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# Simultaneous uptake of arsenic and lead using Chinese brake ferns (*Pteris vittata*) with EDTA and electrodics

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**Abstract:** Chinese brake fern (*Pteris vittata*) has potential for application in the phytoremediation of arsenic introduced by lead arsenate-based pesticides. In this study, Chinese brake ferns were used to extract arsenic, mainly in field and greenhouse experiments, and to assess the performance of simultaneous phytoaccumulation of arsenic and lead from homogenized soil in the greenhouse, with the application of EDTA and electric potential. The ferns have been shown to be effective in accumulating high concentrations of arsenic, and extracting both arsenic and lead from the contaminated soil, with the addition of a chelating agent, EDTA. The maximum increase in lead accumulation in the ferns was 9.2 fold, with a 10 mmol/kg addition of EDTA. In addition, the application of EDTA in combination with electric potential increased the lead accumulation in ferns by 10.6 fold at 5 mmol/kg of EDTA and 40 V (dc), compared to controls. Therefore, under application of EDTA and electric potential, Chinese brake fern is able to extract arsenic and lead simultaneously from soil contaminated by lead arsenate.

**Key words:** Phytoremediation, Arsenic, Lead, Lead arsenate, Chinese brake fern, EDTA, Electrodics, ICP-OES

## 1. Introduction

Phytoremediation is the use of living plants for risk reduction of hazardous materials in the environment. The phytoremediation technology is a cost-effective cleanup technique with by using the ability of plants to remove and degrade harmful chemicals and has become a widely considered alternative for use in contaminated soil and groundwater remediation.[1,2]

Lead arsenate ($PbHAsO_4$, LA) was widely and internationally used of the arsenical insecticides for the effective control of insect pests on apples and other fruit tree from 1905 to 1947.[3] The use of lead arsenate was completely terminated in 1984 in Washington State as the last state and all insecticidal uses of lead arsenate were officially banned on 1988 in the USA.[4] Soil contaminated with arsenic and lead which are hazardous heavy metals is a major health concern leading to cancers and other health issues. In the early 1900s, concern arose about the retention of excessive pesticide residues on plants treated with lead arsenate.[5]

The Barber Orchard, Environmental Protection Agency (EPA) ID No. NCSFN0406989, is located

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along U.S. Highway 74 in Waynesville, Haywood County, North Carolina. The property is about 400 acres in size and was used as a commercial apple orchard by the Barber family. The apple orchard operated from about 1908 to 1988; a portion of the property is still used to grow apples. From 1903 until the 1940's, lead arsenate was used as an insecticide in the apple orchard. In 1999, it was determined that there is extensive groundwater and soil contamination on the home sites, and the area was declared a Superfund site by the EPA in late 1999.[6,7]

Several remediation technologies have been proposed and used for remediating lead arsenate contaminated soils by phytoremediation of arsenic and lead using ferns and Indian mustards (*Brassica juncea*), respectively.[8-12] The fern can accumulate much greater than arsenic by other plants indicating that ferns are equipped with efficient arsenic-uptake in translocation systems. Many reports suggest that phytoremediation of arsenic using Chinese brake ferns as an arsenic hyperaccumulator is a viable in situ remediation technology.[13-15] Indian mustard was used to demonstrate the capability of plants to accumulate high tissue concentrations of lead when grown in lead-contaminated soil.[16] Indian mustard, the metal hyperaccmulating plant, is effective in depleting lead from the soil and has been shown to possess a number of adaptive traits that enhance the rate and efficiency. In addition, application of chelating agents such as ethylenediaminetetraacetic acid (EDTA) to contaminated soils has been shown to induce the uptake of metals by plants.[17-18] It has also been documented that the addition of EDTA increases significantly lead accumulation in Indian mustard. Another technology to remove lead from contaminated soil has been reported by the combination of EDTA and electrodic phytoaccumulation.[11,12]

