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Ameliorative Effects of Ethylenediamine Tetraacetic Acid and Nitrilo Triacetic Acid on Lead Toxicity 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 use of sewage may introduce heavy metals which could be accumulated (Srinivas 1993) by plants, causing serious injuries. Lead is considered quite hazardous to plants since it affects a number of physiological and metabolic aspects of plants (Koeppe 1980). Plants can absorb lead from soil, water and air through their roots and leaves (Carlson et al. 1975).

In spite of indiscriminate dumping of industrial effluents onto croplands, no serious efforts have been made to eliminate the heavy metals in sewage. Synthetic compounds like ethylenediamine tetraacetic acid (EDTA) and nitrilo triacetic acid (NTA) are known as effective chelating agents as to heavy metals. Considerable speculation exists about whether metal-chelate complexes reduce toxicity of heavy metals. One hypothesis is that chelation formed in the soil reduces metal toxicity and metal uptake dramatically (Halvorson and Lindsay 1977). Another emphasizes that the metal-chelate complex formed in the soil can increase metal solubility and promote diffusion, and, hence, potential uptake and toxicity (Wallace et al. 1974).

The objective of this study was to determine if chelating agents reduce or enhance metal toxicity in plants. Okra (Abelmoschus esculentus L.), an important vegetable crop in India as well as in other tropical parts of the world, was used as the test plant. Chlorophyll, protein content and nitrate reductase activity were the variables measured as phytotoxic end points.

MATERIALS AND METHODS

Soil used in the experiment was clay-like in texture,

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with a pH of 8.1; EC 220.1 m mhos/cm; organic carbon, 1.67%; cation exchange capacity, 16.7 meq/100g; manganese, 3.5 μg/g; and zinc, 2.0 μg/g. The sewage water was black 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 mins and washed thoroughly with sterilized distilled water. They were planted in buckets treated with various concentrations 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 adjusted to 7.0 by titrating with alkali. Controls of lead, EDTA and NTA alone were maintained throughout the experiment. 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 16:8 h light: dark. Temperature during the day or light period was between 22-25^0 C, and during the dark 17-18^0 C. Relative humidity was maintained between 55 - 65% during the light period and 75-80% during the dark. Leaf pigments were extracted with 80% acetone and the amount of total chlorophyll (mg/g fresh weight) was calculated using the expression of Arnon (1949). Protein and nitrate reductase activity were analyzed following the methods of Lowry et al. (1951) and Shrivastava and Mathur (1980), respectively. Anova and Studentized range tests were used in analysing the data.

RESULTS AND DISCUSSION

Plants treated with lead inhibited all the variables (chlorophyll, protein and NR activity) studied and the inhibition was directly related to the concentrations of the lead from 50 to 1000 μg/g (Figs.1a, 2a and 3a). High concentrations of lead affected chlorophyll content and a maximum reduction of total chlorophyll was observed as 4.3 mg/g fresh weight at 1000 μg/g lead (Fig 1a). In controls (without lead, with EDTA and with NTA) the total chlorophyll was 6.8, 6.3 and 6.6 mg/g, respectively. Total chlorophyll content was not significantly different between the three controls and 50 μg/g lead concentration, but a significant reduction was noticed at higher concentrations of lead after the application of chelating agents. Tongaria et al.(1989) reported that impaired chlorophyll development of plants may be due to interference of heavy metals with the structural component of chloroplasts. Reduced chlorophyll formation in rice seedlings has also been reported due to lead

