

In-situ Phytoremediation of Arsenic from Contaminated Soil

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Abstract

Arsenic contamination in the world is worrisome, excessive and increasing. In this study, the research was designed for the remediation of arsenic polluted soil site in Beijing China using *in situ* treatment method (phytoremediation). Chelating agents ethylenediaminedisuccinic (EDDS), and citric acid (CA) were used to aid the hyper accumulator to enhance quicker extraction rate. A hyper accumulator Chinese brake fern (*Pteris vittata*) was used with the additions of EDDS and CA at different ratios. The result obtained from the treated soil were analyzed and compared. It showed significant increase in the extraction of Arsenic (As). About 3.55 mg/kg of As was detected in the treated plant biomass whereas the control has 0.98 mg/kg. It was suggested that solubility in the soil after treatment readily for uptake was 3.9 mg/kg. The control plants and the Corn however, had little absorption. It will be more promising, to select hyper accumulator which naturally possess higher biomass and are plants of local and natural origin to peculiar location.

Keywords: Soil contamination; Phytoremediation; Arsenic; Hyper accumulator

Introduction

Soil pollution is a major environmental problem faced by human and as such a great threat to human and environment. Previously, cases of rice Arsenic were reported in Asia. It has become a global concern like other environmental pollution issues, with implications ranges from effects to the ecosystems, ecology, human health etc. Due to the presence of heavy metals, other inorganic and organic contaminants heavy metal and its contamination in soil environment are usually related to human activities such as industrialization, applications of fertilizers and pesticides on farm land, generation of energy and production of fuels, mining and metallurgical processes and waste disposal which causes toxicity to all living organisms [1]. In China, it is estimated that more than 20 million hectares of land or soil environments have been contaminated, accounting for about 20% of the total land. Some of the major heavy metals that causes soil contamination and subsequent environmental pollution includes As, Cd, Cr, Cu, Co, Hg, Mn, Ni, Pb and Zn. Generally, heavy metal pollution is a combination of these several heavy metals [2]. Arsenic is a naturally occurring element widely distributed in the earth's crust. Arsenic is very toxic when found in large quantities in drinking water and food sources. In some part of India and Bangladesh, it is found that arsenic contaminates the groundwater supply. Arsenic is widely distributed in the biosphere. It occurs in sea water at a level of 2 µg/kg [3]. The use of arsenic containing pesticides in the past has left large areas of agricultural land contaminated [4]. The use of arsenic in the preservation of timber has also led to contamination of the environment. Arsenic and its compounds are naturally present in low concentration at places with high geothermal activities [5].

Phytoremediation can be defined as the removal of a substance from the air, soil or water via the natural ability of plants to take up metals as nutrients. Some of them are essential mineral nutrients needed for the growth and development such as Cu, Co, Fe, Mn and Zn whereas other heavy metals, like the Cd, As and Pb, have no physiological benefits. On the other hand, this natural potential of plants is a problem for human health when these elements are found in higher amount in food crops. Based on this findings, many researchers have taken to phytoremediation method. Phytoremediation includes several subsets such as phytoextraction, phytomining, phytostabilization, rhizofiltration and phytovolatilization.

When plants are referred to as hyper accumulator, there must accumulate at least 100 mg/kg (0.01% dry weight) of As, Cd, in their root and particularly be able to translocate them to their shoots. Hyper accumulating plants include *Pteris* ferns, *Pityrogramma calomelanos*, *Lemna gibba* (duckweed), *Lepidium sativum* (watercress), *Lupinus albus* (white lupin), mustard plants [6]. Biomass producing plants are studied following the approach of assisted phytoremediation, in which plants have to be managed with practices to enhance the element bioavailability for the plant uptake [7]. Synthetic chelating agents have the potential to remobilize metals and to form strong soluble complexes [8,9].

Heavy metal hyper accumulators are sometimes low biomass and slow growing plant species that are highly metal specific. Chelating agents (EDDS and CA) were used to aid the hyper accumulators to enhance the extraction rate. The addition of chelating agents Ethylenediaminedisuccinic (EDDS) and Citric acid (CA) to the soil help to enhance the concentration of these metals in the above the ground harvestable plant parts (solubility) and translocation from roots to shoots [10].

This study was done in two fold. First, to investigate and analyze the usage of an in-site remediation technique (phytoremediation) to remediate and control Arsenic contaminated soil to the acceptable National Standard. Secondly, to use the method with the aid of chelating agents to enhance the efficiency of the process. This is envisaged as being an effective method and a more environmentally friendly available technique for such unique condition of contamination to be effectively controlled.

