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PHYTOEXTRACTION OF LEAD (Pb) BY EDTA APPLICATION THROUGH SUNFLOWER (*HELIANTHUS ANNUUS* L.) CULTIVATION: SEEDLING GROWTH STUDIES

NAZILA AZHAR[1], M. YASIN ASHRAF[2*], M. HUSSAIN[1] AND F. HUSSAIN[2]

[1] Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2] Salinity and Environmental Management Division, Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang Road, Faisalabad, Pakistan

Abstract

A series of lab experiments were conducted to assess the toxicity of lead (Pb) and its phyto-extraction through sunflower cultivation. Seedling growth as shoot length, root length and dry matter stress tolerance indices were reduced with increasing concentration of lead (Pb) or EDTA. Very severe effect of Pb and EDTA was noted in case of root length stress tolerance index however, Pb affected root length stress tolerance index more adversely than that of EDTA. The toxicity of Pb for sunflower plants was reduced when applied in combination with EDTA at 1.0-1.0 and 2-1.5 Pb-EDTA levels. Lead @ 3.5 m*M* and EDTA @ 3 m*M* in the growth medium showed 50% reduction in shoot length when applied separately. EDTA also reduced shoot, root length and dry matter stress tolerance indices and the effect was more pronounced at higher levels of EDTA. Root length and dry matter stress tolerance index increased when Pb was applied in combination of EDTA. The results showed that addition of EDTA was effective in enhancing the uptake of Pb in sunflower plants.
Keywords: heavy metal stress, oilseed crop, stress tolerance index, metal chelating agents

Introduction

Metals exist in soils and water as both as natural components or due to industrial and human activity such as metal-rich mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, down-wash from power lines, intensive agriculture, sludge dumping etc. (Ra-skin *et al*., 1994). The flow of heavy metals through different pathways to food stuffs and man is vital in relation to human health (Yamagata & Shigmatsu, 1970; Takijama & Katsumi, 1973, Khan *et al* 1999), because different types of human, animal and plant diseases or physiological disorders are becoming more intricate with industrialization. Due to heavy metal toxicity different types of cancers are frequently being observed in human beings and animals.

Of various heavy metals, lead (Pb) is a major anthropogenic pollutant that has been released to the environment since the industrial revolution and accumulated in different terrestrial and aquatic ecosystems (Verma & Dubey, 2003). It is an extremely toxic metal whose effects on human health have been widely described (Juberg *et al.*, 1997). For example excessive lead (Pb) exposure can cause mental retardation and behavioral disorder and its exposure can occur through multiple pathways, through inhalation of air, water, soil or dust, as it is emitted in the environment from vehicles and automobiles. It can also enter the food chain via plants (Wierzbicka & Antosiewiez, 1993). In plants, its accumulation has been reported in stem, leaves, roots and seeds that increase with

[*] Corresponding Author: Nazila Azhar; gudya_1@hotmail.com

increase in exogenous lead levels (Singh *et al*., 1998). It detrimentally influences plant growth particularly by hampering enzymatic activities (Javed & Saher, 1987).

Numerous efforts have been made to develop technologies for the remediation of contaminated soils, including ex-situ washing with physio-chemical methods, and in-situ immobilization of metal pollutants (Rulkins *et al*., 1995). These methods of cleanup are generally very costly and often harmful to soil properties (i.e. texture, organic matter, microorganisms) that are important for the restoration of contaminated soils. Recently, phyto-extraction of contaminated soils has attracted considerable attention for its low cost of implementation and multiple environmental benefits (McGrath *et al*., 1993; Salt *et al*., 1998).

Two approaches have been proposed for the phyto-extraction of heavy metals (HMs), the use of natural hyper-accumulator plants with exceptional metal accumulating capacity, and the utilization of high biomass plants with a chemically enhanced method of phyto-extraction (Salt *et al*., 1998). Hyper-accumulating plants tend to grow slowly and usually produce low biomass. Many of them are not suitable for use in phyto-remediation in the field. Most of the metals accumulated by plants are often retained in the roots, with relatively limited amount being translocated to the shoots. It has been suggested that higher biomass producing crops can be used such as sunflower, maize, rapeseed, mustard etc. with appropriate chemical treatments to enhance the translocation of an element from roots to shoots (Huang & Cunningham, 1996; Blaylock *et al*., 1997; Huang *et al*., 1997; Shen *et al*., 2002).

