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Reaserch Paper

EDTA increases the phytoremediation and translocation factor of Pb^{2+} in the medicinal plant *Calendula officinalis*

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Abstract

Phytoremediation of lead using plants in lead-contaminated soils is a new and safe environmental technology. By adding chelators and increasing plant extraction, the efficiency of this technology can be increased. In this regard, we evaluated the effect of adding EDTA chelates to lead-contaminated soils to investigate the amount of lead accumulation in a medicinal plant, *Calendula officinalis*. We designed a factorial experiment in the form of a completely randomized, with three replicates in pots and two factors including EDTA at two levels (0, 50 mg kg^{-1}) and lead at four levels (0, 30, 90, and 270 mg kg^{-1}). In this plant, the accumulation of lead was accompanied by an increase in the amount of lead in the soil due to the addition of EDTA to the soil. The results showed that EDTA significantly increased the lead translocation of lead from roots to the aerial part of the plant. Total Chl. and shoot dry weight decrease significantly in EDTA treatment than control specific at a high level of Pb in the soil. Also, the results showed that EDTA increased lead removal from soil to soil solution and increased lead translocation from roots to the aerial part of the plant of *Calendula officinalis*. In general, the results of this research showed that with the careful management and EDTA use in lead extraction, it has provided a cost-effective and safe environmentally strategy.

Introduction

Nowadays, heavy metals including lead are of the most common of considerably environmental and are the subject of much environmental research (Wang et al., 2023). Lead contamination may cause many environmental problems, including contamination of drinking and groundwater, reducing the plant vegetation due to lead toxicity in the environment and affected on the human's, animals and microorganisms (Osman et al., 2023). In addition to the origin of natural earth, industrial and mining activities, lead contamination has occurred in nature due to the use of lead in paints, fuels, as well as the disposal of lead-enriched urban sewage sludge, which has also caused an increase in soil lead levels (Bača and Vanýsek 2023). Remediation and purification of lead-contaminated soils always brings significant costs for many industries and government organizations. (Mielke and Egendorf 2023). Over the past two decades, there has been a growing interest in developing a plant-based

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technology that called phytoremediation, to remediate heavy metal-contaminated soils (Mocek-Płóciniak et al., 2023). Plant extraction and plant stabilization are two different strategies for plant remediation of lead-contaminated soils (Jiao et al., 2023).

In general, plant stabilization is the use of plants or other soil amendment materials to reduce the inherent risk of lead-contaminated soil by reducing the bioavailability of lead in the soil (Jiao et al., 2023). Plant extraction is the use of plants to remove lead from contaminated soils. By continuing to cultivate lead-accumulating plant species in lead-contaminated lands, the soils can eventually be decontaminated. Considering that plant cultivation and harvesting are relatively cheap processes, plant extraction with suitable plant species can be an attractive way to clean up lead-contaminated soils. The timing of the plant lead extraction process depends on soil quality, initial and final concentrations and forms of lead in soil, the type and potential of lead-absorbing plants and the degree of risk it may have for human health and the environment.

In general, the two major limitations for plant extraction of lead are low bioavailability of lead in soil and poor transfer of lead from roots to shoots. One of the important aspects of plant extraction of lead is to increase and maintain the concentration of lead in the soil solution. It has been reported that chelates have significant effects on metal accumulation in plants and have been widely used to increase the solubility of metal cations in plant growth environments (Mirbolook et al., 2023). It has also been documented that chelates can significantly increase lead accumulation in plants. For example, in bromegrass and found that added EDTA increased aerial lead concentrations from 5 to 35 mg kg^{-1}(Huang et al., 1997).