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Materials and Methods

Soil description and location

The soil sample used in this study was collected from Agricultural farm lands in China. This is an area with over 20-years irrigation history from wastewater containing heavy metal. The soil in the test area is said to be heavily polluted by As and to a lesser degree by Cd, farmers had in the past irrigated farmland from the polluted river water in 1970s to 1990s. The area north temperate climate is continental monsoon climate, hot and rainy in summer, winter; cold and dry, dry and windy spring, autumn and short fall. The annual average temperature is 100°C ~ 120°C, where the average temperature of mountainous northwestern is 10.80°C, frost-free period is about 150 days; south eastern plains with the annual average temperature of 11.60°C, frost-free period 190 to 200 days. Average annual precipitation is about 655 mm of precipitation concentrated in the 6 to 8 months, accounting for 85% of annual precipitation, rainfall intensity, more hail, and strong winds. Sandy and slightly loamy soils are the major soil types found here. The pH of the soil was determined as 7.34 and 7.31 at ratio of 1:2.5.

Sample collection

Soil sample were collected randomly from the contaminated sites using a simple collection tools and big sample bags. Over 500 kg of soil were taken from the various farmlands within the range of 0 cm to 30 cm depth, in the late autumn season. This point of collection was monitored by Hand GPS system. The coordinates of the site are represented in Table 1. The collected soil was naturally air dried inside the greenhouse for 7 days.

Experimental setup

The soil was weighed equally into each plastic pot of different diameter of 10 cm depth/height and 15 cm diameter circumference for the planting of Chinese brake fern (*Pteris vittata*). The *Pteris vittata* were transplanted. However, local farm crops (corn) was also planted and monitored alongside in the greenhouse. Two low molecular weight organic acids and Citric acids were used in treating the soil samples

Point no	Coordinates
227	39°37'46.84" N, 115°56'35.98" E
228	39°37'46.53" N, 115°56'37.88" E
229	39°37'42.36" N, 115°56'43.89" E
230	39°37'41.91" N, 115°56'42.27" E
231	39°37'39.92" N, 115°56'42.77" E
232	39°37'40.04" N, 115°56'44.36" E
233	39°37'38.40" N, 115°56'45.98" E
234	39°37'40.29" N, 115°56'48.32" E
235	39°37'38.03" N, 115°56'34.93" E
236	39 ° 37 '34.93" N, 115°56'35.50" E)

Table 1: Shows GPS coordinates of the soil collection sites.

applied. Five replicate pots with Chinese brake fern (*Pteris vittata*) for controlling the arsenic (As) were used. After 4 weeks of growth for a period of 30 days from the planting date, both soil and plant samples were analyzed, 5 mmol kg⁻¹ of EDDS and CA citric acid solution were added at the ratio of 1:1, 2:1, 1:2 to each of the replicates. After 15 more days, totaling 45 days from planting time, sample of soil and plants was extracted from the pots and analyzed. The process continued for 90 days. The pots were kept at about the same percentage of water content throughout the experiment using the leached water and drainage water as water source which was added back to the pots. The experiment was carried out in the greenhouse with no control on sourcing for light intensity for the plants; the soil was generally treated with artificial farmland fertilizers after sieving and weighed into each pot.

Soil characterization

The field water holding capacity of 75% was maintained by giving in much quantity twice a week until the last month of final harvest where it was given once a week. Other parameters such as the temperature were generally controlled by the green house administrator which was set at 13°C and 15°C degree, although this varies between day and night. Other soil parameters such as moisture contents, weight, density, TN and TP, with organic matters were all determined as Soil pH was measured with a glass electrode in distilled water. To 20 g of soil in a 50 mL beaker, 20 mL of reagent water was added, covered and continuously stirred for 5 min. The soil suspension was allowed to stand for about 1hr to allow most of the suspended clay to settle out from the suspension and the pH was measured using the pH meter. Soil: solution=1:2.5 which was 7.34 and 7.40. Total organic C (TOC) was analysed by dry combustion, using a TOC 5000 total C analyser (Shimadzu, Japan). TN was determined by Kjeldahl digestion, available N was analysed by alkali-hydrolytic diffusion method, total P was measured calorimetrically after H₂SO₄-HClO₄ digestion, available P was determined with Olsen method. Each pot was moistened to about 75% of the water holding capacity (WHC) by the addition of water from the greenhouse facilities.

