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Effect of selenite and selenate on plant uptake and translocation of mercury by tomato (*Lycopersicum esculentum*)

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Abstract

Pot culture experiments containing sand and soil, at two levels of mercury (2 and 5 μg mL^{-1}) added through irrigation with increasing supplementation of selenium (selenite and selenate) led to a decrease in the uptake of mercury by tomato (*Lycopersicum esculentum*) plant. Both the forms of selenium (selenite and selenate) were found to be equally effective in reducing the mercury accumulation by plants. The observed reduction pattern of mercury accumulation in plant tissues has been discussed on the basis of the formation of insoluble HgSe complex in soil-root environment.

Introduction

The uptake and translocation of root-absorbed mercury in different parts of variety of plants have been actively investigated (Barghigiani and Ristori, 1994; Godbold, 1991; Kabata and Pendias, 1984). Mercury bioaccumulation seems to be affected by several additive, synergistic and antagonistic interactions with other species present in the soil-root environment. Selenium has been reported to counteract the toxicity, chemical carcinogenesis and also reduce the plant uptake of some toxic metals (Cary, 1981; Whanger, 1981). Selenium-mercury interactions have been studied in zooplankton (Pelletier, 1985; Rouleau et al., 1992). Apparently, no studies have been reported on the possibility of Hg-Se interactions in the soil-plant system, particularly those with different oxidation states of selenium.

Studies on Cr-Se, Cd-Se and Hg-Se interactions in various plants have recently been reported by us (Shanker et al., 1995a, b, 1996 a, b). The present communication describes the antagonistic effect of selenite and selenate species on the uptake of mercury in *Lyropersicum esculentum* grown on sand and soil cultures, using ^{203}Hg as tracer.

Materials and methods

Pot culture experiments under laboratory conditions were performed on the tomato (*Lycopersicum esculentum*) plant for a growth period of 100 days in sand and soil (2.5 kg) using plastic containers. Quartz sand was used after standard washing. Plants grown on sand culture were irrigated with complete nutrient solution and iron citrate (Hoagland and Arnon, 1950). The soil used in the experiments had the following characteristics: pH-8.2; 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 applied., About eight seeds were sown in each pot and after ten days of germination four plants per pot were retained for the study. The plants were irrigated with distilled water. After 90 days of growth, the plants were transferred to the fumehood of our radiochemical laboratory. Sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4) and mercuric chloride ($HgCl_2$) AR grade were used for the treatments. Selenite and selenate (inactive) at concentrations (0, 0.5, 1.0, 2.0, 4.0, 6.0 mg L^{-1} and mercury (2 and 5 mg L^{-1}), labelled with ^{203}Hg were supplied to the plants separately (Exp. I and II). ^{203}Hg was obtained from the Board of Radiation and Isotope

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Technology (BRIT), BARC, Bombay. Quality control of ^{203}Hg activity in stock solutions used for each treatment was monitored in feeding solutions. The total Hg concentration in the ^{203}Hg solution was not verified analytically, as the nominal specific activity was used to calculate the mass of Hg added with ^{203}Hg spikes. Any errors in quantification of Hg residue due to variations in specific activity of ^{203}Hg stock would be small due to the large ratio of nonradioactive to radioactive mercury in the feed solution. To determine the possibility of Hg accumulation in the plants from the atmospheric environment in which it grows via dry and/or wet deposition as foliar uptake, plants with no Hg and Se treatments (Exp. III) were placed in identical environments along with those of treated plants (Exp. I and II).

Plants were kept in the fumehood for 10 days with a daily illumination of fourteen hours (600 W m^{-2}). The upper surface of sand and soil was kept wet, in order to avoid the mercury volatilization during the treatment. Plants were harvested and washed thoroughly with water, HNO_3 (pH 2), finally with distilled water and then placed on filter paper. Plant tissues were separated into root, shoot and fruit and then packed into plastic vials of standard geometry. Accurately weighed amounts of material were counted over a planar NaI (Tl) detector coupled to a 4K MCA (Canberra Accuspec Card). After counting, dried weight of the samples were obtained by bringing them to constant weight at 50 °C in an oven (Barghigiani and Ristori, 1995). The counting geometry was pre-calibrated for efficiency with known amounts of ^{203}Hg activity from the 0.279 MeV photopeak area. The activity of ^{203}Hg was calculated and converted to total amount of mercury in different parts of the plants per gram of dry weight. The data represent mean values obtained after performing analyses in duplicate.