For more than four decades, synthetic chelates are being widely used to supply plants with micronutrients in both soil and hydroponics, which can enhance phytoextraction by increasing HMs bioavailability thus enhancing plant uptake and translocation of HMs from roots to green parts of the plants (Huang *et al*., 1997; Epstein *et al*., 1999). Among these, ethylene diamine tetraacetic acid (EDTA) was often found to be the most effective phytoextraction (Blaylock *et al*., 1997) in enhancing the accumulation of heavy metals in the aerial parts of plants. Sunflower can accumulate a significant amount of Pb when induced in combination with chelating agents such as EDTA (Huang & Cunningham 1996, Blaylock *et al*., 1997). Thus this crop could potentially tolerate excessive amount of Pb translocated from roots to shoots.

The present study assesses the tolerance potential of sunflower against deleterious effects of Pb on plant growth and role of EDTA in order to increase this tolerance index.

Materials and Methods

The test plant was *Helianthus annuus* L. (cv. FH-330). Morphologically healthy selected achenes were first sterilized with 0.1% Mercuric chloride for 5 min. and then subsequently washed in distilled water three times. The achenes were soaked in deionized water for 6 h and allowed to germinate in dark at 28±2^{o}C for three days. The achenes were germinated in Petri plates containing 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 m*M* $Pb(NO_3)_2$ and 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 m*M* Na-EDTA solutions. All these treatments were also applied in combinations of Pb and EDTA. Each experiment had three replications. The seedlings were harvested after 10 days and their shoot and root lengths were recorded. The plants were then dried at 70^{o} C for 48 h in an oven. Stress tolerance indices for different growth parameters were calculated in percentage reduction over control under all the above mentioned stresses following Wilkin's method (1957).

Seedling growth or shoot length stress tolerance index (SLSTI), root length stress tolerance index (RLSTI) and dry matter stress tolerance index (DMSTI) under stress over control were also calculated as follows:

SLSTI = (Shoot length of stressed plants/ shoot length of control plants) x 100
RLSTI = (Root length of stressed plants/ root length of control plants) x 100
DMSTI = (Dry matter of stressed plants/dry matter of control plants) x 100

Results

Growth performance of sunflower under Pb, EDTA and Pb+EDTA indicated that lead (Pb) caused a gradual decrease in shoot length from 0.5 m*M* to 5 m*M* $Pb(NO_3)_2$. The concentration 3.5 m*M* of Pb in the growth medium showed 50% reduction in shoot length. However, higher than this concentration caused severe reduction in seedling growth. The comparison between Pb and EDTA indicated that Pb reduced the root length more severely than that of shoot length. The highest value (85.86 %) for SLSTI was recorded at 0.5 m*M* Pb and the lowest (39.08 %) at 5 m*M* Pb. Similarly, maximum RLSTI (77.05 %) was recorded at 0.5 m*M* Pb that was severely reduced at 1m*M* (28.58 %) and the lowest (3.95 %) at 5 m*M* Pb. Dry matter stress tolerance (DMSTI) of sunflower plants were adversely affected with Pb application. Maximum DMSTI (83.72 %) was noted at 0.5 m*M* and the least (21.73%) at 5 m*M* Pb (Fig. 1 A).

Growth performance under EDTA stress also showed a continuous decline with increase in EDTA concentrations. The highest SLSTI (67.95 %) was maintained at 0.5 m*M* EDTA which was the least (33.34 %) in 5 m*M* EDTA. Maximum RLSTI (68.77%) was observed in 0.5 m*M*, which was minimum (5.26%) at 5.0 m*M* EDTA treated sunflower plants. A very similar trend was recorded for DMSTI with the highest value 67.57 % at 0.5 m*M* EDTA and the lowest (25.71 %) at 5 m*M* EDTA. The severe decrease under EDTA stress was recorded in root length. However, the comparison between Pb and EDTA showed that reduction in shoot is more in EDTA than that in Pb (Fig. 1 B).

