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RESEARCH ARTICLE - BIOLOGICAL SCIENCES

Dietary Toxicity of Lead and Hyper-Accumulation in *Petroselinum crispum*

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Abstract The current study investigated the bioaccumulation potential of *Petroselinum crispum* (Parsley) for lead (Pb) contaminated soils. Different concentrations of lead nitrate (0, 200, 600, 1,000 and 1,200 mg kg^{-1} soil) were applied to the soil, and Parsley plants were grown in contaminated soil for a period of 3 months. One set of treatments was supplemented with EDTA as chelating agent to enhance the Pb uptake. The growth parameters, phytotoxic effects and Pb accumulation in different parts of experimental plants were recorded. Increasing Pb concentrations in soil caused delay in germination rate, decrease in plant height, root length and fresh and dry weight. The Pb accumulation in roots, shoots and seeds of treated plants linearly increased with Pb concentration in soil. For the highest treatment, Pb accumulation in roots and leaves was recorded to be 641 and 439.5 mg kg^{-1} dry weight (DW) over 20.5 and 17.25 mg kg^{-1} DW of control plants, respectively. At higher Pb treatment, the addition of 10 mmol EDTA had a significant effect on Pb accumulation in plants. Although Parsley seems to be a promising candidate to reclaim Pb-contaminated sites, being edible plant dietary toxicity of the plant needs to be seriously considered.

Keywords Lead pollution · Phytoextraction · Parsley · Phytotoxicity · Translocation factor

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1 Introduction

Deleterious heavy metals are eluted from a range of native and anthropogenic activities resulting in an increased contamination rate of biosphere [1,2]. Heavy metal concentration in polluted soil ranges from 1 mg kg^{-1} to as high as 100,000 mg kg^{-1} and the increased levels affect environment and human health at large scale [1]. Among heavy metals, Lead (Pb) is considered to be the most prevailing contaminant of soil deposits, air, and water because it is nondegradable once deposited in soil [3]. Based on studies in animal, Pb compounds like lead acetate and lead phosphate have been precluded to be carcinogens [4]. Aggravated levels of Pb and its salts result in its poisoning of food chain and tend to restrict the plant chlorophyll production, growth and metabolism. Moreover, higher Pb levels cause alteration in membrane permeability, disturbs mineral nutrition, water imbalance and inhibition of enzymes activities along with oxidative stress in plants [5].

Reclamation of polluted sites is necessary to prevent phytotoxicity in plants and mobilization of heavy metals into water bodies and subsequent bioaccumulation [6]. Diverse techniques including variable agronomical practices are being applied for soil restoration depending on soil type and level of contamination but such conventional methods of soil remediation are effective for only moderate level of metal concentrations. Phytoremediation is a revolutionized, novel and exotic green technology for persistent remediation of surface soils contaminated with heavy metals [7,8]. Phytoremediation techniques are adopted for in situ bioremediation of sites, where metal contamination is very high. The technique is assigned to a variable collection of plant-based strategies that use indigenous or genetically modified hyperaccumulators for decontamination and restoration of environments [1].

For an ideal hyperaccumulator, large shoot biomass, effective root system, robust growth habitat, ability to survive and uptake wide range of metals and harvested biomass in terms of economic returns are required parameters for efficient metal cleanup [9]. However, potential hyperaccumulators investigated until now mostly possess low biomass and are slow growing plants [10].

Phytoextraction of Pb from contaminated sites is restricted by low solubility and bioavailability of Pb in soil, which limits its uptake by plants and unavailability of potential hyperaccumulators. Soil amendments like EDTA, organic acids such as citrate, oxalate, malate and succinate are being used as chelators functioning as mobility boosters and enhance metals uptake from contaminated soils. Chelators' addition has significantly increased the metal concentration and bioavailability in the soil, therefore mediating the metal uptake by the plant [11].

