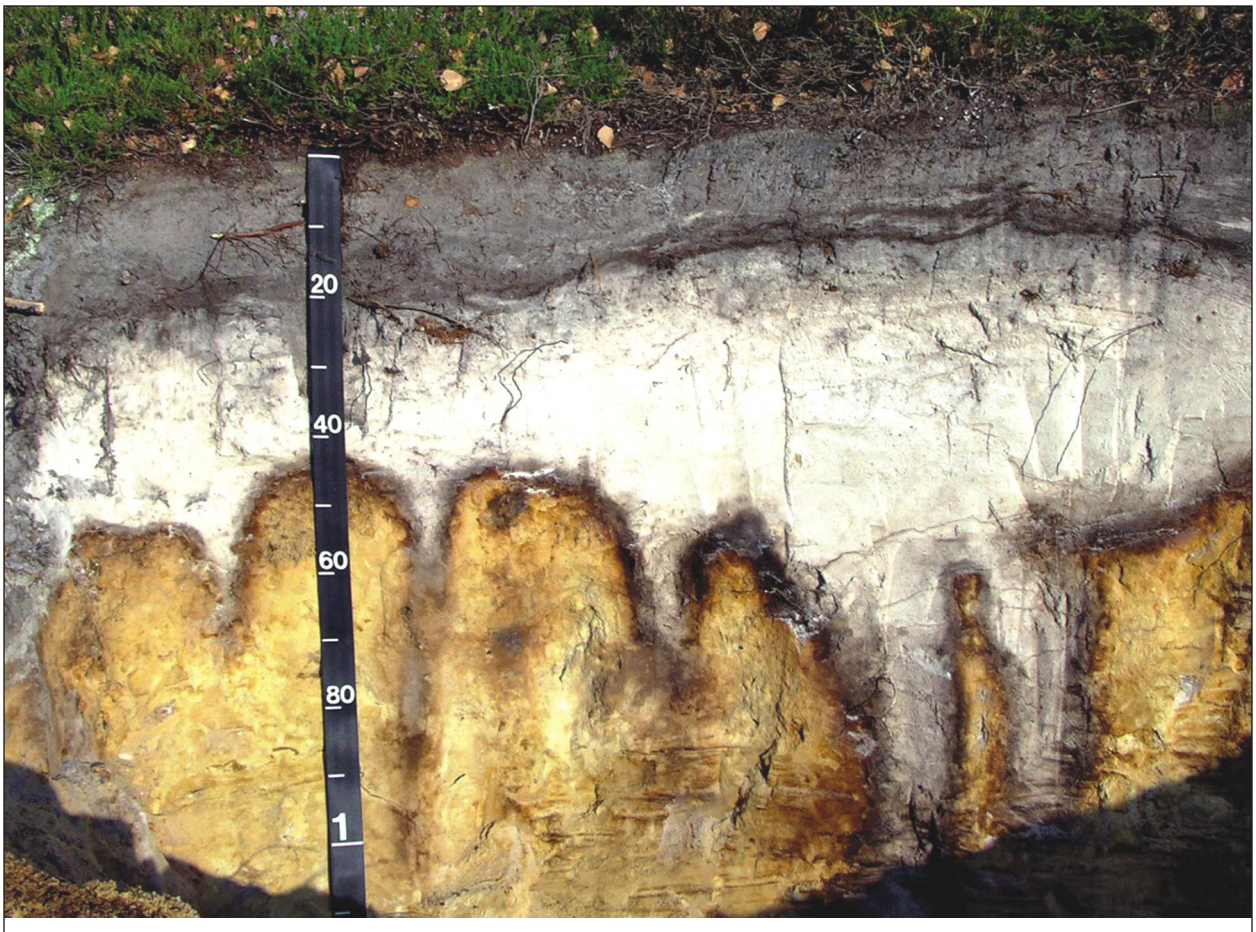

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Research Article
Phytoextraction Efficiency of Lead by Arum (*Colocasia esculenta* L.) Grown in Soil