The purpose of this study is to investigate the phytoremediation potential of Chinese brake ferns for simultaneous accumulation of arsenic and lead from lead arsenate-contaminated Barber Orchard soil. A model illustrates the fundamental information for electrokinetics as well as its combination with phytoremediation in the presence of EDTA. In the previously described method,[11,12] the application of an electric field was enough to have the potential to be combined effectively and synergistically with EDTA-enhanced phytoremediation of lead using Indian mustard. In this study, the introduction of electric potential and EDTA into the soil around grown-up Chinese brake ferns helped removal of arsenic and lead in soil simultaneously. The experimental results showed that lead removal efficiencies depend on the electrical treatment and the concentration of EDTA by ferns.

## 2. Experimental

### 2.1. Soil collection and homogenization

Arsenic (As) and lead (Pb)-contaminated soil was collected from a plot of Barber Orchard 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 which composes a set of a test. The homogenized soil in each pot was monitored by an inductively coupled plasma optical emission spectrometry (ICP-OES) to demonstrate the homogenization of arsenic and lead and obtained approximately the same level of arsenic and lead concentration on each pot.

### 2.2. Field and lab cultivation

Chinese brake ferns (10 cm height) were planted at Barber Orchard approximately 50 cm apart on 7 columns with 8 plants. The soil was tilled to a depth of 30 cm. Gardening fabric and mulch was used to prevent weeds and maintain moisture. Fern shoots were harvested after two months by cutting the shoots 1 cm above the soil level and subsequently harvested after two months from the first harvest as the second harvest. Shoots were washed with distilled-deionized water to prevent contamination from soil and dust. Harvested shoots were air dried in the university greenhouse and homogenized using a ball-mill mixer (Spex 8000, Spex Certiprep, Metuchen, NJ).

The Chinese brake ferns were also planted to pots containing 1.2 kg of contaminated Barber Orchard soil. The plants were grown for 8 weeks after transferring to the pots and watered daily with weekly fertilization treatments. The plants were harvested by cutting the stem 1 cm above the soil surface according to the selected parameters after EDTA treatment and electric potential application. After the shoots were washed and dried, the samples were homogenized using a ball-mill for the analysis.

### 2.3. Experimental apparatus for electrodic phytoaccumulation

The electrodic and EDTA-enhanced phytoremediation (EEEP) system basically consists of an array of electrodes, a power supply, EDTA, and plants. *Fig.* 1 illustrates the array of the EEEP system with six graphite rods 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 dc and 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.

### 2.4. Sample preparation and analysis

0.2 g samples of the homogenized plants (shoots of Chinese brake ferns) were digested on a heating

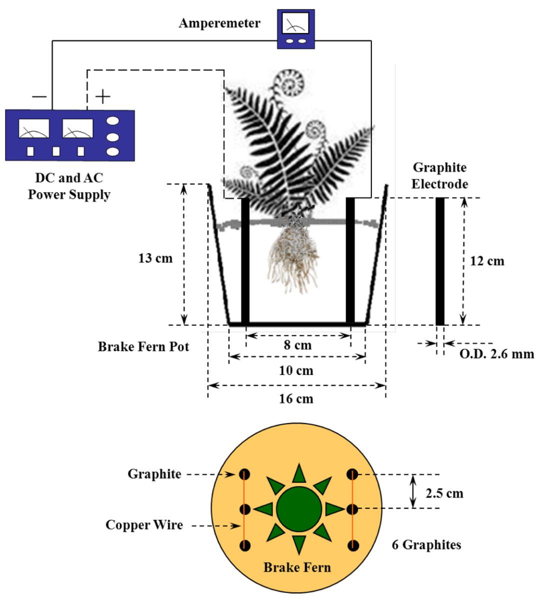


*Fig.* 1. Schematic diagram of the electrodic phytoremediation.