Parsley (*Petroselinum crispum)* or Garden Parsley*y* is a biannual plant/ herb of family *Apiaceae*, which has ability to grow vigorously in harsh environment with scarcity of nutrients. The presence of massive root system and large biomass production of Parsley prompted us to investigate its potential for phytoextraction of Pb from contaminated soil. In the current study, we determined the natural and chelate-assisted Pb accumulation potential and potential dietary toxicity of Parsley.

2 Materials and Methods

2.1 Soil Sampling and Preparation

The soil samples were collected from the research form of COMSATS Institute of Information Technology (CIIT), Abbottabad, Pakistan. The chemical and physical properties of soil were determined before planting (Table 1). The soil was loamy in texture with an initial pH of 8.7, which was adjusted to pH 5. The original Pb concentration in the soil sample was determined by acid digestion method and was found to be 41 mg kg^{-1} soil (Table 1).

Table 1 Chemical and physical properties of soil before planting

Soil parameters	Values
pH	8.7
Sand (g kg^{-1})	300
Clay (g kg^{-1})	710
Texture	Loamy
Organic matter (g kg^{-1})	17
AB DTPA-extractable NO^3N (mg kg^{-1})	0.023
AB DTPA-extractable *P* (mg kg^{-1})	1.78
AB DTPA-extractable K (mg kg^{-1})	56
Lead (mg kg^{-1})	41
EC (dsm^{-1})	0.33

2.2 Experimental Design

Fifty seedlings of Parsley (*Petroselinum crispum)* were grown in non-contaminated soil supplemented with biological fertilizer and 200 mg kg^{-1} of NPK. After 45 days of growth, healthy plants were selected to set up the experiment. For germination data, 10 seeds per pot were sown in experimental contaminated soil.

For assessment of lead accumulation, experiment was set up in randomized fashion with three replicates. In each pot, 700 g kg^{-1} clay, 300 g kg^{-1} sand, 17.87 g kg^{-1} NPK and 17.87 g kg^{-1} of farm yard manure was added. The soil was contaminated with four different concentrations of lead nitrate ($PbNO_3$), i.e., 200, 600, 1,000, 1,200 mg kg^{-1} soil. The control set of plants was grown in non-contaminated soil. To analyze the effect of chelating agent, another set of plants was grown in Pb-contaminated soil supplemented with 10 mmol EDTA.

2.3 Determination of Growth Parameters

Germination data were recorded from the day of sowing until complete germination in all treatments. Plant height was measured with meter ruler at two time points; the first reading was recorded at 45th day and the second one at 90th day after plantation. After three and a half months of growth, the plants were harvested with roots. The fresh plant material was weighed by using top loading balance. The collected samples were dried in oven at 70 °C for 36 h, and dry weight of the samples was calculated.

2.4 Analytical Procedures

Soil pH was determined by Thomas method [12]. Total nitrate nitrogen (NO_3-N), phosphate phosphorus (PO_4-P) and cation exchange capacity (CEC) were determined by ammonium bicarbonate diethylene triamine penta acetic acid (AB DTPA) extraction method [13], respectively. Pb concentration in soil samples was determined by atomic absorption spectrophotometer after acid digestion of samples [14]. To determine lead accumulation in different parts of the Parsley, the plant material was separated carefully into roots and shoots. The plant material was immersed in 0.01 M HCl followed by thorough washing with deionized water. Both soil and plant samples were dried at 90 °C for 24 h. The material was ground to a fine powder and passed through a sieve. One g fine powder was suspended in 20 ml of HNO_3:$HClO_4$(3:1 ratio) solution. 10 % $HClO_4$ was added to the digested and filtered soil samples; the final volume was adjusted to 50 ml. Pb concentration was determined in the digested samples by flame atomic absorption spectrophotometry (Perkin Elmer Analyst 700). The phytoextraction ability of Parsley plants was assessed using both the translocation factor ($TF = ([Pb]_{shoot}/[Pb]_{root})$ and the bioaccumulation factor ($BF = [Pb]_{shoot}/[Pb]_{solution}$).