Calendula officinalis, is a flowering plant in the daisy family Asteraceae. *Calendula officinalis* is widely cultivated and can grow easily in sunny locations in most soil types. This plant has been studied in the process of phytoremediation of heavy metals from soil and has been successful in this regard (Soni and Sharma 2015). Although it is a perennial, it is usually considered an annual, especially in colder regions where it has poor winter survival, and in hot summer regions where it does not survive as well (Leach 2008). It is a medicinal plant that absorbs lead, and if it is used medicinally after being collected from lead-contaminated soil, one should be aware of the amount of lead in it so that it does not overshadow its medicinal properties. In this regard, the amount of lead accumulation in this plant and the effective factors in the absorption and transfer of lead to its aerial parts were investigated. Thus, the important objectives of this study were (i) to evaluate the potential of *Calendula officinalis* in lead absorption, (ii) to compare the relative efficiency of EDTA chelate in enhancing pb phytoextraction and calculate the lead translocation factor of root to shoot.

Materials and Methods

Lead-contaminated soil used in this study was collected from a mining area in Kerman province. At the time of collection, the soil was sieved using a 1.0 cm sieve. Then the soil was air-dried to about 10% water and mixed and uniform in a mixer. Then the collected soil was tested to determine the physical and chemical factors.

Soil physical parameters include: Soil pH was measured using a ratio of 1:5 soil to water. Cation exchange capacity (CEC) was determined by exchange base (Rhoades 1982). The organic carbon content was measured by the Walkley Black method (Nelson 1982) and the particle size was determined by the hydrometer method (Day 1982). Total soil lead was determined by acid digestion with 500 mg of soil in a mixture of concentrated $HNO3/HClO4$ (V: V, 10:7) and the digested samples were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Ettler et al., 2004). Selected physical and chemical properties of lead-contaminated soil are presented in Table 1.

Table 1. Physical and chemical characteristics of soil in this research

Soil texture	Organic matter (%)	pH (1:5 soil/ water ratio)	EC (μ mho/cm) (1:5 soil/ water ratio)	CEC (mequiv/100g)	Pb (mg kg^{-1})
Sandy loam	3.347	6.2	12.42	38.67	245

Caendula officinalis seeds were soaked in concentrated sulfuric acid for 15 minutes, washed with deionized water and soaked in water for 10 hours. Then the seeds of the acid treated plant were planted in the mixture of the pots with three replications. Seedlings were watered daily with nutrient solution for 2 weeks.

Chelator Treatments

The lead-contaminated soil was fertilized with N and K respectively with 150 (mg kg^{-1}) $(NH_4)_2SO_4$, and 150 (mg kg^{-1}) K_2SO_4. EDTA treatments were applied after nitrogen and potassium treatments.
One experiment designed to determine the effects of EDTA levels on the lead desorption from the soil to soil solution and lead accumulation in plants. Another experiment designed to compare the relative efficiency of EDTA chelate in Pb phytoextraction from contaminated soils, the fertilized soil was treated with the (0 or 0.5 g kg^{-1}) of EDTA chelate. After planting the seeds into lead-contaminated soil treated with or without chelate, all pots were kept in a growth chamber with a 14 h, 22 °C/10 h, 18 °C day-night regime. The amount of soil water was maintained at the field capacity by adding water if needed.

Plants were harvested 1 week after being transferred to lead-contaminated soils. During harvesting, the plants were cut at a height of 1 cm from the soil and the shoots were washed with deionized water. The roots were washed in tap water until the soil was free and then washed with deionized water. Then the plant samples were dried in an oven at 70°C.
Plant samples (400 mg) were digested in a mixture of concentrated $HNO_3/HClO_4$ (10:7, V: V), using two-step digestion. The digested samples were brought to constant volume with deionized water and the digested samples were analyzed for lead content by ICP-AES (Etler et al., 2004).
The lead TF was computed in this formula: TF (translocation factor) =C shoot/C root, where C is the concentration (mg g^{-1}) of lead in shoots and roots.
The data reported in this article were analyzed using SPSS software. A probability of 0.05 was considered statistically significant.

Results and discussion

To investigate the effect of EDTA on the release of lead from soil, the concentration of lead in soil solution was examined in a lead-contaminated and non-contaminated soil.