Inhibition of root elongation and browning of roots occurred in plants grown in lead solutions. However, on addition of chelator (EDTA) in the growth medium an improvement in seedling growth was recorded at 1.0-1.0, 2.0-1.5 Pb-EDTA combinations. The addition of EDTA reduced the toxic effects of Pb and root browning, and promoted the generation of number of side roots (visual observations). Under 2.0-1.5 m*M* Pb-EDTA shoot and root stress tolerance indices values were 89.27% and 76.11%, respectively which were followed by 1.0-1.0 m*M* Pb-EDTA with 86.79% and 64.38% for SLSTI and RLSTI, respectively. The results showed that a combination of Pb and EDTA was beneficial for enhancing growth of sunflower plants as compared with Pb or EDTA alone. Addition of EDTA in the growth medium maintained SLSTI, as 95.92, 89.27, 63.01 and 86.79 % at 2.5-1.5, 2.0-1.5, 1.5-1.5 and 1.0-1.0 m*M* Pb-EDTA, respectively, which were 56.45, 66.65, 71.05 and 78.08 % under 2.5, 2.0, 1.5 and 1 m*M* Pb, respectively. Similarly RLSTI at 2.5-1.5, 2.0-1.5, 1.5-1.5 and 1.0-1.0 m*M* Pb-EDTA was 50.05, 76.11, 16.99 and 65.38 %, respectively against 12.68, 23.04, 25.68 and 28.56 % RLSTI under 2.5, 2.0, 1.5 and 1.0 m*M* Pb, respectively. Dry matter stress tolerance index was also improved by the addition of EDTA along with Pb i.e. 74.16, 63.16, 72.03 and

