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Effect of pH and EDTA on Pb Accumulation in *Zea mays* Seedlings

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Abstract: In this research we examined the effect of 0.25, 0.5 and 1 mM $Pb(No_3)_2$ with and without 0.5 mM EDTA in pH 4 and 6 on the growth and Pb accumulation in the root and shoot of four day old seedlings of *Zea mays* at the controlled condition in the period of 72 h. At the end of treatment we determined the length and dry weight of the root and Pb content in the root and shoot of the seedlings. In the second test we considered specially the effect of 0.25, 0.5, 1, 1.5 and 2 mM EDTA with 0.5 mM $Pb(No_3)_2$ on the seedlings of *Zea mays* at the same condition. After 72 h we determined the amount of Pb by Atomic Absorption and EDTA by HPLC and water content of shoot. We concluded (from the results of two tests) that: Pb absorption has grown up parallel to its concentration in growth solution and has inhibited root growth and biomass significantly. Pb taking up in pH 4 was higher than pH 6 and EDTA enhanced Pb accumulation in shoot. But water content of shoot decreased at the concentrations more than 0.5 mM EDTA in growth solution. Maximum level of EDTA accumulated in shoot of plants was at the concentration of 1.5 mM EDTA in the culture, but the highest level of Pb and the least water loss of shoot was at the equimolar Pb and EDTA (0.5 mM Pb with 0.5 mM ETDA). It may concluded that EDTA is taken up by plants, accumulated in shoots in the form of Pb-EDTA or protonated form and enhanced Pb accumulation in shoots of seedlings. So it can be used for phytoextraction of Pb and other metals not only by accumulators but also by tolerant plants such as *Zea mays*. But if EDTA is used for supplying plant micronutrients, its concentration should be minimized and its damage to plants should be clearly considered.

Key words: Pb toxicity, *Zea mays*, EDTA, HPLC

INTRODUCTION

Natural Pb content of soils is strongly related to the composition of the bedrock, but Pb originating from different sources may finally reach the surface soil. Pb is a major chemical pollutant of the soil and environment. It's concentration in soil and vegetation in many countries has increased in recent decades owing to man's activities[1,2]. It is usually insoluble in soils and is bound to soil particles such as clay minerals[3,4]. But many factors like soil pH and chelates increase Pb phytoavailability[5]. Because of many adverse effects of Pb on soil biological activities[6], plant metabolism[7] and the health of human and animals[8] phytoremediation is growing as a potential industry, that uses living green plants for risk reduction by contaminant removal[9,10]. To succeed in this strategy addition of chelates such as EDTA (Ethylenediamintetraaceticacid) and pH adjustment are usually used to enhance phytoextraction[11,12]. EDTA as a synthetic chelate, forms a soluble complex with heavy metals including Pb[13]. It has been shown that EDTA has induced heavy metal accumulation in shoot[12,14], but it's actual mechanism in plants has not been realized well[15]. In this research we investigated the effects of different concentrations of Pb on plant growth, effects of low pH and EDTA in Pb accumulation in the plant of *Zea mays*. We also determined the amount of Pb and EDTA in shoot of seedlings. We suggest that Pb is absorbed in the form of Pb-EDTA or Pb by plant, in spite of the report[16], that describes Fe is absorbed by plant root only when first being split from Fe-chelate complex by a plasma membrane-bound Fe-chelate reductase. EDTA is also absorbed in the form of Pb-EDTA or protonated form.

MATERIALS AND METHODS

Seeds of *Zea mays* Cv. single cross 704 were obtained from Agricultural Research Center of Urmia. They were cleaned, selected by the size, washed with water and detergent and finally with distilled water three times and incubated in 25°C to germinate. Four day-old seedlings with the same size were selected again and nine seedlings (as a replicate) were transferred to a plastic glass containing 50 mL of 0.25, 0.5, 1 mM $Pb(NO_3)_2$ with

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and without 0.5 mM EDTA adjusted to pH 4 and 6. After 72 h growing in an aerated (with air pump) and controlled condition (light density 16000 lux and day/night temperature 24/18°C and humidity 60%), root and shoot of seedlings were harvested separately. To show the effect of Pb on plant growth, effect of pH and EDTA on Pb toxicity, the length, fresh weight and dry weight of the roots and shoots were determined.