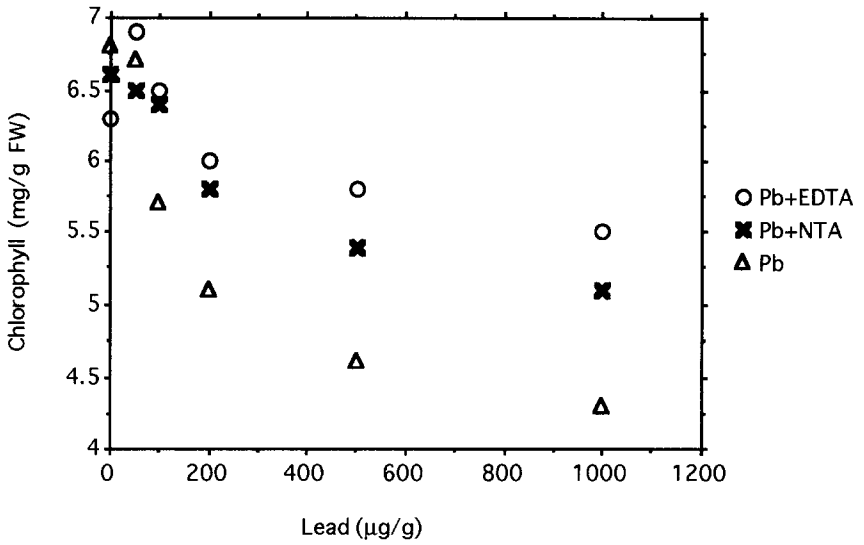

Figure 1a. Chlorophyll content in leaves of okra after the application of lead with EDTA and NTA. Means were separated by Studentized range test (k= 0.71).

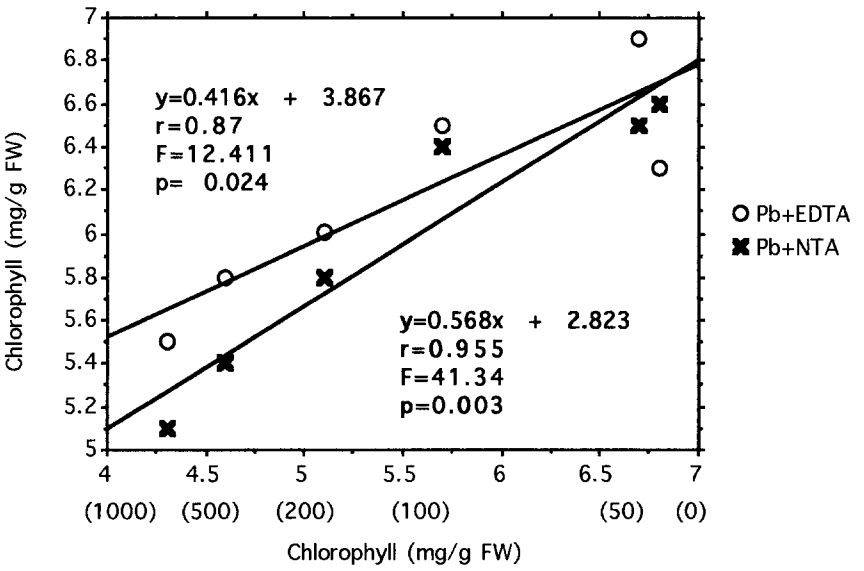

Figure 1b. Regression line of chlorophyll content (mg/g FW) of okra plants grown in different concentrations of lead (µg/g) (X-axis) versus chlorophyll content of plants grown in different concentrations of lead with chelating agents (Y-axis). Numbers in parentheses indicate the concentration of lead (µg/g).

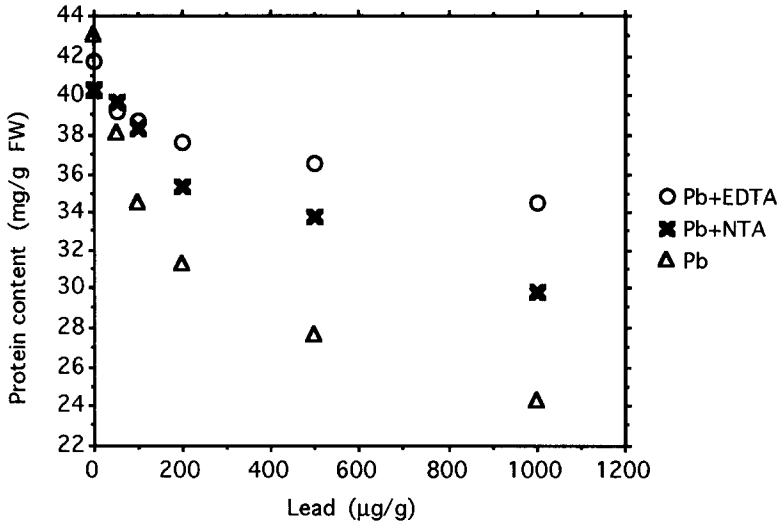

Fig. 2a. Protein content in leaves of okra after the application of lead with EDTA and NTA. Means were separated by Studentized range test (k= 3.64).