Statistical analysis

Statistical analysis was performed using SPSS/PC+$_{TM}$ software package. Tests for non normal data were computed by the Mann-Whitney (independent) U-test to compare individual means using least significant differences at $p \leq 0.05$. Correlation coefficients were used to relate Hg concentration in root, shoot and fruit of the tomato plant and to selenite and selenate treatments supplied to plants grown on sand and soil. Linear regression statistics were applied to the biomass data.

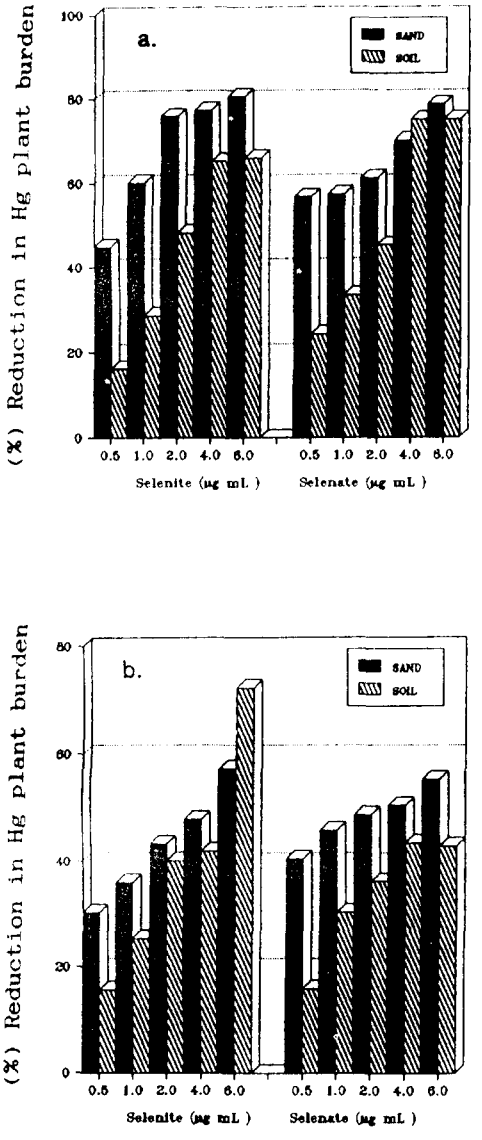

Figure 1. Percentage reduction in mercury burden of whole plant (tomato) in the presence of selenite and selenate, irrigated with (**a**) 2 mg L^{-1} (**b**) and 5 mg L^{-1} of Hg.

Results

Table 1 and 2 represent the accumulation of mercury in different parts of the tomato plant, grown on sand and soil cultures, irrigated with (2 and 5 mg L^{-1}) of mercury as $HgCl_2$ with increasing concentrations of selenite and selenate (0.5–6.0 mg L^{-1}). Statistically significant decrease in the uptake of mercury in root and fruit with increasing concentrations of selenite and selenate (0.5–6.0 mg L^{-1}) in both the culture media was observed, shoot, on the other hand exhibited increased uptake of mercury. It indicates the possible existence