Pb accumulation in root and shoot: Four day old seedlings were selected and exposed to different concentrations of Pb and 0.5 mM EDTA at pH 4 and 6 as mentioned before. After 72 h root and shoot of seedlings from each of three replicate (nine seedlings per replicate) were harvested separately. Pb accumulation was determined by the method of Vassil *et al.*[17] with some modification and oven dried for three days at 70°C. They were ground to a powder. 0.1 g dry matter of each replicate was digested with 5 mL concentrated HNO_3 at 180°C for 1.5 h. Then cooled at room temperature. One milliliter of 30% H_2O_2 was added to the samples and heated 180°C for 20 min. They were cooled again. Dionized water was added to the final volume of 50 mL. Pb concentration was determined using Atomic Absorption Spectrophotometer (model: 6300 Shimadzu Japan).

The effect of EDTA on Pb accumulation in shoots: Four day-old seedlings were exposed to 0.25, 0.5, 1, 1.5 and 2 mM EDTA with 0.5 mM $Pb(NO_3)_2$ or only 0.5 mM $Pb(NO_3)_2$ adjusted to pH 4. After 72 h shoots of seedlings were harvested and their Pb content was determined as described before[17].

Effect of EDTA on water content of shoot: Water content was calculated by subtracting dry weight from fresh weight of the shoots of seedlings for each of three replicates (nine seedlings in each replicate) of different concentrations.

EDTA accumulation in shoot: Four day-old *Zea mays* seedlings were exposed to 0.25, 0.5, 1, 1.5 and 2 mM EDTA and 0.5 mM $Pb(NO_3)_2$ or only 0.5 mM $Pb(NO_3)_2$ adjusted to pH 4. After 72 h shoot of seedlings were harvested, frozen at -80°C. Then these materials homogenized (Heidolph Diax 900) and extracted with 1 mM of 50%(V/V) methanol, heated at 80°C for 10 min, then centrifuged at 6000 rpm for 20 min at room temperature and the supernatant was removed. The extraction process was repeated 3 times with the remaining pellet. Supernatants from all 3 extractions were pooled, dried in a speed vacuum (Heidolph LABOROTA 4003, Germany) and resuspended in 0.5 mL dionized water. These samples were then analyzed by HPLC for EDTA.

HPLC analysis of EDTA: Dried 50% methanol shoot extracts dissolved in 0.5 mL iron (III) chloride 7 mM in 0.01 M acetic acid and 0.5 mL dionized water, centrifuged at 10000 rpm for 5 min and filtered through 0.45 μm filter. HPLC analysis was performed by injection of 20 μL of sample and chromatographed on RP-C_{18} column (30 cm, 4 mm ID., 5 μm P.S KNAUER). The column was run isocratically at 0.5 mL min^{-1} with 10% methanol, 8 mM tetrabutyl ammonium hydroxide and 1mM Fe (III) chloride in 50 mM Sodium acetate/acetic acid buffer (pH 4). EDTA was detected at 254 nm using UV-vis. detector (K-2501, KNAUER) according to the method of Bergers and Degroot[18], with some modifications.

Statistical analysis: Data analysis was done using Analysis of Variance Method (ANOVA; proc. GLM. SOSS) and ANOVA, comparing the mean of data by Dankun test and the values indicate the mean of three replicates±SE[19].