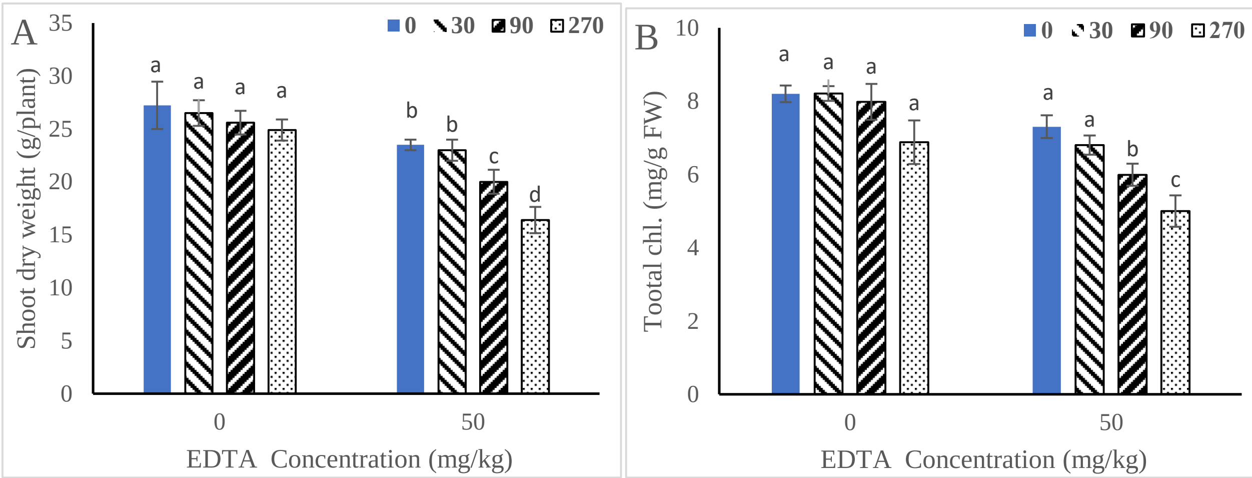

Figure 1: The effect of different doses of lead and EDTA on shoot dry weight (A) and total chlorophyll content (B) of *calendula officinalis*.

Results showed that the shoots dry weights and total Chl. of *Calendula officinalis* decreased under 90 and 270 mg kg^{-1} Pb in the soil (Figure 1). Soil treatment with EDTA had negative favorable effects on the plant growth.

In the lead-contaminated soil with adding EDTA, shoot lead concentrations in *Calendula officinalis* increased significantly. Shoots lead concentrations of *Calendula officinalis* increased linearly with increasing levels of EDTA in the soil (Figure 2 C).

