-0.83*	-0.90*	-0.95*	-0.90*	-0.87*	-0.80*	-0.86*	-0.89*

*p<0.05
Values in parenthesis(SD)

Table 2 Plant-tissue concentrations of chromium (µg/g, dry weight) in spinach plant supplied with chromium (III) and chromium (VI) at 5 µg/ml.

Treatment Selenite/ Selenate (µg/ml)	Chromium concentrations (µg/g, DW) in spinach grown on							
	CrIII - Source				CrVI - Source			
	Presence of selenite		Presence of selenate		Presence of selenite		Presence of selenate	
	Root*	Shoot*	Root*	Shoot*	Root*	Shoot*	Root*	Shoot*
				SAND CULTURE				
0.5	37.9	13.7	19.4	12.5	16.1	05.6	17.5	05.4
	(1.31)	*(0.87)*	*(0.93)*	*(0.89)*	*(0.89)*	*(0.41)*	*(0.93)*	*(0.41)*
1.0	31.9	13.7	19.2	11.5	07.4	04.5	14.7	05.1
	(1.25)	(0.86)	(0.94)	(0.88)	(0.60)	(0.31)	(0.92)	(0.39)
2.0	29.4	07.0	15.8	06.5	04.9	04.4	12.4	03.4
	(1.10)	*(0.61)*	*(0.92)*	*(0.51)*	*(0.33)*	*(0.29)*	*(0.90)*	*(0.25)*
4.0	21.9	06.7	12.5	05.0	04.7	03.4	04.4	02.1
	(0.90)	*(0.53)*	*(0.91)*	*(0.40)*	*(0.32)*	*(0.23)*	*(0.30)*	*(0.21)*
6.0	04.0	01.3	03.0	03.1	01.8	03.2	01.6	01.6
	(0.31)	*(0.17)*	*(0.23)*	*(0.24)*	*(0.23)*	*(0.22)*	*(0.21)*	*(0.20)*
Blank without Se. supplementation			Root* 38.4 *(1.30)*	Shoot* 14.0 *(0.89)*		Root* 19.7 *(0.90)*		Shoot* 05.7 *(0.45)*
Corr. Coeff.	-0.83*	-0.90*	-0.95*	-0.90*	-0.87*	-0.80*	-0.86*	-0.89*
				SOIL CULTURE				
0.5	05.6	05.9	06.4	05.9	05.2	04.6	05.5	04.1
	(0.42)	*(0.50)*	*(0.52)*	*(0.47)*	*(0.43)*	*(0.34)*	*(0.47)*	*(0.31)*
1.0	05.1	03.7	05.9	05.7	04.8	04.6	02.2	03.4
	(0.40)	*(0.30)*	*(0.49)*	*(0.44)*	*(0.35)*	*(0.32)*	*(0.21)*	*(0.29)*
2.0	04.6	03.7	05.3	05.3	04.2	04.4	01.3	01.8
	(0.31)	*(0.29)*	*(0.43)*	*(0.42)*	*(0.32)*	*(0.31)*	*(0.20)*	*(0.21)*
4.0	04.1	03.3	03.7	02.8	03.7	04.3	01.2	01.7
	(0.30)	*(0.27)*	*(0.29)*	*(0.24)*	*(0.29)*	*(0.32)*	*(0.18)*	*(0.23)*
6.0	03.1	02.3	01.9	01.2	03.6	04.1	01.1	01.2
	(0.27)	*(0.21)*	*(0.25)*	*(0.20)*	*(0.29)*	*(0.30)*	*(0.19)*	*(0.18)*
Blank without Se. supplementation			Root* 08.7 (0.76)	Shoot* 07.6 (0.64)		Root* 06.0 (0.49)		Shoot* 05.4 (0.45)
Corr. Coeff.	-0.81*	-0.81*	-0.95*	-0.98*	-0.88*	-0.79*	-0.76*	-0.86*

* $p<0.05$

Values in parenthesis (SD)

Table 3 Source to plant transfer coefficients for chromium in spinach treated with CrIII and CrVI at 2 and 5 µg/ml in presence of increasing selenium supplementation.