RESULTS

We considered the effect of different concentrations of $Pb(NO_3)_2$ with and without 0.5 mM EDTA on *Zea mays* seedlings growth in pH 4 and 6. The results showed that:

Pb toxicity inhibited root elongation and biomass significantly (Fig. 1 and 2). Inhibition effect of Pb increased according to Pb concentration in growth solution (Fig. 1 and 2). Pb accumulation in root of

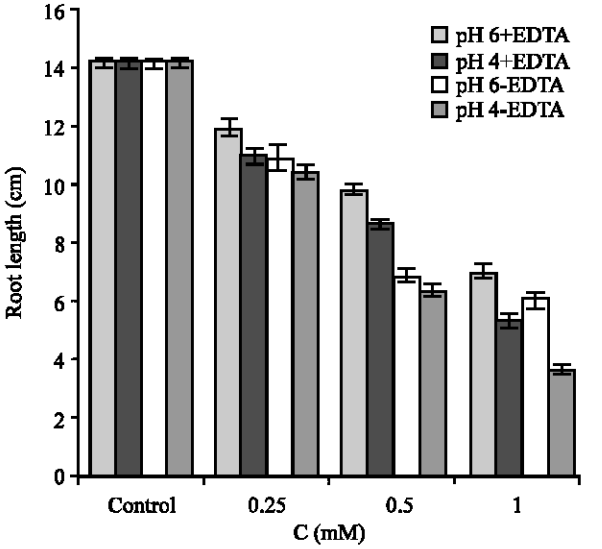

Fig. 1: The effect of different concentrations of $pb(NO_3)_2$ with and without 0.5 mM EDTA at pH,4 and 6 on root growth of *Zea mays* seedlings during 72 h. (Values represent the mean±SE of three replicates)

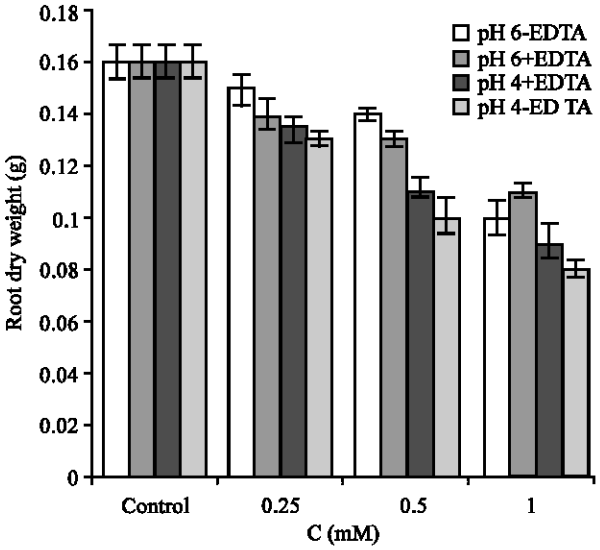

Fig. 2: The effect of different concentrations of $pb(NO_3)_2$ with and without 0.5 mM EDTA on dry weight of roots of *Zea mays* seedlings at pH 4 and 6 during 72 h. (Values represent the mean±SE of three replicates)

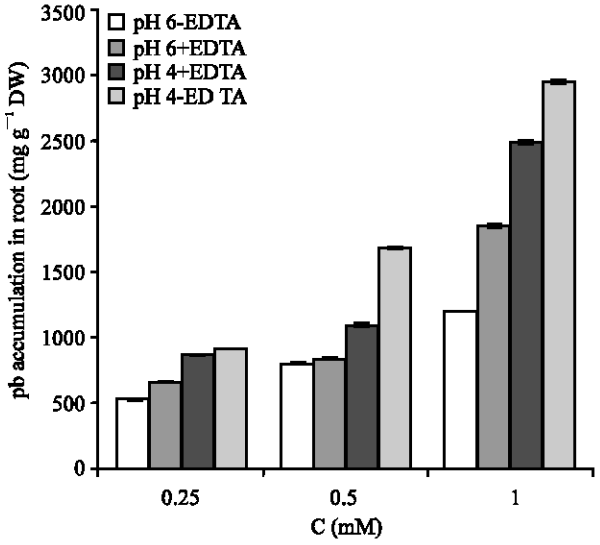

Fig. 3: Pb accumulation in roots of *Zea mays* seedlings exposed to different concentrations of $pb(NO_3)_2$ with and without 0.5 mM EDTA at pH 4 and 6 for 72 h. (Values represent the mean±SE of three replicates)

seedlings in pH 4 was higher than pH 6 (Fig. 3) and the effect of low pH on Pb accumulation in root of plants was greater than EDTA (Fig. 3). As Pb content in shoot of seedlings increased by EDTA (Fig. 4), to know how much EDTA is necessary to accumulate maximum Pb in shoot of plants and what its effect is, we considered specially.