*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 |

block (lab manufactured) with 5 mL of concentrated $HNO_3$ followed by 1 mL of 30 % $H_2O_2$. The digested samples were diluted to 100 mL with distilled-deionized water. Pb and As concentration for Chinese brake fern sample were determined by an ICP-OES (Perkin Elmer, Optima, 4100 DV). Operating conditions for the ICP-OES are listed in *Table* 1.

## 3. Results and Discussion

### 3.1. Arsenic accumulation by Chinese brake ferns

Phytoextraction of arsenic using Chinese brake ferns from the Barber Orchard lot established successfully in seven experimental plots at the field site. *Fig.* 2 shows the analytical results with the amount of arsenic uptake from ferns planted at Barber Orchard. The average of arsenic concentration in ferns on each seven column with eight plants was resulted in 620~1816 μg/g at the first harvest as shown in *Fig.* 2(a). In a previous study, the average of arsenic concentration in the homogenized soil was about 103 mg/kg.[11] An average enrichment of approximately 6~18 times in ferns at the field site was observed. After two months from the first harvest, the consecutive second harvest was carried out for the analysis of arsenic phytoaccumulation. *Fig.* 2(b) shows the average of arsenic concentration in ferns ranged from 524 to 1486 μg/g. The amount of arsenic extracted decreases in 0.40~0.87 folds on the most columns during the second harvest when compared with the first. This is evidence that concentration of arsenic in fern is proportional to concentration of arsenic in the soil and arsenic in the soil was

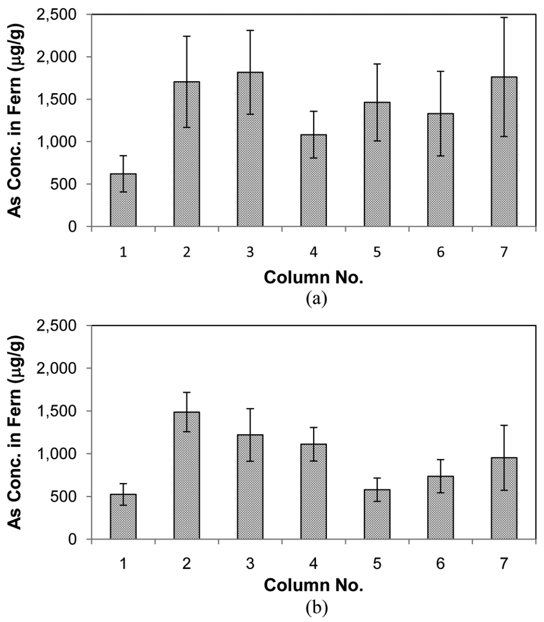


*Fig* 2. Concentration of arsenic in ferns harvested from Barber Orchard for two subsequent harvests: (a) first harvest and (b) second harvest. Values represent the mean ± S.D. of eight fern samples (n = 8) in each column on the field of Barber Orchard.

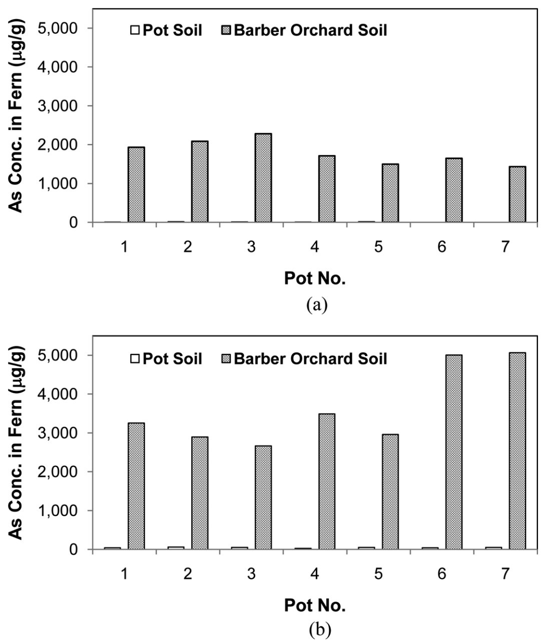


*Fig*. 3. Concentration of arsenic in greenhouse-grown ferns with Barber Orchard soil: (a) the first batch with Barber Orchard soil in seven columns and (b) the second batch with Barber Orchard soil in seven columns.

depleted by fern phytoextraction.