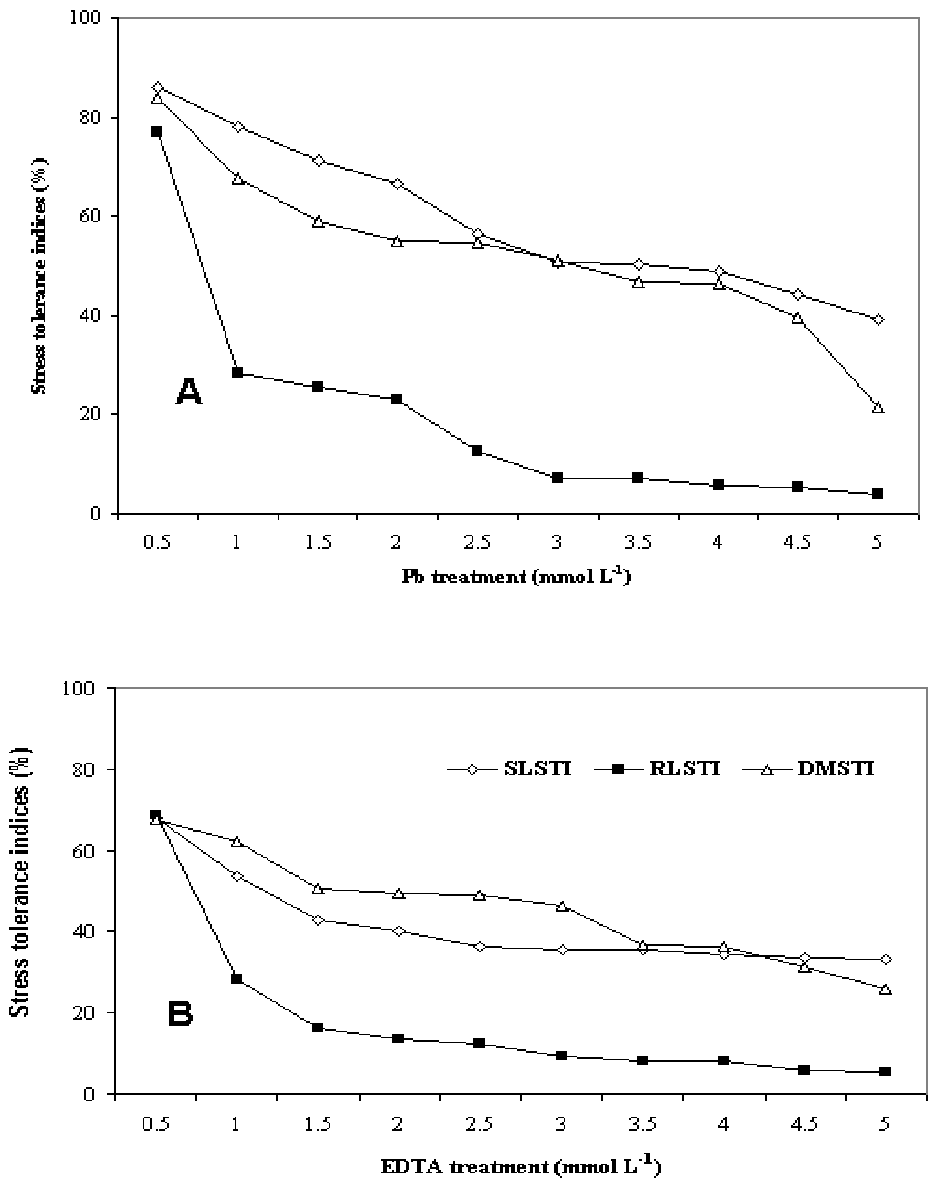

Fig. 1. Effect of different levels of Pb (A) and EDTA (B) on shoot length, root length and dry matter stress tolerance indices.

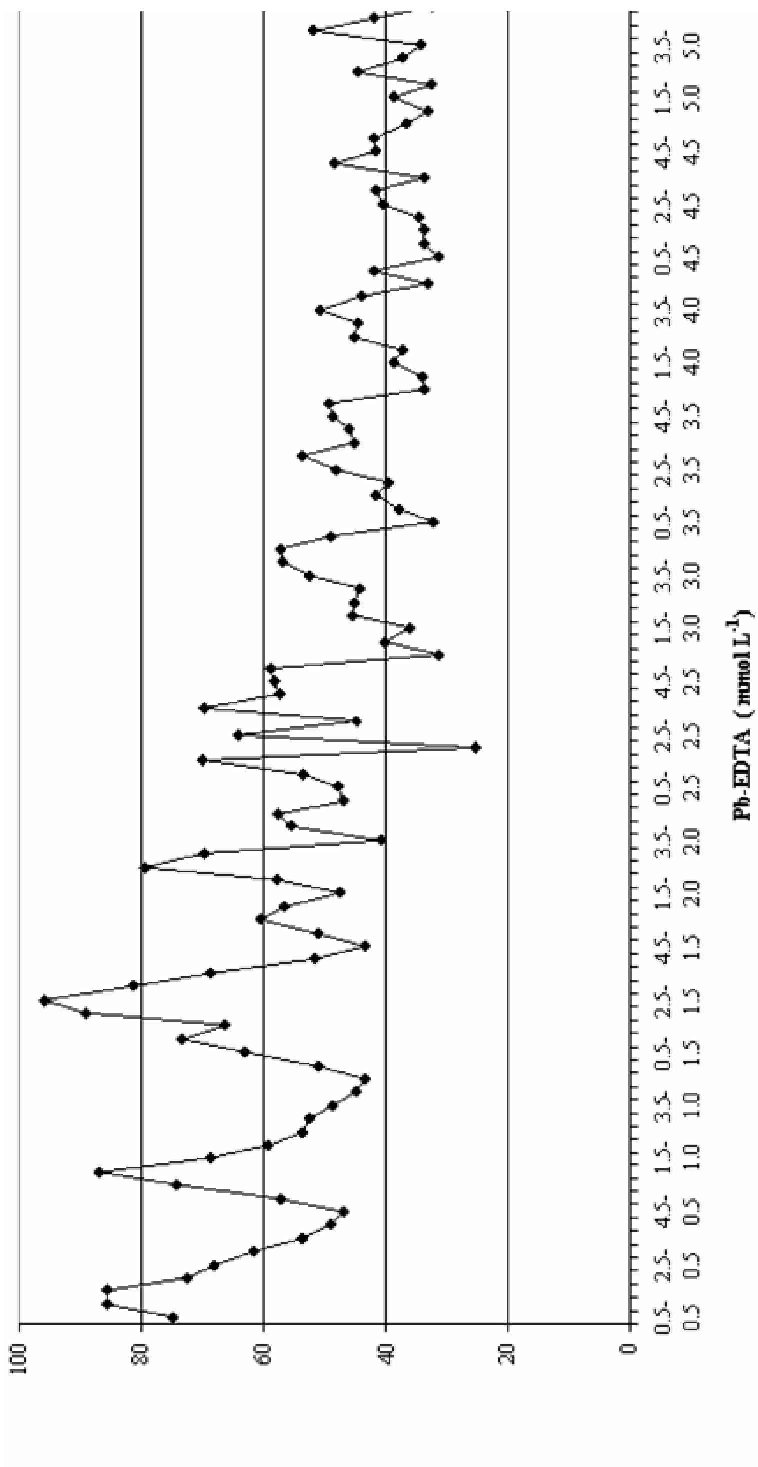

Fig. 2. Effect of different combinations of Pb and EDTA on shoot length stress tolerance index in sunflower plants.

