

A Comparison of Electrical Stimulation for Electrode and EDTA-Enhanced Phytoremediation of Lead using Indian Mustard (*Brassica juncea*)

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The use of plants to remove toxic metals from soil (phytoremediation) is emerging as a cost-effective alternative to conventional methods for the removal of heavy metals from contaminated soil. Indian mustard (*Brassica juncea*) was used as the plant to accumulate high tissue concentrations of lead when grown in contaminated soil. For this study, the application of an electric field combined effectively with EDTA-enhanced phytoremediation. A stimulation of direct and alternating electric potential was compared and EDTA-enhanced phytoremediation of lead using Indian mustard has been performed. The effects of experimental parameters such as operating voltage with different concentration of EDTA, the number of graphite electrodes, and cultivation period on the removal of toxic metal were studied. Shoot lead accumulations in Indian mustard increased as the concentration of EDTA and dc electric potential was increased. Two to four folds was increased when EDTA plus a dc electric potential was applied, compared to an ac electric potential. The maximum lead accumulation in the shoots was achieved by applying EDTA plus dc electric potential with 6 graphite electrodes.

Key Words : Phytoremediation, Lead, Indian mustard, Electrode, EDTA

Introduction

Phytoremediation is the use of living plants and the plant processes to reduce hazardous materials in environment.^{1,2} This emerging technology may offer a cost-effective, non-intrusive, and safe alternative to conventional soil cleanup techniques by using the ability to remove, degrade, or immobilize harmful chemicals in the soil. The science of phytoremediation arose from the study of heavy metal tolerance in plants in the late 1980s. The discovery of hyperaccumulator plants, which contain levels of heavy metals that would be highly toxic to other plants, prompted the idea of using certain plant species to extract metals from the soil and, in the process, clean up soil for other less tolerant plants. The ability to cultivate a high biomass plant with a high content of toxic metals on a contaminated soil will be a determining factor in the success of phytoremediation. Therefore, enhancing metal accumulation in existing high yielding plants without diminishing their yield is the most feasible strategy in the development of phytoremediation. For this reason, recent research on phytoremediation focused on crop species and plants such as oat (*Avena sativa*), barley (*Hordeum vulgare*), Indian mustard (*Brassica juncea*), alpine penny-cress (*Thlaspi caerulescens*), and garden bean (*Phaseolus vulgaris*) which have been shown to accumulate zinc and lead.³⁻⁵

Lead contamination in soils, one of the most important environmental pollutants, causes a variety of environmental

problems, including loss of vegetation, groundwater contamination, and lead toxicity in plants, animals, and humans. Lead has limited solubility in soil and availability for plant uptake due to complexation and precipitation.⁶ Because those contaminants have limited bioavailability in the soil, a method of remediating lead in the soil and facilitating its transport to the shoots and roots of plants is vital to the success of phytoremediation.

Indian mustard (*Brassica juncea*) has been used to demonstrate the capability of plants to accumulate high concentrations of lead in tissue when grown in lead-contaminated soil. It, as a metal hyperaccumulating plant, is also effective in depleting lead in soil. Several cultivars of the *Brassica* species including *B. nigra*, *B. oleracea*, *B. campestris*, *B. carinata*, *B. juncea*, and *B. napus* were investigated for their ability to efficiently accumulate lead and other heavy metals such as chromium, cadmium, zinc, nickel, and copper.⁷⁻⁹ In addition, application of chelating agents such as EDTA to contaminated soils has been shown to induce the uptake of metals by plants.^{3,10,11} It has also been documented that the addition of EDTA significantly increases lead accumulation in Indian mustard. Another technology to remove lead using Indian mustard from contaminated soil has been reported by the combination of electric potential and EDTA.¹²⁻¹⁵

The purpose of this study is to investigate the phytoremediation of Indian mustard for accumulation of lead from lead arsenate-contaminated soil by direct and alternating electric potential with EDTA. In the previously described method, the application of an electric field was enough to have the potential to be combined effectively and synergistically with EDTA-enhanced phytoremediation of lead

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using Indian mustard. In this study, the introduction of the electric potentials and EDTA into the soil around grown-up Indian mustard helped to remove lead in soil. The experimental results showed that lead removal efficiencies by Indian mustard depend on the dc electrical treatment and the concentration of EDTA.