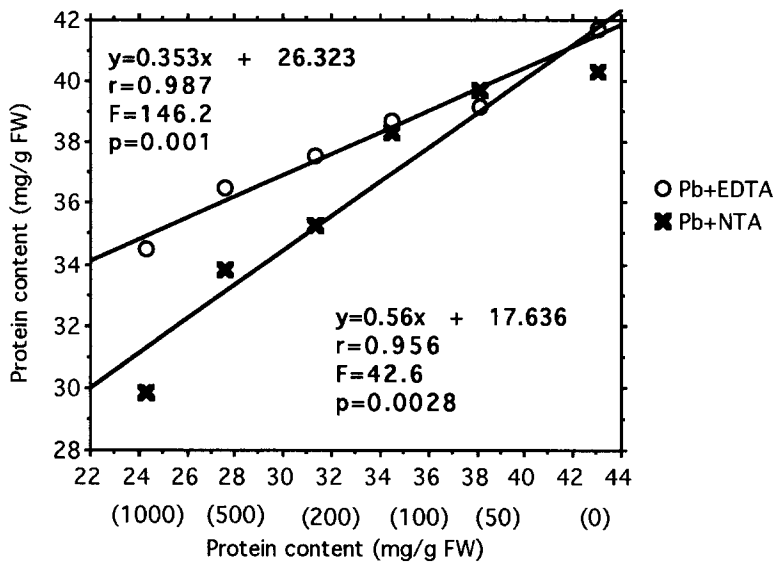

Fig. 2b. Regression line of protein content of okra plants grown in different concentrations of lead (µg/g) (X-axis) versus protein content of plants grown in different concentrations of lead with chelating agents (Y-axis). Numbers in parentheses indicate concentration of lead (µg/g).

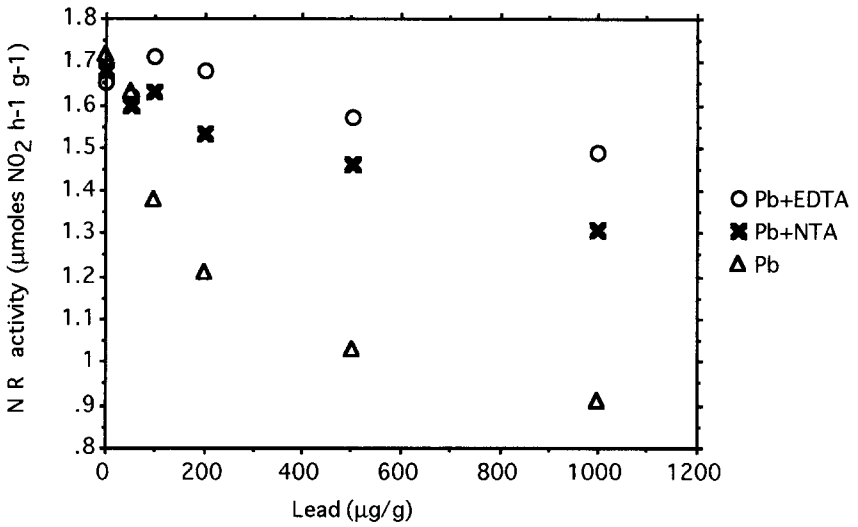

Fig. 3a. Nitrate reductase activity in leaves of okra after the application of lead with EDTA and NTA. Means were separated by Studentized range test (k= 0.25).