2.5 Statistical Analysis

Paired *t* test was used to compare the parameters of control and experimental plants by using SPSS 11.5 (statistical package for social sciences) at 5 % level of significance.

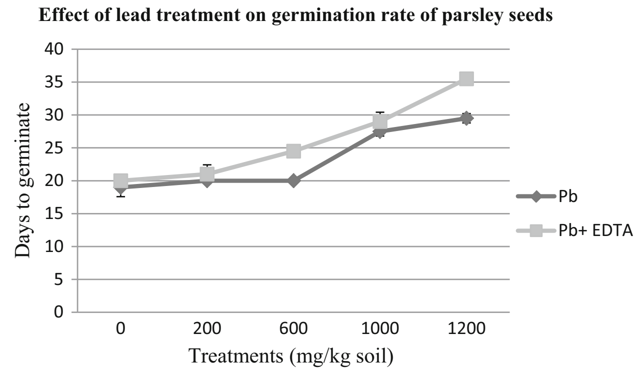

Fig. 1 Effect of lead treatment on germination rate of parsley seeds

3 Results

3.1 Response of Parsley's Growth to Different Pb Levels

Parsley plants were grown in various concentrations of Pb, and different growth parameters like germination index, plant height, root length, and fresh and dry weight of the plants were recorded (Table 2). Pb treatments seemed to delay the germination rate of parsley seeds. At lower concentrations (200 and 600 mg kg^{-1} soil), no significant difference ($p < 0.05$) was noted in germination rate of control and treated plants. However, at highest concentrations of lead (1,200 mg kg^{-1} soil), statistically significant differences ($p < 0.05$) were found in germination rate (Fig. 1).

Plant height was recorded on 45th and 90th day after the growth of plants in lead contaminated soil. A similar trend was observed in readings at both time points; plant height significantly decreased ($p < 0.05$) with increase in Pb concentration (Table 2). On 45th day, the decrease in plant height at lowest and highest Pb concentration was 21 and 50 %, respectively. On 90th day, almost similar trend was observed with reduction in plant height being 25 and 52 % at lowest and highest lead concentration, respectively (Table 2).

Root length was also recorded after 90 days of the experiment. A bell-shaped trend was observed for root growth; the initial Pb concentrations caused a slight increase in root length as compared to control plants. The highest value was recorded for plants treated with 600 mg Pb kg^{-1} soil, being 151 % of the control plants. This was followed by decrease in root length, which became almost same as that of control plants (Table 2). The fresh weight (FW) and dry weight (DW) of control and Pb-treated plants was recorded at the end of experiment. Increase in Pb concentrations caused a significant ($p < 0.05$) reduction in FW and DW. At highest Pb treatment, the reduction in F.W and DW was 69.2 and 65.2 %, respectively (Table 2).

3.2 Pb Concentration and Accumulation in Different Parts of the Plant

Pb accumulation was measured in the roots, shoots and seeds of the plants grown in Pb-contaminated soils for a period of 90 days. A set of plants was grown in soil supplemented with Pb+ EDTA (10 mmol), to see the chelating effects of EDTA on metal uptake. A sharp and linear increase in Pb accumulation in roots of the plants was observed with increasing

Table 2 Effect of lead accumulation on plant height, root length, and fresh and dry weight

Pb treatments	Plant height after 45 days (cm)	Plant height after 90 days (cm)	Root length (cm)	Fresh weight (mg kg^{-1})	Dry weight (mg kg^{-1})
Control	18.5F	54.5D	12.25A	34.75F	5.92C
Control+ EDTA	15.0E	39.86C	13.75A	27.0E	5.64C
200 mg kg^{-1}	14.25DE	38.00C	12.75A	21.5D	4.11B
200 mg kg^{-1}+ EDTA	15.5E	31.85B	15.0AB	17.5CD	3.66B
600 mg kg^{-1}	12.75CD	30.0B	36.5C	17.75CD	3.63B
600 mg kg^{-1}+ EDTA	12.25BC	29.5B	18.5B	19.5D	2.89A
1,000 mg kg^{-1}	12.25BC	28.25AB	12.0A	14.0BC	2.71A
1,000 mg kg^{-1}+ EDTA	13.25CD	29.1B	15.25AB	10.0AB	2.30A
1,200 mg kg^{-1}	10.75AB	24.85A	15.75AB	8.0A	2.42A
1,200 mg kg^{-1}+ EDTA	9.75A	28.0AB	14.0A	11.0AB	2.195A