Experimental

Soil Collection and Homogenization. Lead (Pb) and arsenic (As)-contaminated soil was collected from a plot of the Barber Orchard¹⁶ (a Superfund site by the Environmental Protection Agency) located in Waynesville, Haywood County, North Carolina, U.S.A. and transferred to the university green house. At the green house, the soil was screened to pass through a 1.0 cm sieve and mixed to homogenize the soil. The homogenized soil (*ca.* 1.2 kg) was transferred to each commercial pot (upper dia.: 16 cm, lower dia.: 10 cm, and height: 13 cm). The homogenized soil in each pot was monitored by the ICP-OES to demonstrate the homogenization of arsenic and lead, and approximately the same level of arsenic and lead concentration on each pot was obtained.

Lab Cultivation. Indian mustard seeds (*B. juncea*) were germinated on filter paper for 2-3 days before being transferred to pots containing 1.2 kg of lead contaminated soil. Before phytoremediation experiments were initiated, the plants were grown for 6 or 9 weeks according to the experimental conditions. They were watered daily and fertilized weekly (Miracle-Gro). The plants were harvested 9 days following treatment with/without electric potential and/or EDTA. The plants were harvested by cutting the stem 1 cm above the soil surface. The shoots were washed with soapy water and rinsed with deionized water. The plant samples were dried in an oven at 60 °C and homogenized using a ball-mill mixer (Spex 8000, Spex Certiprep, Metuchen, NJ) for the analysis.

Experimental Apparatus for Electrode Phytoremediation. The electrodic and EDTA-enhanced phytoremediation (EEEP) system basically consists of an array of electrodes, a power supply, EDTA, and plants. Figure 1 illustrates the array of the EEEP system with four or six graphite rods

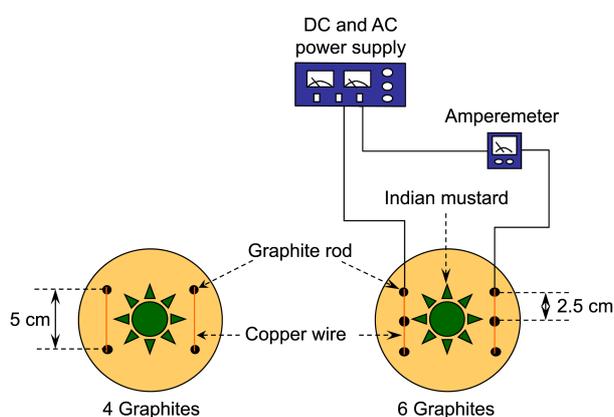


Figure 1. Schematic diagram of the electrodic phytoremediation of Indian mustard.

Table 1. Operating conditions for ICP-OES

Model	Optima, 4100 DV, Perkin Elmer
Power	1.3 kW
Frequency	40 MHz
Ar flow rates:	
Nebulizer gas	0.8 L/min
Auxiliary gas	0.2 L/min
Plasma gas	15 L/min
Emission line:	
Pb	220.353, 217.000, and 283.306 nm
As	188.979, 193.696, and 197.197 nm

in 12 cm long and 0.26 mm O.D. obtained from pencils (Musgrave Pencil Co., TN). Electrical power is applied to the electrode array by direct current (dc) and alternating current (ac) power supplies. Each of these power supplies is capable of delivering up to 400 V at up to 1 A. Application of the electric power to the electrode array was selected by experimental parameters.

Sample Preparation and Analysis. 0.2 g samples of the homogenized plants (shoots of Indian mustard) were digested on a heating block (lab manufactured) with 5 mL of concentrated HNO₃ followed by 1 mL of 30% H₂O₂. The digested samples were diluted to result in 100 mL with distilled-deionized water. Pb concentration for Indian mustard samples were determined by an inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer, Optima, 4100DV). Operating conditions for the ICP-OES are listed in Table 1.