Samples analysis and arsenic detection in soil and plant

The plants were harvested after the addition of EDDS and citric acid by cutting the stem above the soil. The shoots were washed with lab tap water and later rinsed with deionized water and freeze dried, using the freeze drying machine for 48 hr and manually grind (mortar/pestle) and sieved through 0.02 mm sieve. The soils from the pots were collected near the plant root or stand and also air dried and grinds using the mini soil/solid grinder and sieved in order to collect the roots.

A CEM cooperation MARS-5 version 194A06 micro wave reaction accelerated system was used for the wet digestion analysis. Soil and plant samples were both analyzed through the wet digestions (WD) method. Each (0.3000 g) of the samples were accurately weighed (± 0.0002 g) and digested in 6 mL of HNO₃ (60%), 2 mL of HF (20%), place into the micro oven at 190°C for 20 min and later treated with 2 mL of HClO₄ (20%) for 8 hr at 150°C. The solution was diluted to 100 mL with deionized water. All sample solutions were filtered with filter paper before final analyses for Arsenic contents; Inductively Coupled Plasma optical/Atomic Emission Spectroscopy was used. Blank reagent and other analytical duplicates were also used when needed in order to ensure accuracy and precision in the analysis.

Shoot metal uptake

The metal uptake was calculated by subtracting the soil pre greenhouse experiment and treatment contents of the soil from

the total metal content from the samples taken from the study site. Concentrations of heavy metals were summed up in the soil originally from the farm site and that from the greenhouse with that which is in the plant shoots for each treatment. A linear increase in As is assumed. Plant shoots showed adverse effects to the addition of the chelating agents (EDDS and Citric acid). In the space time of two days after the addition of EDDS to the soil the shoots started to show signs of toxicity and by 3 days they were necrotic. EDDS also seemed to reduce the shoot dry weight. In previous investigations signs of toxicity were seen for the application of chelating. Over a period of time, shoot dry weight was not adversely affected by the treatment as both shoot total dry weights were much higher on the heavily contaminated soils. The heavy metals became more soluble and significantly increased after the addition of the chelating agents.

Results

Heavy metal accumulation

Arsenic concentrations in the above ground parts of the Chinese brake fern were between 3.16 mg/kg and 2.23 mg/kg based on all samples and control with the local plant (corn). For the arsenic concentration in plant, there was little significant difference in the metal up-take among the different treatments with the exception of the control. The highest concentration was in the treated sample which has an average of 3.16 mg/kg in its shoot followed by the corn with an average of 2.32 mg/kg while the lowest concentration was in the control plants with 2.23 mg/kg. Table 2 shows the amount (mg/kg) of heavy metals uptake by Chinese brake fern. Figure 1 depicts the amount of heavy metals uptake by Chinese brake fern.

Application of EDDS and CA on growth and metal absorption

The treatments with 5 mmol kg⁻¹ of EDDS and CA significantly depressed the growth of the plants. When the EDDS was applied to the soil, it appeared to be toxic to plants as compared to when CA was applied. A significantly lower biomass was observed following the application of EDDS within the first two days of application; however these changes were overcome by the plants after four to five days. Plants with the combined treatments of EDDS and CA also exhibited a slight decrease in biomass compared to those that had received a treatment of 5 mmol kg⁻¹ of CA alone. Among the combined treatments of EDDS and CA at the ratios of 1:1, 1:2 and 2:1 respectively, there were no significant differences in dry mass yields. Compared with the control group, the application of EDDS and CA at different ratio to the soil significantly increased the concentrations of Cd and As in the biomass of Indian mustard and fern respectively.

When EDDS and CA were applied in combination at different ratios, the concentrations of heavy metals in both plant biomass and their bioavailability in soil were significantly lower than in EDDS applied alone. The combined application of EDDS and CA at the ratio of 2:1 produced the highest heavy metal concentration of 2.98 mg/kg of As in all of its hyper accumulators plants and Cadmium 1.68 mg/kg was found in the soil and 0.98 mg/kg in the shoot of Indian mustard concentration which was significantly lower than that of the 5 mmol kg⁻¹ of EDDS treatment alone on the 90th day. Even though it was observed that the dry matter yield of plants was highly affected when these chelating agents were applied. Table 3 shows the rate of combined application of chelating agents. The rate of heavy metals concentration and accumulation in plants were significantly influenced by these treatments, the accumulation rates were expected to have increased with an increase in the amount of chelating applied, and all plants had the highest accumulation rate proportional to treatments over time. Figure 2 shows the solubility of heavy metals in soil after treatment.