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Abstract

Background and Objective: Lead (Pb) contamination of agricultural soil and water via anthropogenic sources have resulted serious problems in the food chain and consequently the health of organisms, including man. Phytoremediation has been touted as a promising alternative for the generally expensive and disruptive conventional remediation techniques to clean up Pb contaminated soil. Therefore, the aim of this study was to investigate the Pb tolerance and phytoextraction efficiency of arum (Pani Kachu; *Colocasia esculenta* L.) grown in soil spiked with different levels of Pb. **Materials and Methods:** The plant was grown for 105 days in soils spiked with Pb at the levels of 0, 300, 600 and 1200 mg kg^{-1}. The plant parts were digested with HNO_3-$HClO_4$ (3:1) acid mixture. Lead in the digests was measured using atomic absorption spectrophotometer. **Results:** Dry weight of plant parts was not affected by any levels of Pb concentration while concentration of Pb increased in plant parts with the increase of Pb levels in soil. In shoots, concentration of Pb was 7748 mg kg^{-1} at the highest level of Pb concentration (1200 mg kg^{-1}) in soil. This concentration (1200 mg kg^{-1}) did not cause any growth retardation of arum plant which indicated that arum was a metal hyperaccumulator plant. However, translocations of Pb in arum parts at this high level was 62% of total Pb which indicated that a major portion of metal was translocated from roots to shoots. Transfer Factor (TF) greater than one as found in the present experiment confirmed the hyperaccumulation characteristics of arum for Pb in soil media. **Conclusion:** This study indicated that the growth of arum was unaffected by application of any levels of Pb in soil. The Pb concentration in the shoots of arum without growth retardation and TF of Pb in arum indicated that this plant was a suitable candidate for the phytoremediation of Pb contaminated soil.

Key words: Metal, contamination, hyperaccumulator, arum, phytoextraction, toxicity, phytoremediation, soil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lead (Pb) is generally recognized as an environmental toxin to wildlife, domestic animals and humans[1]. Despite efforts by many industrialized countries to reduce Pb emission (e.g., Pb alkyls in gasoline) worldwide Pb emissions are still increasing[2]. Though Pb occur naturally in rocks, soils and water, environmental contamination via anthropogenic activities such as mining and smelting, combustion of gasoline containing anti-knock additives tetraethyl and tetramethyl Pb, use of Pb-based paints and explosives, land application of sewage sludge, use of some fertilizers and pesticides, battery recycling and use of Pb bullets have resulted in serious problems in the food chain and consequently, the health of organisms, including man[2,3]. Lead concentrations in some industrial sites of Bangladesh are found to range from 17-99 mg kg^{-1} [4] which are above the background level for Pb in soil (12-20 mg kg^{-1})[5]. However, the concentrations of Pb in vegetables grown in agricultural soils adjacent to the industrial areas of Bangladesh were observed in the range of 10-26 mg kg^{-1} dry weight by Ahmad and Goni[6] and 13-45 mg kg^{-1} dry weight by Kashem and Singh[4]. These values exceed the acceptable tolerance level for FAO/WHO standard of 5 mg Pb kg^{-1} dry weight[7] and of 10-20 mg Pb kg^{-1} dry weight recommended by Sauerbeck[8].

It is therefore, important to develop methods to cleanup Pb contaminated soils. Phytoremediation, where hyperaccumulators are used to take up large quantities of pollutants from contaminated soils has been touted as a promising alternative for the generally expensive and disruptive conventional remediation techniques to reduce environmental health risks posed by Pb-contaminated sites[9]. To date, about 700 species of plants have been reported to be hyperaccumulators of different contaminants[10] of which only few have been considered as Pb hyperaccumulators[11]. However, successful phytoextraction requires that these plants are capable of producing high biomass while accumulating large amounts of contaminants in the biomass from the soil[12]. In the present investigation, we select a common and locally popular plant species arum (*Colocasia esculenta* L.). This plant is widely distributed in Bangladesh and can grow in both dry and marshy conditions. It has deep roots and long shoots. It possesses the characteristics of high biomass, easy cultivation, extensive competitive ability and strong resistance to environmental stresses. Kashem *et al.*[13,14] conducted both hydroponics and soil experiment in Japan with a different arum species (*Colocasia antiqourum*) and found that this plant had strong tolerance to Cd in the nutrient solution and soil culture and strong accumulation capability of Cd in its body. Chayapan *et al.*[15] reported phytoremediation potential of Cd and Zn by wetland plants, *Colocasia esculenta* L., Schott., *Cyperus malaccensis* Lam. and *Typha angustifolia* L., grown in hydroponics. Although, there are few researches on phytoextraction of Pb by some species[12,16-20] but till date, there is no study to investigate the Pb tolerance and phytoextraction efficiency of arum species of *C. esculenta* L. neither in hydroponics nor in soil cultures until the study conducted by Islam *et al.*[21]. In the previous hydroponics study, it was shown that this plant had strong tolerance to Pb in the nutrient solution and strong accumulation capabilities of this metal in its body[21]. Metal accumulators should be selected under standard and repeatable conditions using both hydroponics and soil cultures[22]. Therefore, the aim of this study was to investigate the Pb tolerance and phytoextraction efficiency of arum grown in soil spiked with different levels of Pb were investigated. The distribution of this metal in different parts of arum was also investigated.