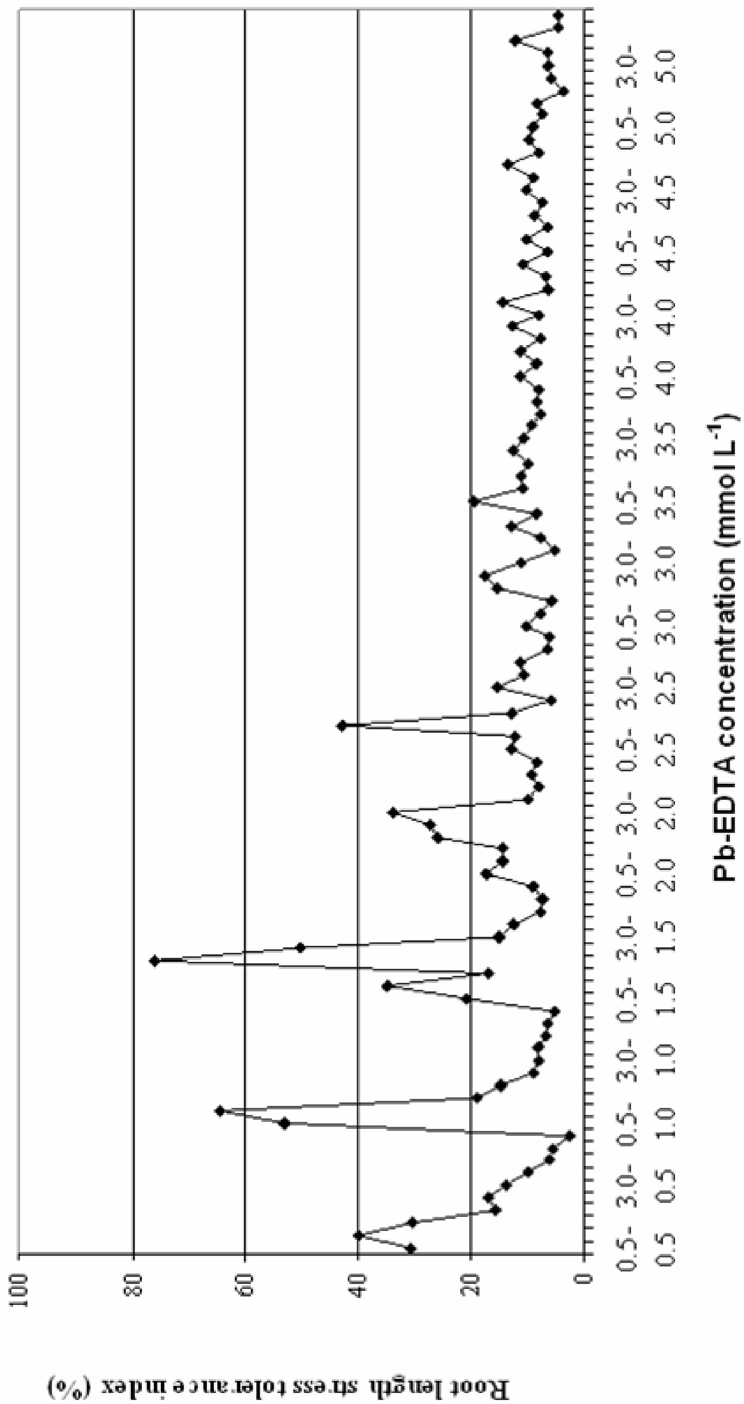

Fig. 3. Effect of different combinations of Pb and EDTA on root length stress tolerance index in sunflower plants.