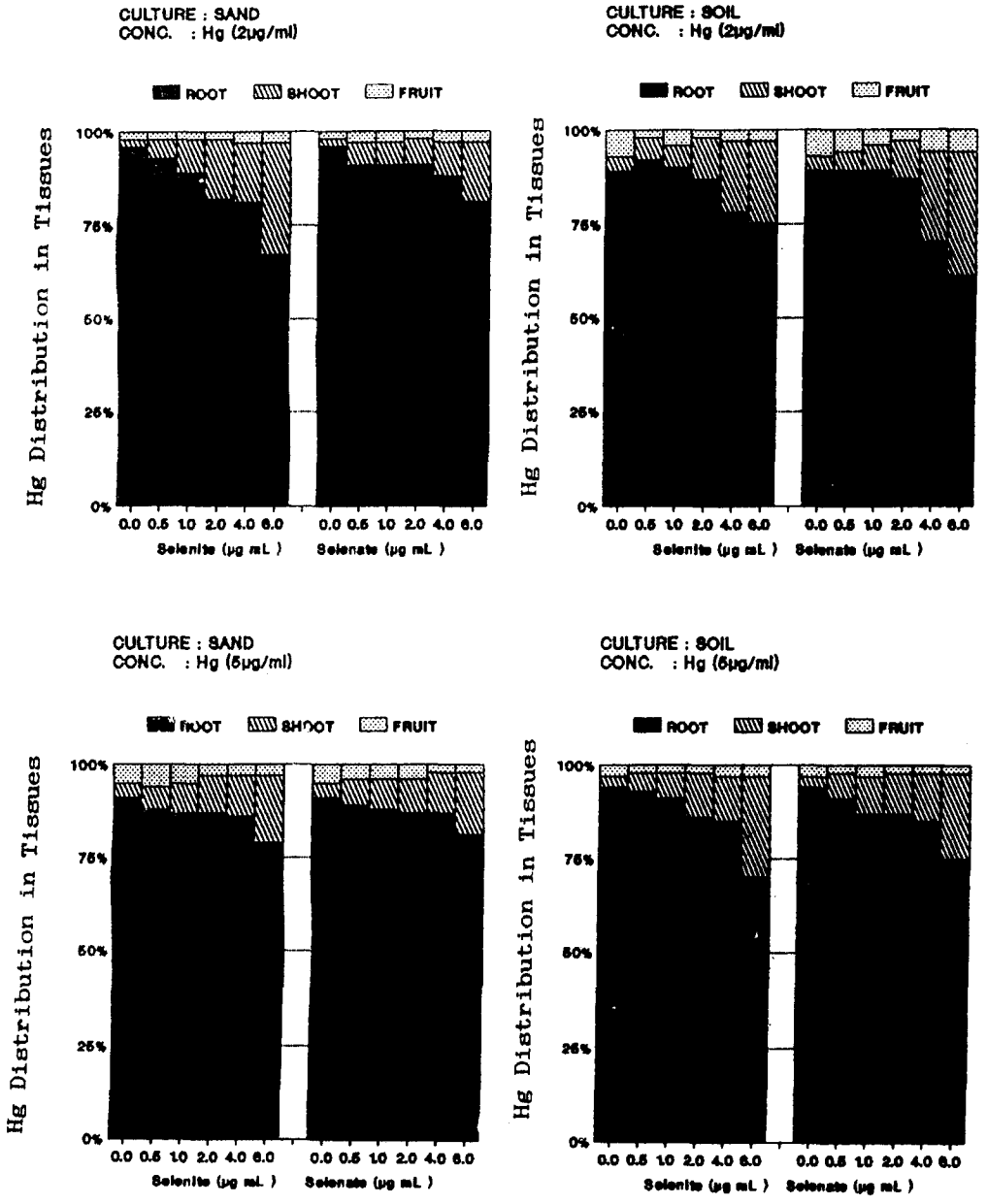

Figure 2. Percentage distribution of mercury in tomato plant tissues in the presence of selenite and selenate supplementation.

of Hg × Se interaction ($p < 0.05$) thereby resulting in the reduction of mercury burden in the whole plant on account of addition of selenium treatments.

The uptake of mercury as a function of selenite and selenate concentrations has been calculated in terms of reduction percentage (whole plant) and distribution percentage in various tissues i.e., root, shoot and fruit. Both the forms of selenium (selenite and selenate) were found equally effective in reducing mercury burden of the plant ($p \geq 0.179$, Mann-Whitney U test). However, in general, these treatments were found to be more effective in reducing mercury burden of the plants grown on sand culture ($p \leq 0.015$, Mann-Whitney U test) (Figure 1).

Linear regressions computed for data were indicative of statistically insignificant difference ($p > 0.05$) in dry matter yields among various selenium treatments (0.05–6.0 mg L^{-1}), suggesting that no selenium toxicity or salt injury occurred to the plant.