Results and Discussion

Effect of Lead Accumulation in Shoots of Indian Mustard by the Electric Potentials and EDTA. Treatment of Indian mustard with EDTA concentrations ranging from 0 to 5 mmol/kg in the lead contaminated soil and with dc and ac electric potentials of 20 and 40 V was conducted with the three sequential harvests. The shoots of Indian mustard were harvested after applying EDTA for nine days and

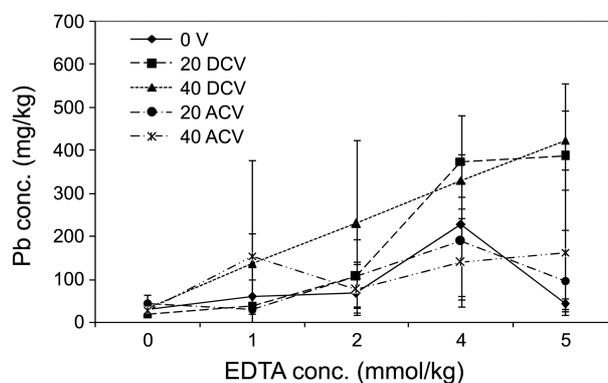


Figure 2. The effect of direct and alternating electric potentials and EDTA concentrations upon lead phytoextraction in shoots of Indian mustard. Values represent the mean \pm S.D. of three replicates from three plants.

simultaneously applying the electric potentials for one hour per day. The concentration profiles of lead in the shoots are shown in Figure 2. The controls were EDTA concentration levels between 0 and 5 mmol/kg of EDTA without electrical potential. The maximum accumulation of lead in shoots of Indian mustard was obtained at the 5 mmol/kg EDTA giving average 386.7 mg/kg and 423.4 mg/kg at both 20 DCV and 40 DCV, respectively. However, relative low lead accumulation (182.2 mg/kg at maximum) was obtained at both 20 and 40 ACV with even high concentration of EDTA.

With dc electrical potentials, accumulation of lead in shoots of Indian mustard was significantly enhanced as the EDTA concentration increases. At 5 mmol/kg of EDTA with dc electric potentials, lead accumulations in shoots reached a maximum lead concentration represented by a two to four-fold increase compared to that in the shoots with ac electric potential treatments. The results of the concentration of lead in the shoots of Indian mustard show the ability of soil remediation and were quite encouraging with dc electric potential and EDTA based on the increase of the mobility of lead in the soil.

The Effect of the Number of Graphite Electrodes on Lead Accumulation with the Electric Potentials and EDTA. A study was performed to determine the effect of the number of graphite electrodes on lead accumulation in shoots of Indian mustard with the electric potentials and EDTA because the number of graphite electrodes affects the electric field density in the soil. The results of this study are shown in Figure 3. Minimal accumulation of lead was observed in the absence of both EDTA and electric potential. However, the combination of EDTA and the electric potentials induced higher accumulations in the plants. For the effect of the number of graphite electrodes, 4 and 6 rods were selected in this study and inserted to the pots containing of Indian mustard. At 20 DCV of electric potential and 2 mmol of EDTA/kg, the six graphite electrodes system showed higher efficiency than the four electrodes for the lead accumulation in shoots of Indian mustard. At 20 ACV of electric potential, in spite of high variation, the six graphite electrodes system was better than the four electrodes for lead accumulation. In general, the concentration of lead in Indian mustard

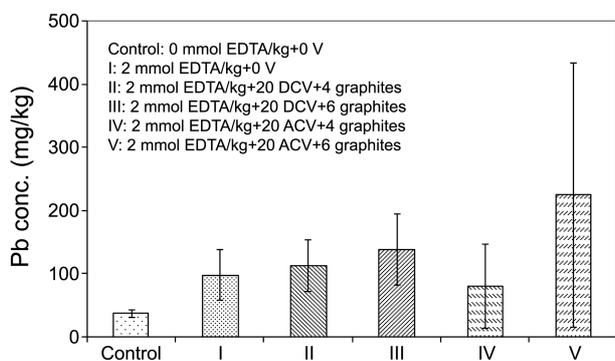


Figure 3. The effect of the number of graphite electrodes for lead accumulation in shoots of Indian mustard with the electric potentials and EDTA. Values represent the mean \pm S.D. of three replicates from three plants.

