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Reduction of Manganese Accumulation by Ethylenediamine Tetraacetic Acid and Nitrilo Triacetic Acid in Okra (*Abelmoschus esculentus* L.) Grown in Sewage-Irrigated Soil

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Disposal of sewage has become a global problem. One alternative disposal operation which is currently receiving rather widespread attention is diverting to cropland to provide water and nutrients to enhance yields. A problem arises since the usage of sewage introduces inadvertently metals which are often accumulated by plants. Metals uptake by plants is important in the study of metal transfer from soil systems to plant systems. Studies have shown greater metal uptake of manganese has resulted in growth reduction in plants where sewage is often used for irrigation (Allison et al. 1981; Cunningham et al. 1975). Plant disorder caused by high concentrations of manganese has been reported in lettuce (Vlamis and Williams 1973) and in maize and barley (Singh and Stenbergik 1974).

Synthetic chelating compounds like ethylenediamine tetraacetic acid (EDTA) and nitrilo triacetic acid (NTA) are known as effective chelating agents, forming stable metal-chelate complexes. There has been considerable speculation about whether metal-chelate complexes will be taken up by plants or remain in the soil. One hypothesis is that chelation formed in the soil reduces metal toxicity and metal uptake dramatically (Halvorson and Lindsay 1977). The alternative emphasizes that the metal chelate-complex formed in the soil can increase metal solubility and promote metal diffusion and, hence, potential uptake and toxicity (Wallace et al. 1974).

Srinivas and Singh (1989) reported that manganese at higher concentrations (> 200 $\mu g/g$) exerts deleterious effects on plants. The objective of this study was to test the hypothesis as to whether chelating agents reduce manganese uptake in plants by forming a stable metal-chelate complex or enhances manganese uptake. The test plant selected was okra, Abelmoschus esculentus, an

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important vegetable crop in India and in other tropical parts of the world. The uptake and accumulation of manganese was estimated with and without chelating agents.

MATERIALS AND METHODS

Soil used in the experiment was claylike in texture, with a pH of 8.1; EC, 220.1 m mhos/cm; organic carbon, 1.67%; cation exchange capacity, 16.7 meq/100g and manganese, 3.5 μg/g. Sewage water used in this study to grow the plants was black in color with a pH of 7.3; conductivity, 222.0 m mhos/cm; total residue, 1.475 mg/1; chloride 402.0 mg/1; nitrogen, 51 mg/1; sodium, 103 mg/1; potassium, 61 mg/1; lead, 0.03 mg/1; and manganese, 8 mg/1.

Seeds of A. esculentus were surface-sterilized with a 0.01% solution of mercuric chloride for 5 min and washed thoroughly with sterilized distilled water. They were planted in buckets treated with various levels of manganese as manganese chloride (0, 100, 200, 500, and 1000 μg Mn/g soil), with and without the addition of EDTA and NTA in 10^{-2}M concentrations. The pH of the chelating agents was brought down to 7.0 by titrating with alkali. Controls of manganese, EDTA and NTA alone were maintained throughout the experiment. After seven days, when germination was normally completed, seedlings were thinned to ten uniform individuals in each bucket.

Plants were grown in a greenhouse irrigated with sewage water for 30 days with a photoperiod of 16:8 hr light:dark. Temperature during the photoperiod was between 22-25° C and during the night 17-18° C. Relative humidity was maintained between 55 and 65% during the light period and 75-80% during the dark. Manganese was extracted by wet digestion with a mixture of nitric acid: perchloric acid: sulphuric acid (Baker and Smith 1974). The estimation of manganese was done by atomic absorption spectrophotometry (AAS) taking five replicates. The reference standards were made by dissolving 1.000 g of manganese metal in a minimum volume of 1:1 nitric acid and diluted to 1 liter to give 1000 mg/1 Mn. The limit of detection by AAS was 3 μg/1 and the recovery was 100 percent. Anova and Studentized range tests were used in analysing the data.

RESULTS AND DISCUSSION

Roots of plants treated with high concentrations of manganese had high levels of accumulation. The accumulation was highest in plant roots treated with 1000 mg/g manganese, and treatments of plants with either EDTA or NTA resulted in considerably lower levels (Table 1).

Table 1. Accumulation of manganese ($\mu g/g$) in roots of okra after application with EDTA and NTA.