The total extraction of the heavy metals in the biomass increased significantly with the application of chelating agent. The maximum extraction of both Cd and As was found in the application of 5 mmol kg⁻¹ in EDDS alone. The combined application of EDDS + CA was more efficient in enhancing the bioavailability in comparison with CA alone. When EDDS and CA were applied at ratios of 1:1 and 2:1, respectively, it was higher in concentrations of Heavy metals both in soil as compared to CA applied alone at 5 mmol kg⁻¹ for plants uptake.

Discussion

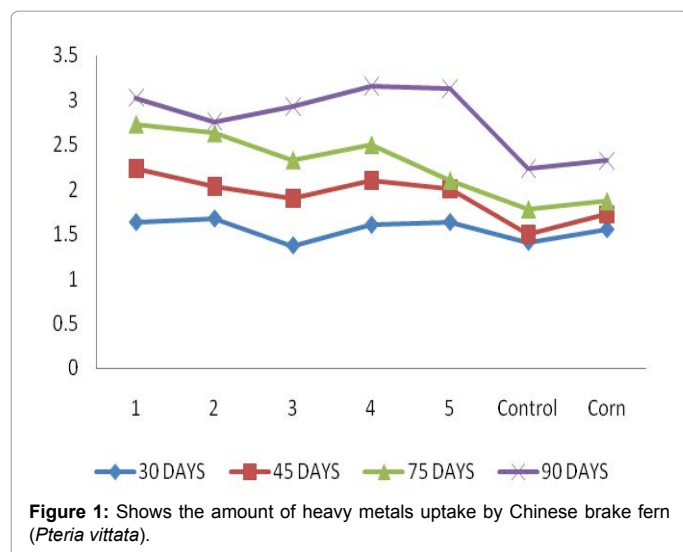
Plant relative growth rate analysis

Relative growth rate (RGR), expressed as grams per kilogram per day, was considered and was calculated as $RGR = \frac{\text{final dry weight} - \text{initial dry weight}}{\text{number of days}}$, and the tolerance index (TI) was calculated to measure the plant capacity to grow in the presence of the heavy metals. $TI = \frac{\text{shoot height in the treatment}}{\text{shoot height in the control}} \times 100$, Bio concentration factor (BCF) was calculated to measure plant uptake of heavy metals: $BCF = \frac{\text{Heavy metal concentration in plant shoot mg/kg}}{\text{Heavy metal concentration in soil mg/kg}}$. The biomass weight and shoot height of plants after been grown in the batch-scale in the greenhouse from 30-90 days in the polluted soil acclimation are shown in Table 4.

As show in Table 2, there was a significant increase in the weight of the biomass and height of the hyper accumulators and other plant (corn) the percentage amounts of heavy metals accumulated in the biomass of these plants as expected is directly proportional to RGR of

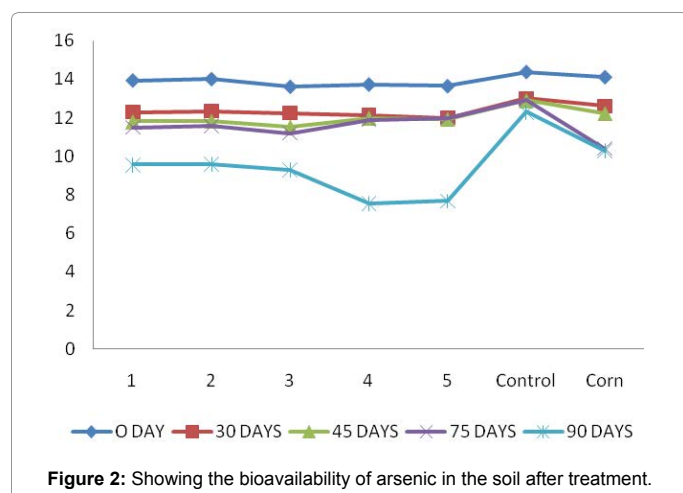
90 days			75 days		40 days		30 days	
Height(m)		Weight(g)	Height(m)	Weight(g)	Height(m)	Weight(g)	Height(m)	Weight(g)
Sample (A)	171	3.47	74	1.12	57	0.98	43	0.5
Sample (B)	79	3.43	48	1.43	35	0.99	23	0.5
Control (A)	87	1.53	43	0.76	39	0.54	30	0.7
Control (B)	43	1.21	30	1	27	0.32	20	0.5
Corn (A)	57	4.1	33	2.61	31	1.97	25	0.9
Corn (B)	33	3.01	33	2.15	30	2	27	1.04

Table 2: Showing the relative growth rate of hyper accumulator in height and weight.