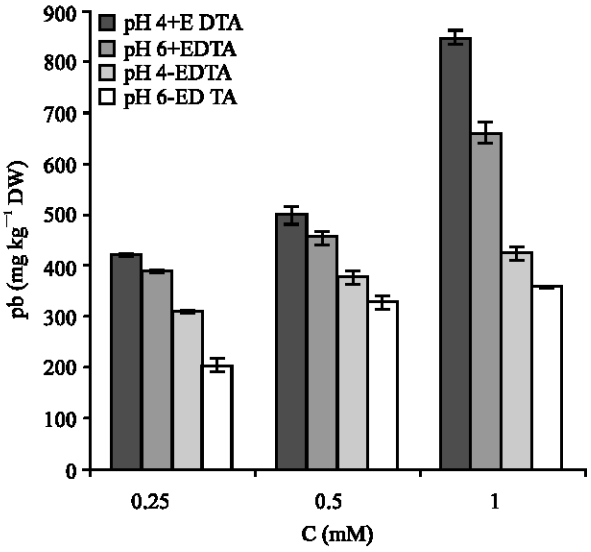

Fig. 4: Pb accumulation in shoot of *Zea mays* seedlings exposed to different concentrations of $pb(NO_3)_2$ with 0.5 mM EDTA at pH4 and 6 during 72 h. (Values represent the mean±SE of three replicates)

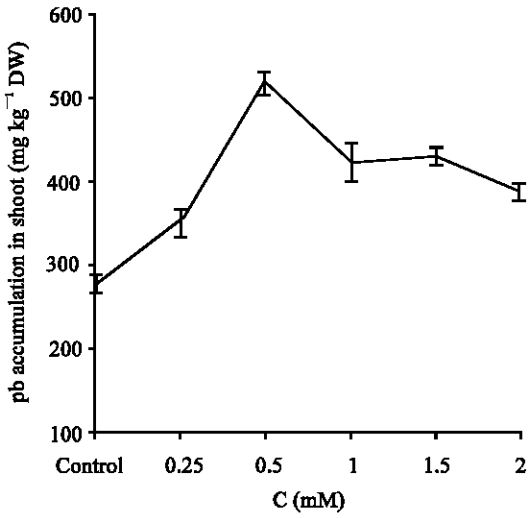

Fig. 5: Pb accumulation in shoots of *Zea mays* seedlings exposed to 0.5 mM pb $(NO_3)_2$ and also 0.5 mM $pb(NO_3)_2$ with different concentrations of EDTA at pH 4 during 72 h. (Values represent the mean±SE of three replicates)

The effect of EDTA in Pb accumulation in shoots of seedlings: Treatment of seedlings with different concentrations of EDTA with 0.5 mM $Pb(NO_3)_2$ showed that EDTA enhanced Pb accumulation in shoots (Fig. 5). but the highest level of Pb was at 0.5 mM EDTA with 0.5 mM $Pb(NO_3)_2$ in growth medium without any apparent metal toxicity effects.

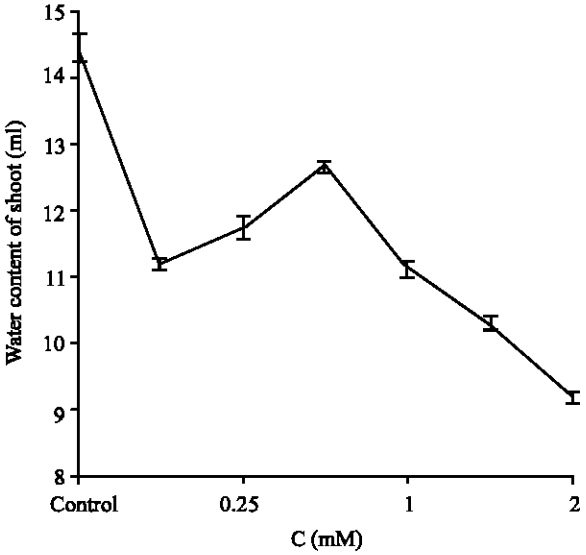

Fig. 6: The effect of only 0.5 mM $pb(NO_3)_2$ or 0.5 mM $pb(NO_3)_2$ with different concentrations of EDTA on water content of shoots of *Zea mays* seedlings at pH 4 during 72 h. (Values represent the mean±SE of three replicates)