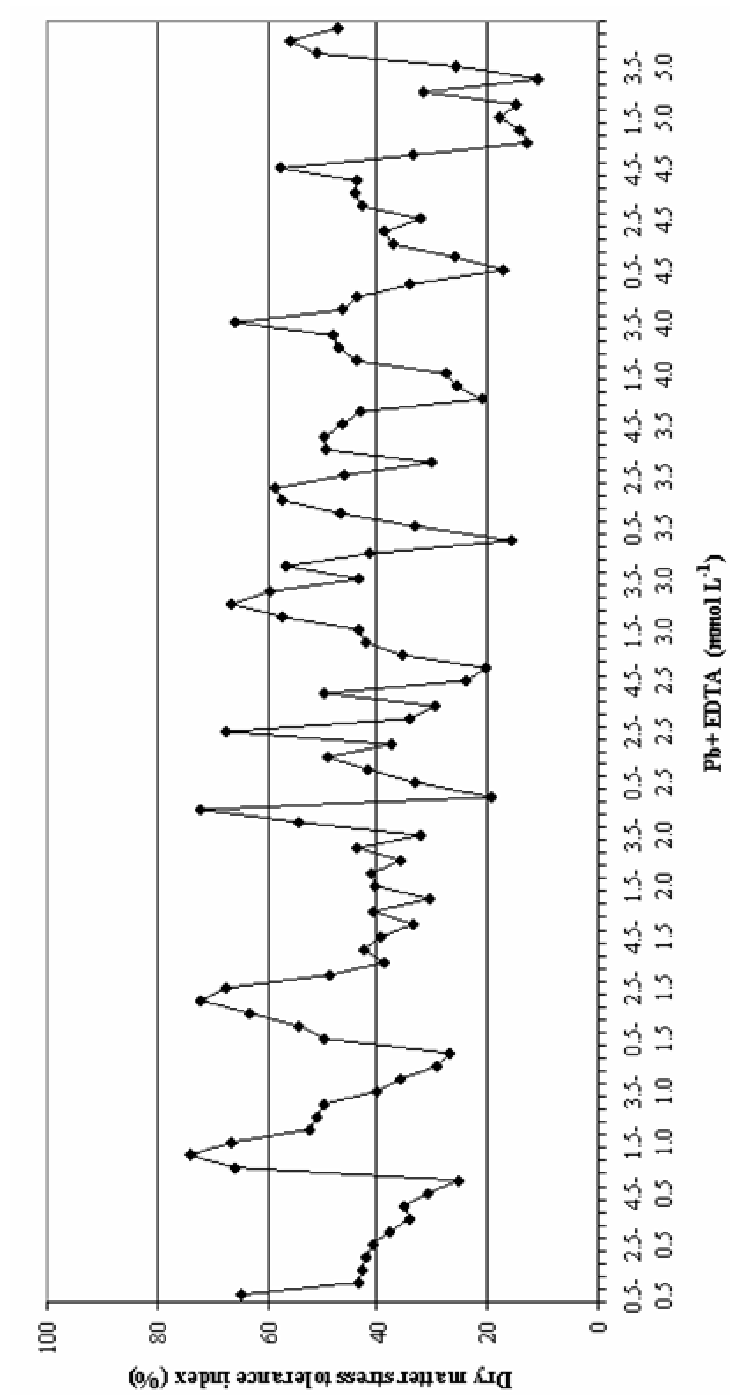

Fig. 4. Effect of different combinations of Pb and EDTA on dry matter stress tolerance index in sunflower plants.

67.51% under 1.0-1.0, 1.5-1.5, 2.0-1.5 and 2.5-1.5 m*M* Pb-EDTA, respectively while DMSTI at 1.0, 1.5, 2.0 and 2.5 m*M* Pb was 67.80, 58.92, 55.03 and 54.51 % respectively. The data proved that addition of EDTA was effective in enhancing the uptake of Pb in sunflower plants. The Pb-EDTA levels at which more than 50 % SLSTI and DMST maintained were 1.5-1.5 and 1.0 1.0 m*M* Pb-EDTA.