Means with no significant difference have share alphabets at confidence level 0.05

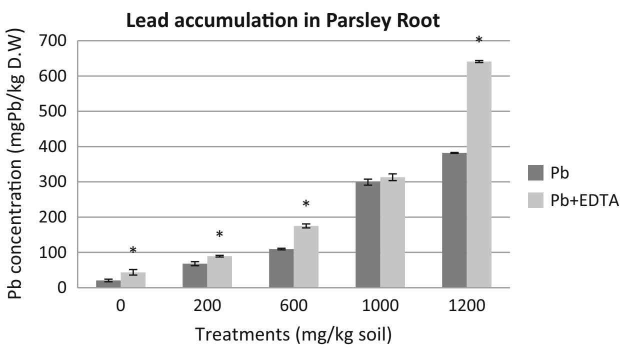

Fig. 2 Lead accumulation in the roots of Parsley, the symbol (*) shows the significant differences ($p < 0.05$) between data obtained at applied treatments

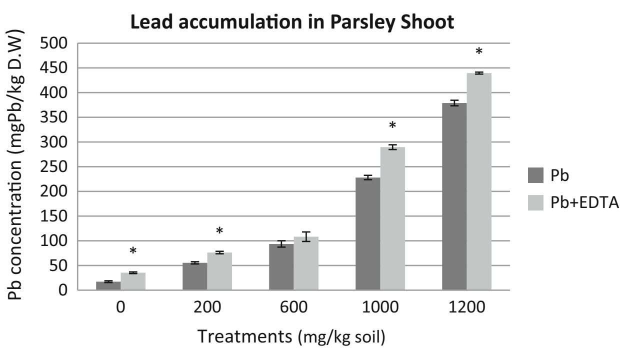

Fig. 3 Lead accumulation in the shoots of Parsley, the symbol (*) shows the significant differences ($p < 0.05$) between data obtained at applied treatments

Pb concentrations in soil. The maximum Pb accumulation in roots was observed in plants grown on highest Pb concentration; the value being 382 mg kg^{-1} DW as compared to 20.5 mg kg^{-1} DW, in the case of control. The addition of EDTA had a pronounced effect; Pb accumulation increased from 382 and peaked to a value as higher as 641 mg kg^{-1} DW, the increase in Pb accumulation being 67.78 % (Fig. 2).

Similarly, Pb accumulation increased to a significantly higher level in the shoots of Pb-treated plants. Pb concentration of 379 mg kg^{-1} DW was observed for the highest Pb concentration, as compared to 17.25 mg kg^{-1} DW in the case of control. Again addition of EDTA had a significant effect; Pb accumulation raised from 379 to 439 mg kg^{-1} DW (16 % increase) (Fig. 3).

A significant level of Pb accumulation was found in Parsley seeds. Pb accumulation upto 86.5 mg kg^{-1} DW was observed for the highest Pb treatment (Fig. 4). EDTA seemed to have no effect on Pb accumulation in seeds.