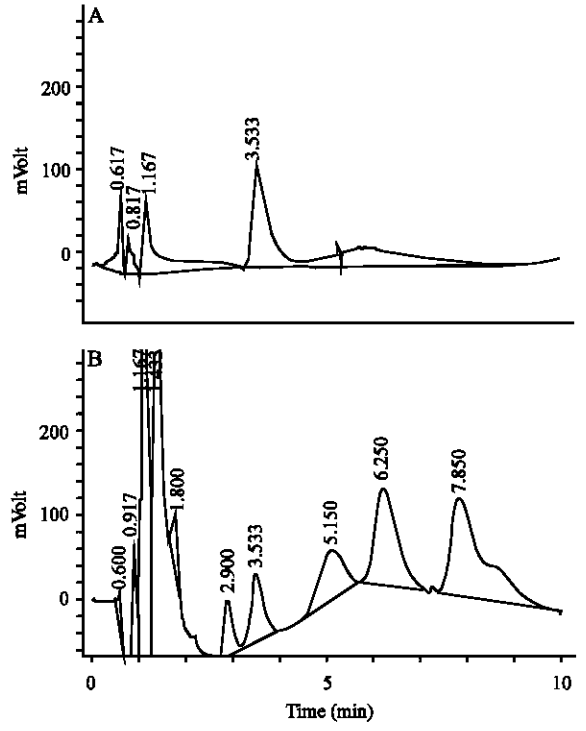

Fig. 7: A. the chromatograph of standard solution of EDTA and B: chromatograph of total EDTA of shoot of *Zea mays* seedlings

Effect of EDTA on water content of shoot: Treatment of *Zea mays* seedlings with different concentrations of EDTA with 0.5 mM $Pb(NO_3)_2$ decreased water content of shoot tissues in the concentrations greater than 0.5 mM EDTA in culture (Fig. 6). But water loss from the shoot was the least in equimolar concentration of Pb and EDTA (0.5 mM Pb with 0.5 mM EDTA) in medium.

EDTA accumulation in shoots: Analyzing the amount of EDTA in shoots of seedlings by HPLC (Fig. 7) indicated that the level of total EDTA increased from 0.25 mM EDTA in growth solution, reached to maximum in 1.5 mM, but its amount decreased at the concentrations more than 1.5 mM (Fig. 8).

DISCUSSION

Treatment of *Zea mays* seedlings with different concentrations of Pb inhibited root growth and biomass (Fig. 1 and 2); As, has been reported in rice by Yell Yang[20]. It is because of Pb accumulation in root and its low mobility in plants[21]. Feng[22] implies that heavy metals decrease viscosity and elasticity of cell wall and inhibit root growth. Considering the effect of pH indicated that root elongation in pH 4 was inhibited greater than pH 6 (Fig. 1). It is due to increased Pb solubility in acidic pH and elevated availability by plant. This confirms the reports which describe that Pb solubility increases in pH ≤ 4[8,23]. EDTA as a synthetic chelate makes a soluble complex with heavy metals including Pb and increases pb accumulation in shoots[17]. For many years it has been used to supply micronutrients for plants but recently it is used to enhance heavy metal accumulation in shoots of accumulators (Indian mustard) and phytoextraction[14]. In this experiment EDTA has also enhanced Pb accumulation in shoot of *Zea mays*, as a Pb-tolerant plant (Fig. 4 and 5). The results of this research indicated >2 mg kg^{-1} dry weight Pb accumulated in root of *Zea mays* seedlings (Fig. 3) and 500 µM Pb treatment inhibited root elongation 50% (Fig. 1) while 10 µM Pb inhibited root elongation of Pb-sensitive varieties of rice 80%[20]; but the effect of EDTA in enhancing Pb accumulation in shoots of *Zea mays* was not as much as Indian mustard (an accumulator)[24]. To understand whether EDTA is absorbed by plant or not, we determined total amount of EDTA in shoots of *Zea mays* seedlings. The results showed that its amount in shoot of seedlings has grown up according to its concentration in growth solution. Shoot EDTA accumulation however does not always appear to be related to physiological stress; at a low

