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

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Waste water is applied to cropland primarily to provide water and nutrients to enhance yields. A problem arises since the usage of sewage introduces inadvertently heavy metals and these often accumulate in plants. Total metal uptake by plants is important in the study of metal transfer from soil system to plant system. Studies have shown greater metal uptake has resulted in growth reduction in plants where sewage is often used for irrigation (Allison et al. 1981).

Synthetic chelating compounds like ethylenediamine tetraacetic acid (EDTA) and nitrilo triacetic acid (NTA) are known as effective chelating agents to form stable metal-chelate complexes. There has been considerable speculation about whether metal-chelate complexes will be taken up by the 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 convection and diffusion and hence potential uptake and toxicity (Wallace et al. 1974).

The objective of this study was to test the hypothesis as to whether chelating agents reduce metal uptake in plants by forming a stable metal-uptake complex or enhances metal uptake. The test plant selected was (okra) Abelmoschus esculentus, an important vegetable crop in India as well as in other tropical parts of the world. The uptake and accumulation of lead was estimated with and without chelating agents.

MATERIALS AND METHODS

Soil used in the experiment was clay like in texture,

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with a pH of around of 8.1; EC, 220.1 m mhos/cm; organic carbon, 1.67%; cation exchange capacity, 16.7 meq/100g; manganese, 3.5 $\mu g/g$; zinc, 2.0 $\mu g/g$. Sewage water which was used in the study to grow the plants was black in color with a pH of around 7.3, conductivity, 222.0 m mhos/cm, total residue, 1.475 mg/l; chloride 402.0 mg/l; nitrogen, 51 mg/l; sodium, 103 mg/l; potassium, 61 mg/l; lead, 0.03 mg/l and manganese, 8 mg/l, respectively.

Seeds of A. esculentus were surface sterilized with a 0.01% solution of mercuric chloride for 5 mins and washed thoroughly with sterilized distilled water. They were planted in buckets treated with various levels of lead as lead chloride (0, 50, 100, 200, 500, and 1000 μg Pb/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 lead, EDTA and NTA alone were maintained throughout the experiment. After seven days, when germination is normally complete, seedlings were thinned to ten uniform individuals in each bucket.

Plants were grown in a greenhouse irrigated with sewage water for 40 days with a photoperiod of 16h a day. Temperature during the photoperiod was between 22-25^0 C and during the night 17-18^0 C. Relative humidity was maintained between 55 and 65% during the light period and 75-80% during the dark. The estimation of lead was done taking five replicates following the methods of Khan et al. (1972) and Baker et al. (1974) using atomic absorption spectrophotometry.

RESULTS AND DISCUSSION:

Plants treated with lead had high levels of accumulation in the roots. The accumulation was highest in plant roots treated with 1000 $\mu g/g$ lead, and treatments of plants with either EDTA or NTA resulted in considerably lower levels (Table 1). Greater accumulation of lead in stems and leaves began started in plants treated with 200 $\mu g/g$ lead or more (Tables 2,3).

The application of chelating agents significantly reduced the uptake of lead, and no lead was detected at the 50 $\mu g/g$ exposure treated with EDTA or NTA. Trace amounts were detected in plants treated with NTA or EDTA at 100 $\mu g/g$. Consistently lower amounts of lead were detected even at concentrations of 200 $\mu g/g$ or more in plants treated with chelating agents. Table 4 shows the amount of lead accumulated in fruits treated with and without the chelating agents in the presence of lead. Less uptake of lead was observed even at 500 and 1000 $\mu g/g$ concentrations in the presence of chelating agents, whereas more than one ppm of lead was accumulated at 500

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

Lead Conc ($\mu g/g$)	Pb+EDTA (10^{-2}M)	Pb+NTA (10^{-2}M)	Pb
0	ND	ND	ND
50	2.6±0.07	3.2±0.15	6.7±0.001
	<	NS	>
100	3.8±0.07	4.5±0.3	10.3±0.15
	<	NS	>
200	6.5±0.09	8.1±1.1	18.6±0.42
	<	NS	>
500	15.7±1.1	13.3±0.75	37.3±0.79
	<	NS	>
1000	19.8±1.2	23.7±1.31	48.3±1.42

ND = not detectable $p< 0.001$.

Means are separated by analysis of variance and Studentized range test (k= 2.28). <NS> indicates means are not significantly different in a row.

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

Lead Conc. ($\mu g/g$)	Pb+EDTA (10^{-2}M)	Pb+NTA (10^{-2}M)	Pb
0	ND	ND	ND
50	ND	ND	2.2±0.015
100	0.5±0.03	1.3±0.07	3.6±0.13
	<	NS	>
200	2.8±0.31	3.1±0.13	8.7±0.94
	<	NS	>
500	3.5±0.72	6.7±0.57	13.7±0.56
1000	6.9±1.1	7.9±1.38	21.2±2.1

ND = not detectable; $p< 0.005$

Means are separated by analysis of variance and Studentized range test (k= 0.94). <NS> indicates means are not significantly different in a row.

and 1000 $\mu g/g$ concentrations in the absence of chelating agents. The toxicity and uptake of any metal can result only if the metal moves from soil to plant roots. Srinivas (1990) reported that metal uptake and accumulation by plants is usually correlated with substrate concentrations. This study also suggests that

Table 3. Accumulation of lead ($\mu g/g$) in leaves of A.esculentus after application with EDTA and NTA.

Lead Conc. ($\mu g/g$)	Pb+EDTA (10^{-2}M)	Pb+NTA (10^{-2}M)	Pb
0	ND	ND	ND
50	ND	ND	0.6±0.001
100	ND	0.3±0.001	1.1±0.03
200	0.7±0.01	1.3±0.11	4.7±0.75
	<	NS >	
500	1.2±0.002	1.7±0.35	8.1±0.13
	<	NS >	
1000	1.7±0.13	2.1±0.14	10.3±1.2
	<	NS >	

ND = not detectable; $p< 0.001$
Means are separated by analysis of variance and Studentized range test (k= 0.66). <NS> indicates means are not significantly different in a row.

Table 4. Accumulation of lead ($\mu g/g$) in fruits of A.esculentus after application with EDTA and NTA.

Lead Conc. ($\mu g/g$)	Pb+EDTA (10^{-2}M)	Pb+NTA (10^{-2}M)	Pb
0	ND	ND	ND
50	ND	ND	ND
100	ND	ND	0.3±0.0
200	ND	ND	0.9±0.03
500	0.3±0.01	0.5±0.02	1.3±0.09
	<	NS	>
1000	0.6±0.03	0.9±0.04	2.1±0.1
	<	NS	>

ND = not dectectable; $p< 0.001$
Means are separated by analysis of variance and Studentized range test (k= 0.57). <NS> indicates means are not significantly different in a row.

lead present in the soil at concentrations higher than 200 $\mu g/g$ could be readily transported to the shoot system unlike results reported by Warren et al. (1970) that most of the lead remains in the soil without being transported to edible portions of the plant. Miller and Koeppe (1970) reported that corn (Zea mays) accumulated large amounts of lead when grown in lead- contaminated soil. The present study also suggests that lead will be accumulated more in roots than in shoots.

Application of chelating agents (EDTA and NTA) was quite successful in significant alleviation of lead 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 also the uptake of lead in the presence of chelation agents was less, probably because of the formation of stable metal-chelate complexes which are poorly absorbed by plants. Of the chelating agents used EDTA was found to be the most effective in reducing the metal uptake followed by NTA. The difference in their efficiency may be due to the (Martell, 1957) difference in their stability constants with the metal.

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