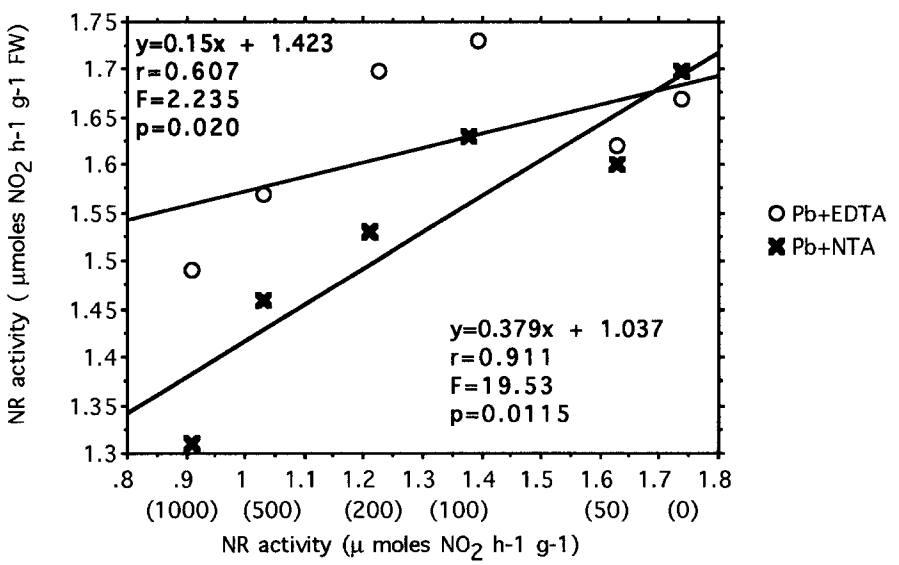

Fig. 3b. Regression line of NR activity of okra plants grown in different concentrations of lead (µg/g) (X-axis) verses NR activity of plants grown in different concentrations of lead with chelating agent (Y-axis). Numbers in parentheses indicate the concentrations of lead (µg/g).

poisoning (Nag et al. 1981).

Lead at concentrations greater than 100 μg/g exerted a deleterious effect on the protein content of the plants. Like chlorophyll, the amount of protein content was also reduced when the concentration of lead increased to 1000 μg/g in the soil (Fig. 2a). The decrease in protein content caused by heavy metals such as lead may be due to the decline in the metabolism of the plant, and this reduced activity can be attributed to a lower level of nucleic acid synthesizing enzymes (Maitra and Mukherji 1979).

Nitrate reductase, one of the most important enzymes in the assimilation of exogenous nitrate, was also affected by lead. Activity of this enzyme in plants gives a good estimate of the nitrogen status of the plant and is very often correlated with growth, yield and protein content (Shrivastava 1980). The present studies showed that the effect of lead on nitrate reductase activity was dependent on the concentration of the metal. At 500 and 1000 μg/g of lead, the enzyme activity was reduced to 1.03 and 0.91 (μ moles NO^2 h^{-1} g^{-1} fresh weight), respectively, but no significant reduction was observed at 50 μg/g. This might have occurred as a result of less uptake and mobilization of lead to the actual site of action (Sinha et al. 1988).

Less reduction of chlorophyll, protein content and nitrate reductase activity was observed in the presence of chelating agents, even at 500 and 1000 μg/g lead (Figs. 1a,2a,3a). Application of chelating agents was quite successful in significant alleviation of lead toxicity. Increased amounts of total chlorophyll, protein content and nitrate reductase activity were observed in plants treated with chelating agents. Regression lines were plotted against the variables (chlorophyll, protein, nitrate reductase activity) treated with chelating agents along with lead at various concentrations (on Y axis in graph) versus the variables treated with lead alone at various concentrations (X axis in graph) (Figs. 1b, 2b, 3b) to show the ameliorative effect of chelating agents.

The relative importance of chelating compounds in reducing metal toxicity in plants was studied by Srinivas (1993) who reported that the uptake of chelated metals is less than that of their ionic forms, hence chelated metals are generally less toxic. In the present study the toxicity of lead 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 the metal toxicity than NTA. The difference in their efficiency may be due to the difference in their

stability constants with the metal (Martell 1957).

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