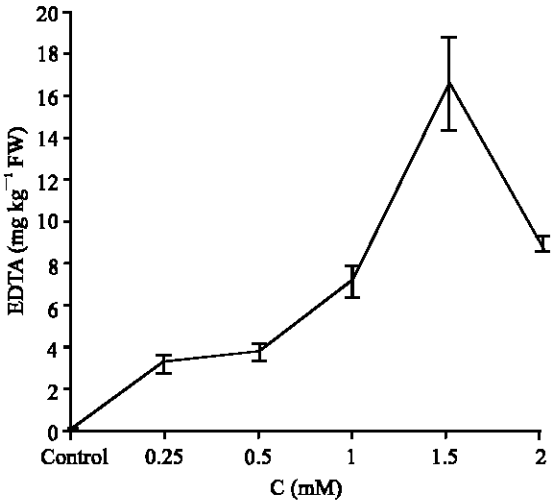

Fig. 8: EDTA accumulation in shoots of *Zea mays* seedlings exposed to different concentrations of EDTA with 0.5 mM $pb(NO_3)_2$ at pH 4 during 72 h. (Values represent the mean±SE of three replicates)

EDTA concentration and neutral pH (pH 6) nearly 0.1±0.02 mM EDTA kg^{-1} dry weigh was observed in shoots of Indian mustard during 48 h[25]. This suggest that, it was taken up by plants but its actual mechanism is not clearly understood. To know how EDTA influences the plants we determined water content of shoots. The results showed that (Fig. 6) EDTA caused reduction in shoot water content at the concentrations more than 0.5 mM EDTA in medium. But there was not any apparent toxicity effect on shoots at the equimolar Pb and EDTA (0.5 mM $Pb(No_3)_2$ with 0.5 mM EDTA). while Pb concentration in shoot was maximum. Figure 8 showed that maximum level of EDTA in shoot of plants was at treatment of 1.5 mM EDTA and this shows that EDTA is absorbed by plants and accumulated in the form of Pb-EDTA and protonated form. So to reduce toxicity effects of EDTA in plants and decrease water content loss we suggest that the rate of EDTA should be minimized or adjusted to heavy metal concentration of growth solution. We conclude from the results of this research: a) Pb is a toxic heavy metal that inhibits plant root elongation, b) *Zea mays* is a Pb-tolerant plant, c) pH is a major factor that influences Pb absorption by plants, d) EDTA enhances Pb accumulation in plants but it is a toxic material and causes water loss and probably some other damages to plants. So it can be used to phytoextraction not only by accumulators but also by tolerant plants. If it is used to supply plant micronutrients, determining the necessary amount and its effects on plants need further investigation[26].