Sample b	Sample weight (g)	30 Days	45 Days	75 Days	90 Days
1	0.3002	1.63	2.23	2.73	3.03
2	0.3003	1.67	2.03	2.63	2.76
3	0.3	1.37	1.9	2.33	2.93
4	0.3002	1.6	2.1	2.5	3.16
5	0.3001	1.63	2	2.1	3.13
Control	0.3	1.41	1.5	1.78	2.23
Corn	0.3	1.5	1.72	1.87	2.32

Table 3: Showing the amount (mg/kg) of heavy metals uptake by Chinese brake fern (*Pteris vittata*).



the plants. Between 75 to 90 days; there was almost 100% increase in the height and weight of the hyper accumulators in the treated soil, hence the amount of metals in them too. Except the corn, all of the treatment and changes in temperature shows great abnormality in its growth rate of only 35-57 m it was not observed between 70-90 days to have also increased in its height over this period of favourable condition as other plants but the body mass weight did increase considerably.

Chelating agents	Applications rate	Soil (mg/kg) as	Plant (mg/kg)
EDDS	5 mmol kg ⁻¹	3.89	1.03
Citric acid(CA)	5 mmol kg ⁻¹	0.98	0.12
EDDS + CA (1:1)	2.5 mmol kg ⁻¹ + 2.5 mmol kg ⁻¹	1.65	0.78
EDDS + CA (2:1)	3.33 mmol kg ⁻¹ + 1.67 mmol kg ⁻¹	2.98	0.98
CA + EDDS (2:1)	3.33 mmol kg ⁻¹ + 1.67 mmol kg ⁻¹	1.09	0.56

Table 4: Showing the rate of combined application of chelating agents.

Application of chelating and the solubility of metals in soil

The relative efficiency of EDDS and CA in enhancing soluble metals in soil was confirmed as compared to that of the control. For the dissolution of As and Cd, EDDS showed higher efficiency than CA. The addition of EDDS at 5 mmol kg⁻¹ produced higher amount of soluble As and Cd in the soil. Applying EDDS and CA in a combined ratios of 1:1, 1:2, and 2:1 totaling 5 mmol kg⁻¹, the concentration for extraction of these heavy metals did not remained the same as with the 5 mmol kg⁻¹ of EDDS treatment alone, this might be so because the total amount of EDDS applied was not being maintained, as it was altar for some percentages of CA. One can conclude here that there was a significant difference observed among the treatments of 5 mmol kg⁻¹ of EDDS alone and three different combined treatments of EDDS and CA during the whole experiment period. EDDS was more effective than CA in solubilizing both the heavy metals. The soil pH remained relatively stable with this application of chelating agents

Conclusion

The result and data analysed from the treated soil compared to control showed significant increase in the extraction of As 3.55 mg/kg of As was in the treated plant biomass whereas its control has 0.98 mg/kg. It was suggested that soluble As in the soil after treatment readily for uptake was 3.9 mg/kg. However, the concentrations of heavy metals (As, Cd) in the soil originally from the farm site, and that from the greenhouse in both plants shoot and soil for each treatment as analysed, a linear increase in the uptake of As concentration with time assumed, this was found to be the case for the hyper accumulator shoots and leaves grown in the polluted soils. The half-life of EDDS in sludge-amended soil was 2.5 days, this implies that residual EDDS in the soil will rapidly be degraded and pose a relatively lower risk with respect to the leaching of metal into groundwater. That draws the conclusion that the EDDS and CA are good alternative to the usage of EDTA for phytoremediation.

This technology is however still in its early stages of development, with laboratory research and limited field trials being conducted to determine processes and refine methods. Additional research, including genetic engineering, need be conducted to improve the natural capabilities of plants to perform remediation functions and to investigate other plants with potential phytoremediation applications and as well strict measures are needed to control to avoid the circulation of these contaminants from entering the food chain.

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