Two independent batches of Chinese brake ferns also planted and cultivated for two months in the green house to study arsenic uptake with the homogenized Barber Orchard soil. The arsenic concentration of ferns on seven pots at the first batch with homogenized Barber Orchard soil in seven columns was ranged from 1433 to 2281 µg/g compared to pot soil as shown in *Fig.* 3(a). The concentration of the second batch was ranged from 2666 to 5056 µg/g as shown in *Fig.* 3(b). The arsenic concentration was increased in 2.0 folds at the second batch as average 1799 µg/g compared to the first batch as 3620 µg/g and was dependent on the contaminated soil samples from Barber Orchard.

### 3.2. Effect of arsenic and lead accumulation in Chinese brake ferns by EDTA

Three harvests of Chinese brake ferns at each condition were conducted to evaluate the potential of EDTA treatment with three different concentrations to enhance the uptake of arsenic and lead from contaminated soils. The ferns were harvested on 9 days after application of EDTA to the contaminated soil. *Fig.* 4 shows the concentration of arsenic and lead in ferns with the increased concentration of EDTA applied to the soil. Arsenic concentration in ferns shows almost same uptake to 2 mmol/kg of EDTA compared to the control. However, arsenic concentration in ferns increased in 1.7 and 1.4 folds with 5 and 10 mmol/kg of EDTA, respectively. The ferns are one of the most well known plants as arsenic accumulator from the many studies with field and lab experiments but are not popular plants for lead accumulation as shown in *Fig.* 4 at the first two bar graphs with pot soil and the Barber Orchard soil. However, when the concentration of EDTA (0 to 10 mmol/kg) increases, a significant increase of lead accumulation in ferns occurred up to maximum 9.2 folds at 10 mmol/kg of EDTA with the Barber Orchard soil. *Fig.* 4 shows accumulation of lead in ferns was correlated with concentration of EDTA. In general, the concentration of lead in ferns increased linearly with increasing EDTA concentration and significantly

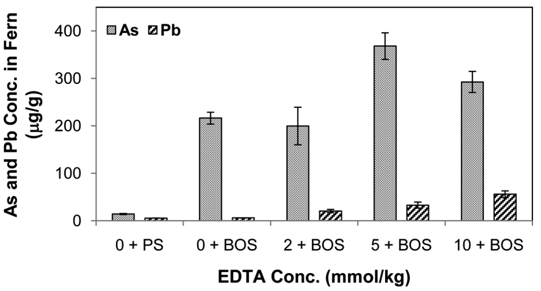


*Fig.* 4. Concentration of arsenic and lead in ferns with EDTA (PS = pot soil and BOS = Barber Orchard soil). The plants were harvested after 9 days of the application of EDTA. Values represent the mean ± S.D. of three replicates.

affected by EDTA.

## 3.3. Effect of arsenic and lead accumulation in ferns by EDTA and electric potential

The investigation of the experimental parameters was conducted to provide the new information required to refine a theoretical model of lead accumulation in ferns with EDTA and electric potential. The ultimate objectives of the new remediation model are to compare the relative efficiency and to identify the synergistic combination of simultaneous arsenic and lead accumulation in ferns with EDTA and electric potential. Chinese brake ferns, two months old grown in the homogenized Barber Orchard soil, were treated with EDTA for 9 days at 5 mmol/kg and an electrical potential of 40 V direct current (dc) or 40 V alternating current (ac) for 1 hr per day for 9 days. Each condition had three ferns with the homogenized Barber Orchard soil. After 9 day treatment, the ferns were harvested for analysis.