Table 1. Plant-tissue concentrations of mercury (ng g^{-1} dry weight) in tomato plant irrigated with 2 mg L^{-1} of mercury as $HgCl_2$

Treatment	Hg uptake in plants grown on sand						Hg uptake in plants grown on soil					
Selenite/	Selenite			Selenate			Selenite			Selenate		
Selenate	Root	Shoot	Fruit	Root	Shoot	Fruit	Root	Shoot	Fruit	Root	Shoot	Fruit
(mg L^{-1})	(ng g^{-1})						(ng g^{-1})					
0.0	404.50	8.13	8.05	404.50	8.13	8.05	120.09	5.21	9.44	120.09	5.21	9.44
0.5	215.59	10.82	5.24	164.88	10.56	6.24	103.81	6.48	2.61	91.31	5.76	5.11
1.0	148.27	14.78	4.43	163.95	11.18	4.56	86.62	7.27	2.26	79.70	6.55	3.57
2.0	83.56	14.79	3.62	147.55	11.40	4.11	60.50	7.60	1.75	64.08	7.53	2.13
4.0	77.26	15.18	3.47	111.76	11.40	3.46	36.64	8.78	1.67	23.73	8.09	2.06
6.0	55.31	24.49	2.52	73.01	14.21	3.23	34.71	10.18	1.11	20.80	11.08	1.97
Corr. coeff.	–0.755*	0.914*	–0.808*	–0.718*	0.870*	–0.816*	–0.926**	0.969***	–0.724*	–0.942**	0.975***	–0.717*

* $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Table 2. Plant-tissue concentrations of mercury (mg g^{-1} dry weight) in tomato plant irrigated with 5 mg L^{-1} of mercury as $HgCl_2$

Treatment	Hg uptake in plants grown on sand						Hg uptake in plants grown on soil					
Selenite/	Selenite			Selenate			Selenite			Selenate		
Selenate	Root	Shoot	Fruit	Root	Shoot	Fruit	Root	Shoot	fruit	Root	Shoot	Fruit
(mg L^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})	(ng g^{-1})
0.0	705.18	28.22	39.59	705.18	28.22	34.59	315.62	9.58	12.13	315.62	9.58	12.13
0.5	475.38	29.80	33.99	411.18	33.35	15.71	263.51	13.56	7.55	256.92	20.08	6.98
1.0	430.07	39.49	25.43	367.95	35.52	15.36	215.88	17.44	5.29	205.59	23.41	6.04
2.0	381.15	44.36	12.95	346.79	36.30	13.38	173.62	23.28	5.15	186.26	23.43	5.61
4.0	345.38	46.07	11.94	333.07	42.17	7.74	167.01	23.79	5.02	161.23	24.92	4.61
6.0	260.51	59.34	10.39	279.18	59.02	7.38	65.27	24.89	3.43	145.36	44.98	2.19
Corr. coeff.	–0.710*	0.715*	–0.685*	–0.796*	0.961**	–0.751*	–0.942**	0.854**	–0.747*	–0.857**	0.902**	–0.839**

* $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Discussion

The reduction in mercury uptake in the presence of selenite and selenate species observed in plant may be attributed to the tendency of the reduced form of selenium to form a HgSe complex, no presently obtained evidence. The complex has been reported to be stable and insoluble (HgSe, $\log K_{sp} = -64.5$) (Kotrly and Sucha, 1985) which appears to be unavailable for plant uptake. To explain the formation of the HgSe complex, we propose a synthesis of different approaches involving the interaction between Hg forms, the role of root exudates in altering the pH and redox capabilities of the soil-root environment.

Mercury and selenium show following course of reduction favoured by low pH in soil root environment (Allaway, 1975; Anderson, 1979).

$$Hg^0 \leftrightharpoons Hg_2^{2+} \leftrightharpoons Hg^{2+} \leftrightharpoons MeHg \leftrightharpoons Me_2Hg$$

$$SeO_4^{2-} - - - > SeO_3^{2-} - - - > Se^0 - - - > Se^{2-}$$

Selenite or selenate added to the culture media tends to get reduced into elemental selenium (Se^0) (Adriano, 1986). The release of organic acids as root exudates is likely to provide low pH (Hale and Griffin, 1974) required for the reduction of selenite or selenate. However, low concentrations of root exudates seem to be capable of reducing only low levels of selenite or selenate in the immediate vicinity of the root. Elemental selenium (Se^0) may interact chemically with mercury,