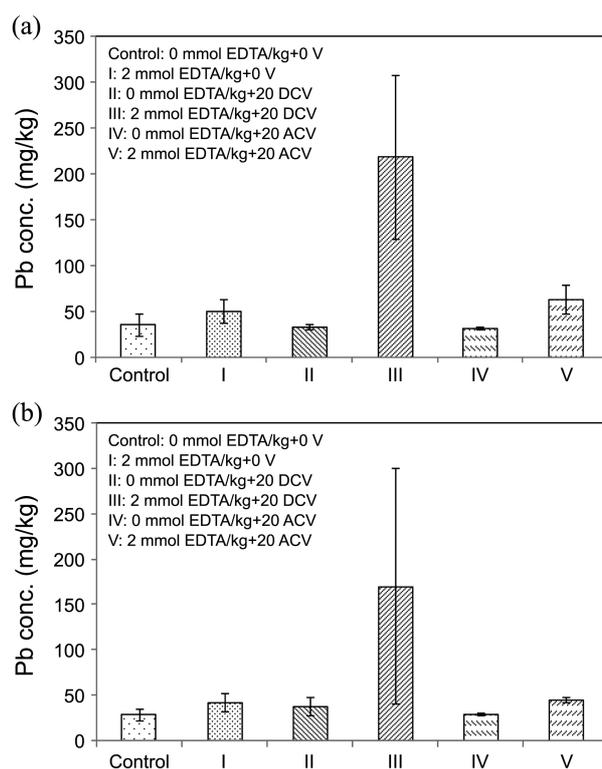


Figure 4. The effect of cultivation periods for lead accumulation in shoots of Indian mustard with/without electric potentials and EDTA: (a) 6 week cultivation and (b) 9 week cultivation. Values represent the mean \pm S.D. of three replicates from three plants.

increased with higher electric field density and EDTA.

The Effect of Cultivation Periods on Lead Accumulation with/without Electric Potentials and EDTA. The investigation of the cultivating experimental parameters was conducted to refine a theoretical model of lead accumulation in shoots of Indian mustard with the electric potentials and EDTA. The ultimate objective of the cultivation periods is to compare the relative efficiency by growing period because the old mustard leaf fall increase. Indian mustards, six and nine weeks old grown in the homogenized Barber Orchard soil, were treated with/without 2 mmol of EDTA/kg for 9 days and electrical potential at 20 DCV and ACV for one hour per day for 9 days. Each condition had three plants with the homogenized Barber Orchard soil. After 9 day treatments, Indian mustards were harvested for analysis.

Lead accumulation in Indian mustard at each experimental condition was shown in Figure 4. For lead accumulation in Indian mustard grown in six weeks as shown in Figure 4(a), the maximum concentration of lead was obtained with 218.8 mg/kg at 2 mmol of EDTA/kg and 20 DCV of electric potential. In Figure 4(b), lead accumulation in Indian mustard grown in nine weeks old was also obtained with maximum 176.6 mg/kg at 2 mmol of EDTA/kg and 20 DCV of electric potential. In spite of a slight decrease of lead accumulation at old Indian mustard, the lead accumulation was not affected at periodic experimental condition between six weeks and nine weeks in general.

Conclusions

A comparison study of the electric potentials and EDTA-enhanced phytoremediation for lead-contaminated soil remediation has been investigated with Indian mustard. For the proposed remediation model, the relative efficiency of lead accumulation in Indian mustard with dc and ac electric potentials and EDTA was studied. After applying dc electric potential and EDTA, lead accumulation in the shoots of Indian mustard was increased rapidly. The synergistic combination of dc electric potential and EDTA-enhanced phytoremediation was defined to maximize the total lead removal ranging from two to four folds compared to ac electric potential. Various experimental parameters including the number of graphite electrodes and cultivation period have been optimized in this experiment. The six graphite electrodes system was more efficient than the four electrodes system due to the higher density of electric field causing the increased mobility of lead in the soils. For the cultivation period experiment, lead accumulation was maximized with dc electric potential and EDTA at both periods and not changed significantly.

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