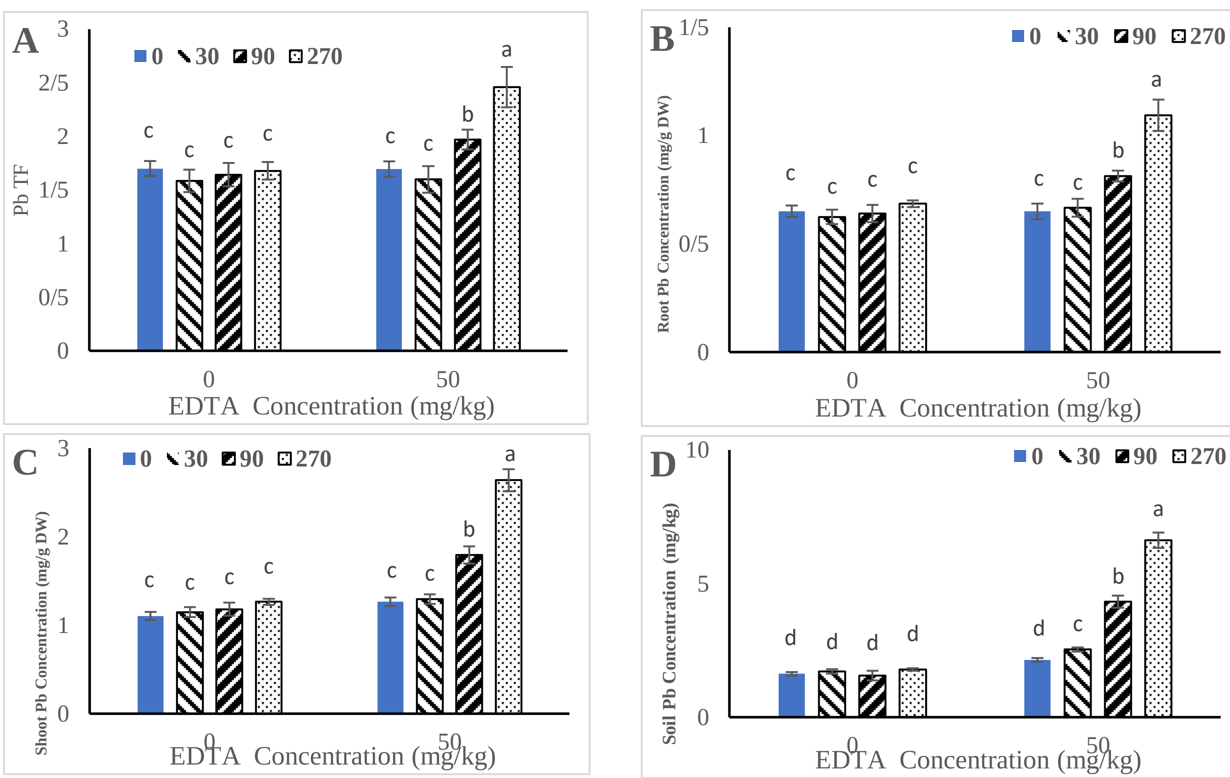

Figure 2: The effect of different doses of lead and EDTA on pb translocation factor (A), root, shoot and soil Pb concentration (B, C and D) of *Calendula officinalis*.

Soil treatment with EDTA significantly increased the lead translocation factor (TF) 70 % approximately, the lead concentration in soil more than 5-time, root and shoot 60 and 55% respectively under 50 mg kg^{-1} EDTA in soil at 270 mg kg^{-1} lead in the soil (Figure 2).

Based on the results of lead concentration of soil solution, it represents a very small part of the total soil lead. In addition, most of the lead absorbed by plant accumulates in the roots. Therefore, a key and important method for plant extraction of lead is to increase the solubility of lead in contaminated soils and transfer lead from roots to aerial parts.

The results of this study showed that EDTA can quickly increase the concentration of lead in the soil solution as well as the transfer of lead from the root to the shoot of *Calendula officinalis* (Figure 2). One of the main factors for increasing the concentration of lead in EDTA added soil, can be the chelation between lead and common chelating agents in the soil, which causes lead to be removed from the soil to the soil solution. In this regard, special and more efficient lead chelate can be found or synthesized with more screening. The more efficient a chelate is at removing soil lead, the less chelate is needed to add to lead-contaminated soil. The increase in soil soluble Pb concentration caused by EDTA is directly related to the increase of Pb transfer from roots to shoots and accumulation of Pb in plants (Figure 2 B -D).

These results suggest that chelates may remove the two main limiting factors in phytoextraction of Pb from contaminated soils: Low bioavailability of lead in soil and low transfer of lead from roots to shoots. The mechanisms involved in this phenomenon are still unanswered. Different mechanisms are probably involved in the excessive accumulation of lead caused by chelation in plants. Increasing the level of lead in the soil solution is one of the main ones. Second, chelates can inhibit Pb activity near the root surface and thus maintain a constant source of free Pb at root Pb uptake sites. Third, Pb, EDTA complex may be absorbed by roots directly and translocated to the shoots (Figure 3).