Arsenic and lead accumulation in ferns at each experimental condition was shown in *Fig.* 5. For arsenic accumulation in ferns as shown in *Fig.* 5(a), an average concentration of arsenic was 247 μg/g in ranged from 164 to 368 μg/g. However, in spite of 1.5 fold increase of arsenic accumulation with EDTA only, arsenic accumulation was not affected at each experimental condition in general.

An interesting aspect was observed in lead accumulation in ferns as shown in *Fig.* 5(b). The first three bar graphs show that the lead concentrations in

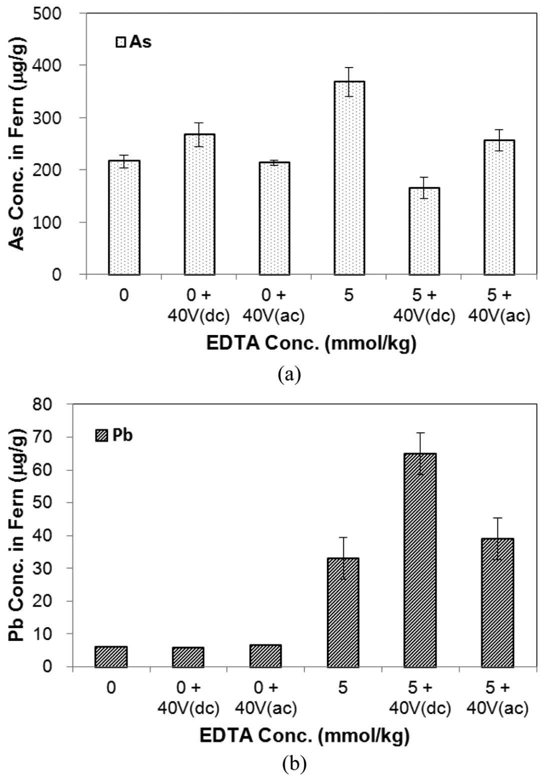


*Fig.* 5. Concentration of arsenic and lead in ferns with EDTA and electric potential (dc = direct current and ac = alternating current): (a) arsenic and (b) lead. The plants were harvested at 9 days after the application of EDTA and electric potential. Electric potential was applied for 1 hr a day. Values represent the mean ± S.D. of three replicates.

ferns were not affected by the dc or ac in the absence of EDTA. However, lead concentration in ferns was increased in 5.4 folds with EDTA such as results shown in *Fig.* 4. In addition, the application of dc electric potential in combination with EDTA caused 10.6 fold enhancements in the amount of lead accumulation in ferns. The combination of EDTA and dc electric potential increased lead accumulation by about 2 folds compared to the treatment of EDTA only. The combination of EDTA and ac electric potential did not cause as large as an increase in lead accumulation in ferns compared to dc electric potential but observed higher lead accumulation than the presence of EDTA only. This result is in good agreement with the previous experiments about the synergistic combination of dc electric potential and

EDTA-enhanced phytoremediation based on the increase of the mobility of lead in the soil.[11,12]

## 4. Conclusions

Chinese brake fern was efficient in the uptake of arsenic from the contaminated soil in both the field and green house experiments and was capable of reducing arsenic concentrations in the contaminated soil by continuously reuse after the first growth period. Arsenic accumulation in the field was decreased in 0.40~0.87 folds during the subsequent second harvest compared to the first. The amount of lead extraction in ferns significantly increased up to maximum 9.2 folds with the amount of EDTA applied to the soil. The combination of EDTA and electric potential for arsenic and lead enhanced phytoextraction in ferns with the contaminated soils. The application of both EDTA and dc electric potential caused 10.6 fold enhancements of lead accumulation in ferns and increased about 2 fold lead accumulations in ferns compared to the treatment of EDTA only.

In summary, successful fern phytoremediation of arsenic and lead was established by the combination of EDTA and electric potential. As shown in the results, EDTA and electric potential-enhanced phytoremediation of arsenic and lead with ferns appears to be a viable solution for soil remediation. On the whole, this application seems to be a very efficient and inexpensive soil remediation method, which really deserves to be thought of when facing the pollution problems.

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