3.3 Translocation and Bioaccumulation Factors

The Pb concentration linearly increased in shoot with the increasing supplied Pb levels. The maximum accumulation

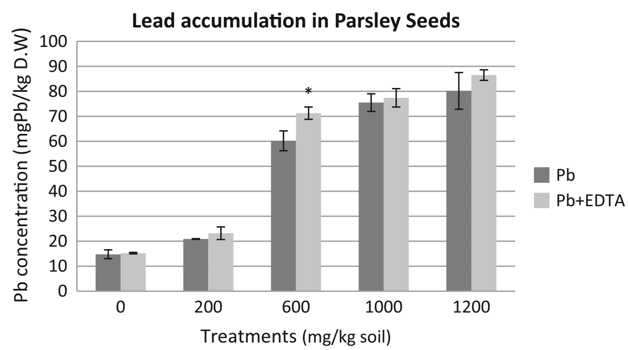

Fig. 4 Lead accumulation in Parsley seeds, the symbol (*) shows the significant differences ($p < 0.05$) between data obtained at applied treatments

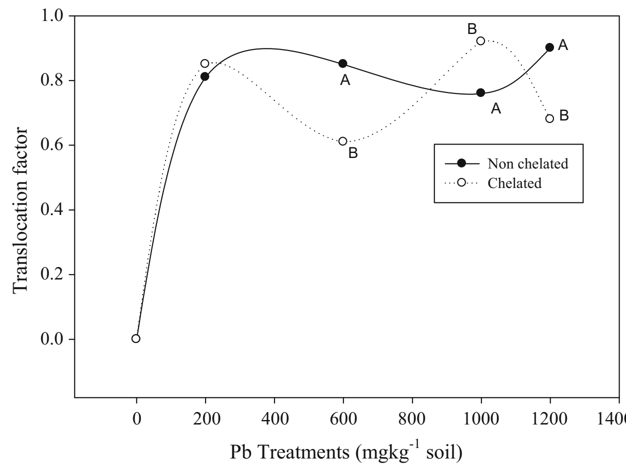

Fig. 5 Effect of EDTA on the translocation factor for Pb treatment. The *letters* (*A* and *B*) show significant differences at various data points

in leaves occurred at 1,200 mg L^{-1} treatment and the order of Pb accumulation was roots <leaves. Bioaccumulation factor suggested that EDTA application significantly affected the Pb removal from the growth medium and caused its accumulation at 1,200 mg L^{-1} (Figs. 5, 6).

4 Discussion

Higher Pb concentrations negatively influenced the plant growth. Detrimental effects of Pb on plants include inhibition of enzymes activities, water imbalance, alterations in membrane permeability, disturbance in mineral nutrition, inhibition of chlorophyll synthesis and oxidative stress [5]. Growth disorders causing reductions in biomass yields are commonly observed in plants subjected to high metal levels [15]. In a study [16], three plant species were grown on lead contaminated soil and growth parameters were recorded. It was found that *M. caesalpiniaefolia* was the only species in which high Pb concentrations had no apparent effect on the mea-

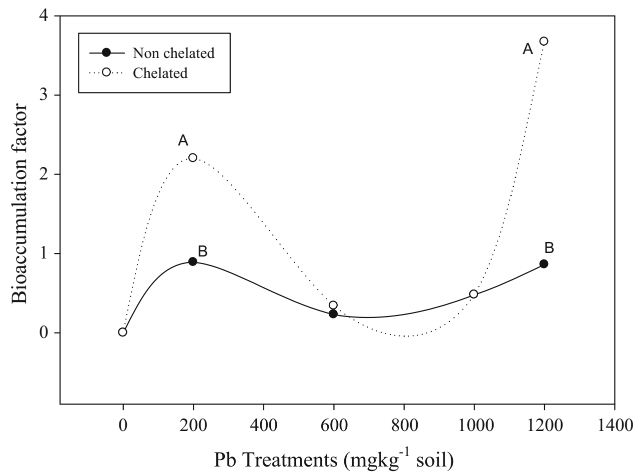

Fig. 6 Effect of EDTA on the bioaccumulation factor for Pb treatment. The *letters* (*A* and *B*) show significant differences at various data points

sured plant growth parameters. While the other two species, *E. speciosa* and *S. parahyba* showed reductions in biomass production, leaf area or plant height relative to plants in non-treated soil [16]. In the present study, higher Pb accumulation in different parts of the Parsley was found to be associated with few effects on growth parameters like reduction in plant height, decrease in fresh and dry biomass. Germination rate was also found to be delayed in Pb-treated Parsley plants. These findings suggest that even at highest concentrations of Pb, Parsley plants were able to survive and continue growth and development.