Manganese Conc ($\mu g/g$)	Mn+EDTA (10^{-2} M)		Mn+NTA (10^{-2}M)	Mn
0	17.3±1.8		22.3±3.2	29.3±1.8
	<	NS		>
50	24.2±1.2		31.2±1.6	43.9±2.2
	<	NS		>
100	34.2±1.3		29.1±1.9	66.3±1.3
	<	NS	>	
200	39.7±1.1		44.3±1.8	87.2±2.4
	<	NS	>	
500	47.3±2.8		54.1±1.8	95.1±3.5
	<	NS	>	
1000	59.3±1.7		67.1±2.4	121.4±4.1
	<	NS	>	

p> 0.005. Means were separated by analysis of variance and Studentized range test (k= 15.10). <NS> indicates means were not significantly different in a row.

Table 2. Accumulation of manganese ($\mu g/g$) in stems of okra after application with EDTA and NTA.

Manganese Conc ($\mu g/g$)	Mn+EDTA(10^{-2}M)		Mn+NTA (10^{-2}M)	Mn
0	15.2±1.2		21.3±1.3	39.4±2.7
	(- 12.1)		(- 4.48)	(+ 34.4)
	<	NS	>	
50	22.4±1.8		29.2±1.4	63.1±3.9
	(- 7.43)		(- 6.41)	(+ 43.7)
	<	NS	>	
100	23.9±2.1		32.8±2.1	74.8±3.2
	(- 30.1)		(- 21.6)	(+ 12.8)
	<	NS	>	
200	33.7±2.4		38.5±1.9	90.3±2.6
	(- 15.1)		(- 13.0)	(+ 35.5)
	<	NS	>	
500	43.8±2.6		47.3±2.5	110.3±3.5
	(- 7.39)		(- 12.5)	(+ 15.6)
	<	NS	>	
1000	52.7±1.8		59.5±2.8	138.4±4.6
	(- 11.1)		(- 11.3)	(+ 14.0)
	<	NS	>	

p> 0.005. Means are separated by analysis of variance and Studentized range test (k= 16.76). <NS> indicates means are not significantly different in a row.
Values in parentheses indicate percent increase (+) or decrease (-) translocation of metal from roots to stems.

Greater accumulation of manganese in stems and leaves ocurred in plants treated with higher concentrations of manganese (< 100 μg/g) (Tables 2,3).

The application of chelating agents reduced significantly the uptake of manganese in roots, stems and leaves even at 500 and 1000 μg/g Mn exposure. Plants treated with

Table 3. Accumulation of manganese (μg/g) in leaves of okra after application with EDTA or NTA.

Manganese Conc (μg/g)	Mn+EDTA (10^{-2}M)		Mn+NTA (10^{-2}M)	Mn
0	11.3±1.8		14.6±1.3	44.2±1.8
	(- 25.6)		(- 31.4)	(+ 12.1)
	<	NS	>	
50	19.9±1.6		23.8±2.1	76.3±2.5
	(- 11.1)		(- 18.5)	(+ 20.9)
	<	NS	>	
100	22.4±2.1		28.2±3.1	83.1±3.2
	(- 6.27)		(- 7.0)	(+ 11.0)
	<	NS	>	
200	30.9±1.3		34.6±1.4	96.3±3.6
	(- 8.3)		(- 10.1)	(+ 6.64)
	<	NS	>	
500	37.1±1.6		41.8±2.8	119.3±4.3
	(- 15.2)		(- 11.6)	(+ 8.1)
	<	NS	>	
1000	40.8±2.6		47.2±3.2	143.9±4.2
	(- 22.5)		(- 20.6)	(+ 3.59)
	<	NS	>	

p< 0.005. Means were separated by analysis of variance and Studentized range test (k= 18.90).
Values in parentheses indicate percent translocation increase (+) or decrease (-) of metal from stems to leaves.

various concentrations of manganese in the absence of EDTA or NTA began to transport high amounts of manganese from roots to stems and leaves and their percent translocation from roots is given in Tables 2 and 3, respectively. Foy et al. (1978) reported also that there was a positive correlation between the amounts of manganese in soil and its uptake by plants. Lower amounts of manganese were transported consistently to stems and leaves, even at concentrations of 200 μg/g or more in plants treated with chelating agents.