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REFERENCES

1. Cannon, H., 1976. Lead in Vegetation in the Environment. Lovering T.G. Ed., US Geol. Surv. Prof. Pop, 967: 23.
2. Hughes, M.K., N.M. Lepp and D.A. Phipps, 1980. Aerial heavy metal pollution and terrestrial ecosystem. Adv. Eco. Res., 11: 217.
3. Kabata-Pendias, 1980. Heavy metal absorption by clay minerals and oxides of iron and manganese. Mineral Pol., 11: 3.
4. Hildebrand, E. and W.E. Blume, 1974. Lead fixation by clay minerals. Naturewissenschaften, 61: 169.
5. Schmidt, D., 2003. Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation and leaching of heavy metals. J. Environ. Qual., 32: 1939-1954.
6. Andersson, A. and K.O. Nillsson, 1976. Influence on the level of heavy metals in soil and plant from sewage sludge used as fertilizers. Wed. J. Agric. Res., 6: 151.
7. Doelman, P., 1978. Effects of Lead on Properties of Lead-sensitive and Lead-tolerant Cells. Environmental Biochemistry and Geomicrobiology, Krumbein, W. Ed., Ann. Arbor. Sci., Ann. Arbor, MI., pp: 98.
8. Smith, K.S. and H.L.O. Huyck, 1999. An overview of the abundance, relative mobility, bioavailability and human toxicity of metals in the environmental geochemistry of mineral deposits. Part A. processes, techniques and health issues. Plum lee G, S.Econ. Geol. Rev. Econ, Geol, A., pp: 29.
9. Kumar, P.B.N.A., V. Dushenkov, R.A. Mottoh and I. Skin, 1995. Phytoextraction: The use of plants to remove heavy metals from soils. Environ. Sci. Technol., 29: 1232-1238.
10. Salt, D.E., R. Smith and I. Raskin, 1998. Phytoremediation. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49: 643-668.
11. Kramer, U. and A.N. Chardonnens, 2001. The use of transgenic plants in the bioremediation of soils contaminated with trace elements. Applied Microbiol. Biotechnol., 55: 661-672.

12. Mitch, M. Lasat, 2002. Phytoextraction of toxic metals, a review of biological mechanisms. J. Environ. Quality, 31: 109-1.
13. Kroschwitz, J.I., 1995. Iron Compounds. In: Kroschwitz, J.I., Ed., Kirkothmer Iron Compound. Encyclopedia of Chemical Technol., Ed. 4, John Wiley and Sons, New York, pp: 887-888.
14. Huang, J.W., J. Chen, W.B. Berti and S.D. Cunningham, 1997. Phytoremediation of lead-contaminated soils; role of synthetic chelates in lead phytoextraction. Environ. Sci. Technol., 31: 800-805.
15. Wallace, A. and G.A. Wallace, 1992. Some of the problems concerning iron nutrition of plants after four decades of synthetic chelating agents. J. Plant Nutr., 15: 1487-1508.
16. Chaney, R.L., J.C. Brown and L.O. Tiftin, 1972. Obligatory reduction of ferric chelates in iron uptake by soy beans. Plant Physiol., 50: 208-213.
17. Vassil, A.D., Y. Kapulnik, I. Raskin and D.E. Salt, 1998. The role of EDTA in lead transport and accumulation by Indian mustard. Plant Physiol., 117: 447-451.
18. Bergers, P.J.M. and A.C. Degroot, 1994. The result analyzing of EDTA in water by HPLC. Water Res., 28: 639-642.
19. Heim, D.C., G.G. Kenedy and J.W. Wancuyn, 1990. Survey of insecticide resistance among north Carolina, Colorado Potato beetle population. J. Econom. Entomol., 83: 1029-1035.
20. Yell Yang, Y., J. Young Jung, W. Yong Song, H. Soo Suh and Y. Lee, 2000. Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. Plant Physiol., 124: 1019-1026.
21. Kovalevskiy, A.L., 1979. Biogeochemical Exploration for Mineral Deposits, Published for the USDI and the NSf, American Publ. Co. Pvt., New Delhi, pp: 136.
22. Feng, M.J., R.F. Shen, S. Nagao and F. Tanimoto, 2004. Aluminum targets elongating cell wall by reducing cell wall extensibility in wheat roots. Plant Physiol., 5: 583-589.
23. Chung, M.C., G.Y. Shu and J.C. Liu, 1996. Solubility of heavy metals in a contaminated soil; effects of redox potential and pH, water, air, soil pollut. Water Air Soil Pollut., 90: 543.
24. Blaylock, M., D.E. Salt, V. Dushenkov, V. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley and I. Raskin, 1997. Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. Environ. Sci. Technol., 31: 800-805.
25. Kapulnik, Y., B.D. Ensley and I. Raskin, 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol., 31: 860-865.
26. Chieh Chang, Y., M. Zouri, Y. Gogorcena, J.J. Lucena and J. Abadia, 2003. Effect of cadmium and lead on ferric chelate reductase activity in sugar beet roots. Plant Physiol. Biochem., 41: 999-1000.