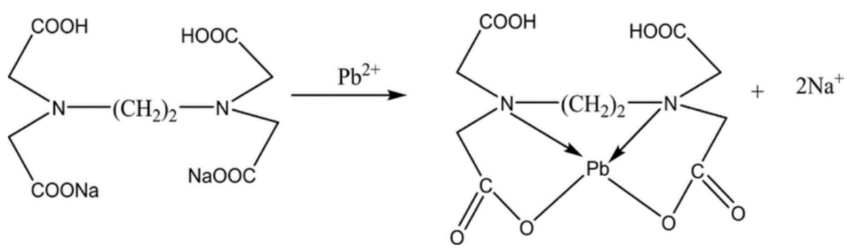

Figure 3: The mechanism EDTA with lead ions (Abdel-Aziz et al., 2020)

It is commonly believed that plants do not absorb or translocate synthetic chelates or combinations of synthetic chelates and ions (Kochian, 1991). This belief is based on the condition that the concentration of synthetic chelates used is low and mostly in the micromolar range. However, in the experimental conditions of this study, the concentration of EDTA in the root zone was in the range of more than micromolar. Experimental evidence shows that chelate is rapidly absorbed by the roots and transported from the roots to the shoots. It has been reported that Pb EDTA was transported into roots and translocated from roots to shoots (Tanton and Crowdy 1971). In addition, it is possible that artificial chelates in high concentrations change the plant's ion transport systems so that lead absorption and translocation is facilitated.

To date, the exact mechanism of increased transport and accumulation of lead due to synthetic chelates such as EDTA has not been determined. More research is needed to understand the mechanisms involved in lead transport and accumulation, to address environmental concerns and to minimize the addition of chelators Economically. This issue may be solved by precise engineering tools or by discovering and using more specific lead chelates. However, our data show that with the same soil used in this study, the concentration of chelate has a significant difference in the ability to absorb and accumulate lead in the plant (Figures 2 and 3). In addition, despite the overall increase in aerial lead concentration by using chelates, attention should be paid to the plant's ability to absorb lead. In this regard, plants with higher lead absorption efficiency can receive more attention (Figure 3). Therefore, the results of this study showed that plant extraction of lead from lead-contaminated soils may be done by growing a plant with high biomass, which is very sensitive to the increase in lead accumulation caused by chelate in plants. An application in this field would be to use a selective and special chelate to facilitate rapid accumulation of lead in the plants after the crop has grown to a significant size. Also, after using chelates, the accumulation of lead in the aerial parts of the plant increased rapidly, the plant should be harvested shortly after adding chelates.

This strategy can have advantages in reducing the risks that may exist by having plants with high levels of lead in the field for an extended period of time. The addition of chelates to lead-contaminated soils as part of phytoremediation of lead may introduce a new phase to field application and health, safety, and environmental concerns. The use of new irrigation techniques and hydroponic cultures that can quantitatively control the flow of water in the root zone of the plant can be useful here. The time of using chelate to prevent the bioavailability of metal caused by chelate into underground water is also very important and of interest. Also, since excessive accumulation of lead from chelate in plants is rapid, chelate can be applied in a controlled manner to the root zone when vegetation is well established in contaminated sites. It is also important to know about rainy days for not using chelate. Before the plant extraction technique with the help of chelate is widely and practically used in polluted places, more and more detailed research is always needed.

It is a step forward in the development of plant lead extraction, regarding to the results reported here, by using plants with high biomass that can accumulate a high proportion of lead in aerial organs. More research is needed on the most suitable plant-chelate-lead combination for practical and economic use. Based on currently available data, the calculated costs of phytoremediation of lead, such as the costs of chemicals and synthetic materials, plant species cultivation, and biomass treatment, are only a small percentage of other alternative processes, including excavating contaminated soil.

Overall, based on our results in many carefully managed applications, chelate-assisted phytoextraction can be a cost-effective soil remediation strategy, at least in the case of Pb.

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