Table 3 shows the amount of manganese accumulated in leaves treated with and without chelating agents. At 500 and 1000 μg/g exposure of manganese the accumulation was 119.3 and 143.9 μg/g, respectively, whereas in the

presence of EDTA and NTA the levels of uptake were 40.8 and 47.2 μg/g, respectively. The toxicity and uptake of any metal can result only if the metal moves from soil to plant roots. Srinivas (1993) reported that metal uptake and accumulation by plants are usually correlated with substrate concentrations. This study also suggests that manganese present in roots could be transported readily to the shoot system, unlike results reported by Oullette and Dessureaux (1958) that most of the manganese remains in roots without being transported to the shoot system. This study suggests also that manganese will be transported more from roots to leaves, where a high percentage of accumulation will take place.

The application of chelating agents (EDTA and NTA) was quite successful in significant alleviation of manganese uptake. The relative importance of chelating compounds in reducing metal uptake was studied by Srinivas and Singh (1992) who reported that the uptake of chelated metals is less than that of their ionic forms, hence chelated metals are generally less toxic. Evidence for the role of chelation in the detoxification of metals also comes from in vitro studies. Neet et al. (1982) found that metal-induced inhibition of yeast hexokinase activity was reversed by EDTA. These authors argued that the formation of metal-ATP complex was responsible for inhibition of hexokinase activity and amelioration was due to the formation of stable complexes. In the present study the uptake of manganese in the presence of chelating agents was also less, probably because of the formation of stable metal-chelate complexes which are poorly absorbed by plants. EDTA was found to be more effective in reducing metal uptake than NTA. The difference in their efficiency may be due to the (Martell 1957) difference in their stability constants with the metal.

REFERENCES

Allison DW, Oziolo C (1981) The influence of lead, cadmium and nickel on the growth of rye grass and oats. Plant Soil 62: 81-89

Baker AS, Smith RL (1974) Preparation of solutions for atomic absorption analysis of Fe, Mn, Zn and Cu in plant tissue. J Agr Fd Chem 22: 103-105

Cunningham JE, Keeney DR, Ryan JA (1975). Phyto-toxicity and uptake of metals added to soils as in-organic salts or in sewage. J Environ Qual 4: 60-42

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Ann Rev Pl Physiol 29: 511-566

Halvorson AD, Lindsay WL (1977) The critical Zn concentration for corn and the non absorption of chelated zinc. Soil Sci Am J 41: 531-34

Martell AE (1957) The chemistry of metal chelates in plant nutrition. Soil Sci 84: 13-26
Neet KE, Furman TC, Hueston WJ (1982) Activation of yeast hexokinase by chelators and the enzymic slow transition due to metal-nucleotide interactions. Arch Biochem Biophysics 213: 14-25
Ouellette GI, Dessureaux L (1958). Chemical composition of alfalfa as related to degree of tolerance to manganese and aluminum. Can J Plant Sci 38: 206-214
Singh BR, Stenbergik (1974) Plant responses to micronutrients. II. Uptake distribution and transformation of manganese in maize and barley. Plant Soil 40: 640-654
Srinivas D (1993) Reduction of lead accumulation by ethylenediamine tetraacetic acid and nitrilo triacetic acid in okra (Abelmoschus esculentus L). grown in sewage irrigated soil. Bull Environ Contam Toxicol 51: 40-45
Srinivas D, Singh VP (1989) Effects of mercury and manganese on physiological aspects of radish (Raphanus sativus L). Proc Nat Young Scientist's Semin. Environ Pollut. Univ. Agrl Sci Banglore pp-100-104
Srinivas D, Singh VP (1992) Ameliorative effects of ethylenediamine tetraacetic acid on lead toxicity in okra (Abelmoschus esculentus L). In: The Canadian Society of Plant Physiologists 34th Annual Meeting. Memorial Univ of Newfoundalnd, NF Canada
Vlamis J, Williams DE (1973) Manganese toxicity and marginal chlorosis of lettuce. Plant Soil 20: 221-231
Wallace A, Mueller RT, Cha JW, Alexander GV (1974) Soil pH, excess lime and chelating agent on micronutrients in soybeans and bush beans. Agron J 66: 698-700