Plants absorb, translocate and accumulate toxic metals primarily through roots, which provide mechanism to prevent contaminant toxicity [17]. In spite of being a non-essential metal for plant, Pb is readily taken up by plants roots. In the current study, Parsley roots accumulated large amount of Pb (641 mg kg^{-1} DW), which demonstrates aggravated tolerance to Pb treatments. Previous studies carried out on *S. procumbens*, *P. granaiflora* and *A. philoxeroides* indicated Pb accumulation of 12.33, 9.77 and 22.49 mg kg^{-1}, respectively, in the roots of the plants. Similarly, Radish root was found to accumulate 0.75 mg kg^{-1} of Pb [18]. Similarly in another study, Sunflowers were found to accumulate Pb at 77.7 mg kg^{-1} DW after 8 weeks of growth on Pb-contaminated soil (400 mg kg^{-1}) [19]. In the current study, we have found a much higher accumulation of Pb by roots of Parsley. These findings indicate amazingly huge potential of this plant for phytoextraction of Pb-contaminated soils. This potential may be attributed to its massive root system.

A higher translocation factor (shoot/root ratio) of heavy metal content in plant is important as for as practical phytoremediation of heavy metal-contaminated soils is concerned. This is an obvious advantage as phytoremediation of the heavy metal-contaminated soils can be carried out only by harvesting the aboveground parts of the plants. It is generally believed that root to shoot translocation of Pb is very slow [20]. Lead hyperaccumulating plants usually have a higher shoot/root ratio of lead content in plant (0.04–0.1) than the non-hyperaccumulators [21]. In present study, the value of translocation factor ranged from lowest as 0.62 (at Pb 600 mg kg^{-1} soil) to as higher as 0.99. This is in fact a significant finding, which shows that at higher Pb concentrations about all the Pb taken up by roots is translocated to shoots of the Parsley plants. Translocation factor values observed in this study are much higher [21]. Similarly, values for bioaccumulation factor have been found to be much elevated at higher Pb treatments.

EDTA has been reported to be the most effective and efficient in phytoextraction [22]. EDTA addition at a concentration of 0.1 mmol L^{-1} made it possible to raise the Pb translocation to shoots from 16.8 to 30.5 % [23]. Parsley showed 15.96 % increase in Pb absorption in shoots and 67.78 % in roots in the presence of EDTA which confirmed mobilization of Pb by the chelating effect of EDTA.

The knowledge on the physiological and biochemical responses may help to adopt different strategies of purification and improvement of the environment through use of plants which can concentrate and tolerate high level of heavy metal solution [24,25]. The results of present study clearly showed native hyperaccumulation ability of Parsley for decontamination and complexion of Pb from soil. Further, it would be interesting to investigate the biochemical and genetic basis of Parsley for Pb tolerance and hyperaccumulation. The edible nature of Parsley may restrict its large scale use as phytoremediation candidate; however, some non-edible plants should be employed to remediate metal-contaminated sites including *Andropogon scoparius*, ribwort plantain *(Plantago lanceolata* L., *Holcus lanatus)*, mosses, lichens, crowberry *(Empetrum nigrum* L.), Tamarix (*Tamarix parviflora*), Eucalyptus (*Eucalyptus camaldulensis*), and Chinese Brake fern (*Pteris vittata* L.) and recently discovered *Arundo donax* L.

5 Conclusion

The current study has proved that parsley has enormous potential to accumulate Pb from contaminated soils. The plant did not show any serious phytotoxic effects and manifested tolerance against Pb stress even at elevated concentrations. However, the dietary toxicity caused by Pb accumulation in Parsley should be seriously considered. The tolerance of Parsley against Pb contamination may be explained on genetic basis, which demands further investigation.

Conflict of interest The authors have neither any conflict of interest nor any financial gain for the present publication.

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