Discussions

Lead (Pb) belongs to a group of elements, not necessary for the life of plants or any living organisms and human beings. It is in fact vital to understand mechanisms due to which plants can live unharmed in the lead contaminated environment. There is a species to species, and even variety to variety differences in tolerance to the harmful effects of heavy metals and their accumulation in different parts of plants (Kataba-Pendias & Pendias, 1999). Plants modify their metabolic activities according to the environmental conditions, plant age and its general physical condition and the vegetation period. There are several possible areas through which lead can penetrate into plants. However, it is understood that roots are the main pathway through which trace metal ions enter into plant body. It was determined that in plants, metal uptake at first stops on root surface and then a portion of ions which penetrate into roots is bound in cell walls and the rest is accumulated in the intercellular space (Malone *et al*., 1974; Wierzbicka, 1987). It was determined that in the sunflower plants examined, roots are the main accumulation site of Pb (Fig. 1 A) because they are severely affected by Pb concentrations in the medium.

EDTA alone has also shown toxic effects by reducing shoot, root and dry matter stress tolerance indices (Fig. 1 B) that may be due to its chelating property. It forms chelate with some micronutrients in the growing medium which is translocated in higher amount i.e. upto toxic level. It is reported that application of EDTA is effective in enhancing the uptake of heavy metals by plants (Wojcik & Tukendorf, 1999a; Wu *et al*., 1999; Sahi *et al*., 2002) and the present study indirectly confirmed it, because with the addition of EDTA in the growth medium containing Pb substantially reduced the toxic effects of Pb by increasing shoot, root and dry matter stress tolerance indices at 1.0-1.0, 1.5-1.5, 2.0-1.5, 2.5-1.5 and 3.5-2.0 m*M* Pb-EDTA (Fig. 2, 3,4).

Differences in growth rate of root length are an important indictor of plant resistance to heavy metals (Wilkins, 1957). This method can effectively compare the growth of roots growing with the addition of the stress factor with that of control plants. The results obtained showed that root elongation of sunflower plants was reduced with the application of 0.5 m*M* $Pb(NO_3)_2$. Similar results were described earlier by Geebelen *et al*. (1999; 2002) who confirmed the phytotoxic effects of Pb. They noted reduction in root length of *P. vulgaris* (cv. Limburgse vroege) at 0.08 µ*M* $Pb(NO_3)_2$, and application of 0.4 µ*M* Pb caused a decrease in the amount of bean fresh weight by up to 90%.

Disturbances in shoot or root growth caused by the toxicity of lead were described also for other crops e.g. onion (Wierzbicka, 1989; 1995), and Indian mustard (Liu *et al*., 2000). Mechanisms initiating inhibition of root elongation are not well understood. This must be a complex process that is affected by many factors. It is thought that the process is correlated with a decrease in dry and fresh weights of plants (Gabara & Golaszewska, 1991). In the present studies, dry matter stress tolerance index was also decreased even at 0.5 m*M* $Pb(NO_3)_2$ application. DMSTI values reduced with increasing Pb concentrations (Fig.1), and the same is true with SLSTI values. It is assumed that one of the reasons can be the disturbance of plant water balance. The resulting water deficit is one of the main factors limiting growth and plant development in the environment contaminated with heavy metal ions (Wierzbicka, 1994).

Numerous reports on the use of synthetic chelates in enhancing uptake and transport of heavy metals by plants have been published in the recent years. It was demonstrated that among the chelators studied, EDTA was the most effective chelator for Pb (Huang *et al.* 1997; Cooper *et al*., 1999; Lasat, 2002). One of the most intensely studied subject at the moment is the possibility of using commercial plants exhibiting tolerance to heavy metal ions and high biomass production in induced phytoremediation e.g. corn, barley, sunflower (Salt *et al*., 1998). In the present experiments, sunflower plants were grown with the addition of different Pb + EDTA combinations. The application of synthetic chelator-EDTA at the concentration of 1 or 1.5 m*M* together with Pb ions in the growth medium resulted in a significant enhancement in plant growth. These concentrations inhibited the toxic effects of Pb on shoot and root elongation, root colour and dry matter. Piechalak et *al*. (2002) also reported similar results with EDTA. The similar results were observed in pea by Wojcik and Tukendorf (1999 a; b).

From the present study, it can be concluded that application of EDTA at 1.0 or 1.5 m*M* is effective in reducing the toxic effect of Pb due to its chelating property. The EDTA application would be beneficial in accelerating the phyto-extraction of Pb through hyper-accumulating sunflower plant.

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