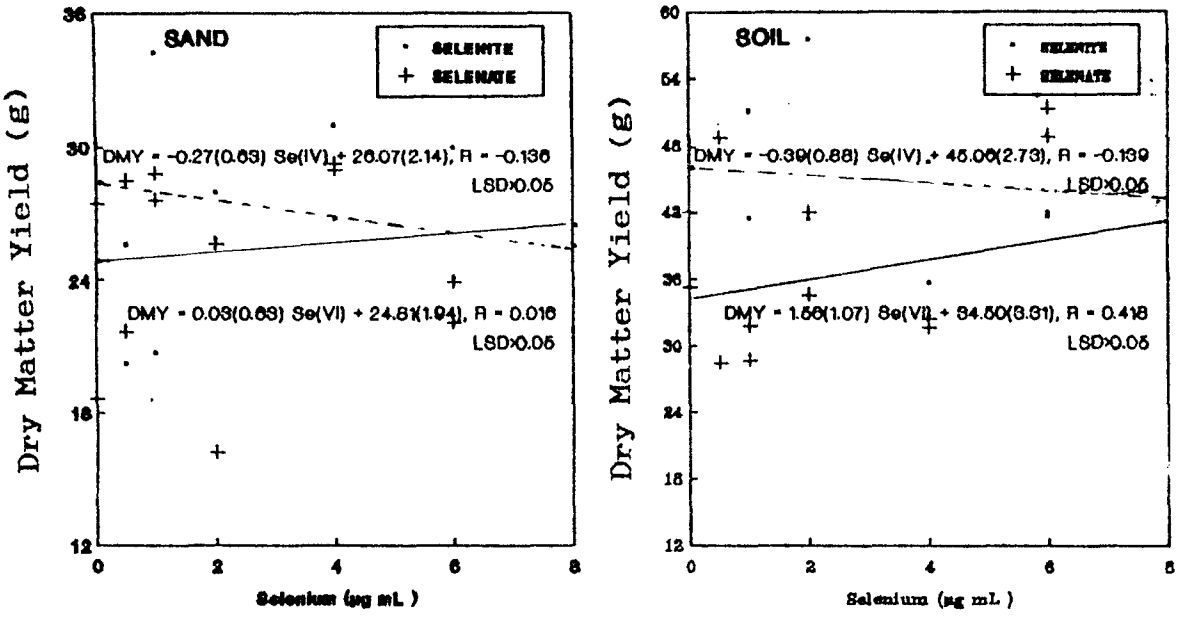

Figure 3. Linear regressions of between selenium supplementation and dry matter yield of the plants grown in sand and soil.

reduced in a similar fashion forming the HgSe complex (Hogland, 1994; Millis, 1974). The possibility of the extension of the reduction course of elemental selenium Se^0 into Se^{2-} might also be considered (Diplock, 1993), for the interaction of Hg^{2+} and Se^{2-} to form Hg-Se complex.

The reduction in uptake of mercury observed in plants grown on sand culture compared to soil culture under similar selenite and selenate treatments can be explained on the basis of the ease of the formation of the Hg-Se complex. Sand being an inert medium with better aeration causes greater root injury and presumably may release greater amounts of root exudates (Hale et al., 1978) providing favorable reducing environment for the formation of elemental selenium (Se^0) or selenide (Se^{2-}).

Selenite and selenate treatments were found to show similar trends of reduction in mercury uptake in both the experiments (Hg treatments; 2 and 5 mg L^{-1}). The level of accumulation of mercury in different plant tissues indicates that most of the mercury (89–96%) is retained in the root and only a small amount of mercury is translocated to aerial part (2–7%). Increasing concentration of selenite and selenate, reduce the overall plant mercury burden, simultaneously affecting its distribution pattern also. Selenium supplementation seems to facilitate root to shoot transfer of mercury not affecting the mercury concentrations in fruit (Figure 2).

The order of mercury accumulation in the plant tissues: shoot > fruit > root > soil or sand, in Experiment III (plant with no Hg treatment) is possibly an indication of the tendency of mercury to enter the plant through the foliar route (Huckabee et al., 1983).

Linear regressions of dry matter yield of tomato plants were computed against selenium supplementation. The poor correlation ($p > 0.05$) for dry matter yield with selenite, selenate treatments in sand and soil culture respectively, indicate that both selenium species have no toxic effects and has not resulted into salt injury to plants grown on contamination sources (Figure 3). The general dry matter yield of plants grown in soil was observed to be higher than plants grown in sand culture which can be ascribed to the higher buffering capacity of the soil.

Further studies on plant uptake and translocation of various forms of selenium as a result of Se-Hg interaction in soil and sand culture is the topic of ongoing research.

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