Treatment Selenite/ Selenate µg/ml	Chromium concentrations (µg/g, DW) in spinach grown on							
	CrIII - Source				CrVI - Source			
	Presence of selenite		Presence of selenate		Presence of selenite		Presence of selenate	
	2µg/ml	5µg/ml	2µg/ml	5µg/ml	2µg/ml	5µg/ml	2µg/ml	5µg/ml
				SAND CULTURE				
0.0	19	10	19	10	6	5	6	5
0.5	19	10	19	6	6	5	6	5
1.0	18	10	17	6	4	4	5	4
2.0	15	7	14	4	4	3	4	3
4.0	14	6	13	3	3	1	4	1
6.0	6	1	6	1	2	1	2	1
Corr. Coeff.	-0.96*	-0.96*	-0.97*	-0.90*	-0.92*	-0.95*	-0.95*	-0.95*
				SOIL CULTURE				
0.0	5	3	5	3	3	2	3	2
0.5	4	2	4	2	3	2	3	2
1.0	2	2	3	2	3	2	3	1
2.0	2	2	3	1	2	2	2	1
4.0	1	1	2	1	2	2	2	1
6.0	1	1	1	1	2	1	1	1
Corr.	-0.82*	-0.88*	-0.94*	-0.79*	-0.82*	-0.79*	-0.95*	-0.66*

* $p < 0.05$

the culture. The finding is in the harmony with contention that plants grown on high chromium level would absorb more chromium than plants grown on low chromium level (Cary and Kubota 1990). However, increase in feed concentration, of course, increase enrichment in the plants but not proportionally.

Effects of selenite and selenate species on the reduction of chromium uptake from its two oxidation states were found in the following order: **Cr III-SeO_4^{2-} ≈ Cr III - SeO_3^{2-} > Cr VI - SeO_4^{2-} ≈ CrVI - SeO_3^{2-}.**

To explain the above order of reduction behaviour in the plant uptake of chromium, attempts have been made to synthesise the possible explanations based on our experimental findings and pertinent information available on the relevant topic.

The reduction in the chromium uptake in the presence of selenite and selenate species in the plant is attributed to the tendency of the reduced form of selenium to form metal - selenium compounds (Cary 1981). Trivalent chromium supplied to the plants might react with selenite species added or reduction product of selenate, to form chromium selenite [$Cr_2(SeO_3)_3$]. Chromium selenite is a very sparingly soluble compound (Mellor 1952) and appears unavailable to plants, thus causing the reduction in chromium uptake. Hexavalent chromium added to the plant is likely to be reduced into trivalent chromium under prevailing conditions and react with selenite species.

$$Cr^{6+} + SeO_3^{2-} \underset{\text{[K overall = 2.08 x } 10^{18}\text{]}}{\rightleftharpoons} Cr^{3+} + SeO_4^{2-}$$ [Bodek et al., 1988]

However, in this redox process selenite is likely to be oxidized into selenate. Therefore, the remaining fraction of selenite left might be only involved in the reaction with the formation of less chromium selenite and thus causing comparatively less reduction in chromium uptake from hexavalent chromium treatment.

The reduction behaviour in plant uptake of chromium in the plants grown on sand and soil has been compared. Soil is a complicated matrix and has various adsorptive components, not present in pure quartz sand, might sequester chromium and reduce its availability although selenium would also be adsorbed as well. The higher reduction in the uptake of chromium observed in the plants grown on sand compared to soil culture under similar selenite and selenate treatment, can be explained on the basis of ease of the formation of chromium selenite. The quartz sand used possesses sharp edges and better aeration conditions can cause greater root injury releasing larger amount of root exudates (Hale et al., 1978) providing favourable reducing environment (lowering of pH) for the formation of interacting selenite species. The release of organic acids (low molecular weight) as root exudates and their degradation products have been reported to provide low pH. Marschner and Romheld, 1983, Yossef and Chino, 1991). However, such low concentration of metabolic products and root exudates might be capable of reducing selenite and selenate species in the immediate vincity of root environment.

Linear regression of dry matter yield of spinach plant were computed against selenium supplementation. The poor correlation ($p>0.05$) for dry matter yield with selenite and selenate treatment in sand and soil culture respectively indicate that the treatments imposed had no toxic effect and were not resulting into salt injury to the plants grown on these contamination sources.

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