MATERIALS AND METHODS

A surface (0-15 cm) sandy loam soil was collected from the crop field near the net house of the Department of Soil Science of the University of Chittagong, Bangladesh. The collected soil was air dried, ground and passed through a 4 mm sieve for using in pot experiment. For laboratory analysis, a sub sample was passed through a 2 mm sieve and stored. Soil pH of 5.0 was measured in a 1:2.5 soil/water suspension. Soil Organic Carbon (OC) of 0.4% was determined by wet oxidation method[23]. Cation Exchange Capacity (CEC) of 4.1 cmol kg^{-1} was determined by extraction with 1 M NH_4OAc (pH 7.0)[24]. The hydrometer method was used for the particle size distribution[25]. The soil had 67.9% sand, 12.7% silt and 19.4% clay.

The pot experiment was conducted under natural light condition in a net house of the Department of Soil Science at the University of Chittagong, Bangladesh. Moist soil equivalent to 7 kg dry mass was placed in a 12 L plastic pot after mixing 0, 300, 600 and 1200 mg kg^{-1} of Pb as $Pb(NO_3)_2$ (ACS grade, Sigma-Aldrich Co.). In each pot, N (as $(NH_4)_2 SO_4$), P (as KH_2PO_4) and K (as KCl) was added as 100 mg kg^{-1} soil as recommended by SRDI Report of Land and Soil Resource Use[26]. Three replications of the treatment were set out in a complete randomized block design. Two healthy and uniform size

plantlets of arum (*Colocasia esculenta* L.) were transplanted in each pot. After one month, one plantlet was left in each pot. From 40 days of growth, water was added daily approximately up to the field capacity during the growth period.

Plants were harvested after 90 days of growth following transplanting. Plant roots were carefully removed from each pot and washed thoroughly to get rid of adhering soil particles followed by quick wash of deionized water. The fresh weights of plants were measured. Then the plants were separated into leaves, stems, stolons, rhizomes and roots and their fresh weights were measured. After drying in an oven at 68°C for 72 h, their dry weights were also measured. The plant parts were grounded using a stainless steel grinder and were then digested with HNO_3-$HClO_4$ (3:1) acid mixture[27]. Lead in the digests was measured using atomic absorption spectrophotometer (Agilent Technologies, 420 AA, Australia). Reagent blanks were processed to ensure Pb was not added during sample preparation. The TF of Pb was measured by dividing the concentration of Pb in shoots to those in roots of arum grown in soil culture[28]. All results are presented on Dry Weight (DW) basis. The results obtained were subjected to one way analysis of variance using Minitab program[29].

RESULTS

Effects of Pb on plant growth: Dry weight of leaves, stems, stolons, rhizomes and roots of arum plant was not affected by any application rates of Pb in soil. There were no visual symptoms of toxicity in any plants parts caused by high application rate of Pb (1200 mg kg^{-1}) in soil. The dry weight of plant in control pots were 11.2, 30.2, 17.8, 26.6 and 13.8 g for the leaves, stems, stolons, rhizomes and roots, respectively. The corresponding values in the pots treated with the highest Pb application (1200 mg kg^{-1}) were 10.3, 29.8, 17.2, 25.9 and 13.3 g (Fig. 1).

Lead concentrations and its accumulations in arum plant: The concentration of Pb in different parts of arum increased significantly ($p<0.05$) with increasing its levels in the growth media. In control treatment, concentrations of Pb could not be detected in plant parts and were not shown in table and figures. Lead concentrations in plant increased from 182-2860, 342-4889, 43-571, 34-360 and 519-5684 mg kg^{-1} in leaves, stems, stolons, rhizomes and roots, respectively when Pb concentrations in the soil were increased from 300-1200 mg kg^{-1}. In the whole plant, Pb concentrations were 210, 1299 and 2798 mg kg^{-1} at soil Pb levels of 300, 600 and 1200 mg kg^{-1}, respectively (Fig. 2).

Accumulations of Pb significantly ($p<0.05$) increased in different parts of plant with the Pb application rates applied in soil (Fig. 3). Lead accumulations in plant increased from 2-29, 10-146, 1-10, 1-9 and 7-76 mg $plant^{-1}$ in leaves, stem, stolons, rhizomes and roots, respectively when Pb concentration in soil increased from 300-1200 mg kg^{-1}. However, accumulations of

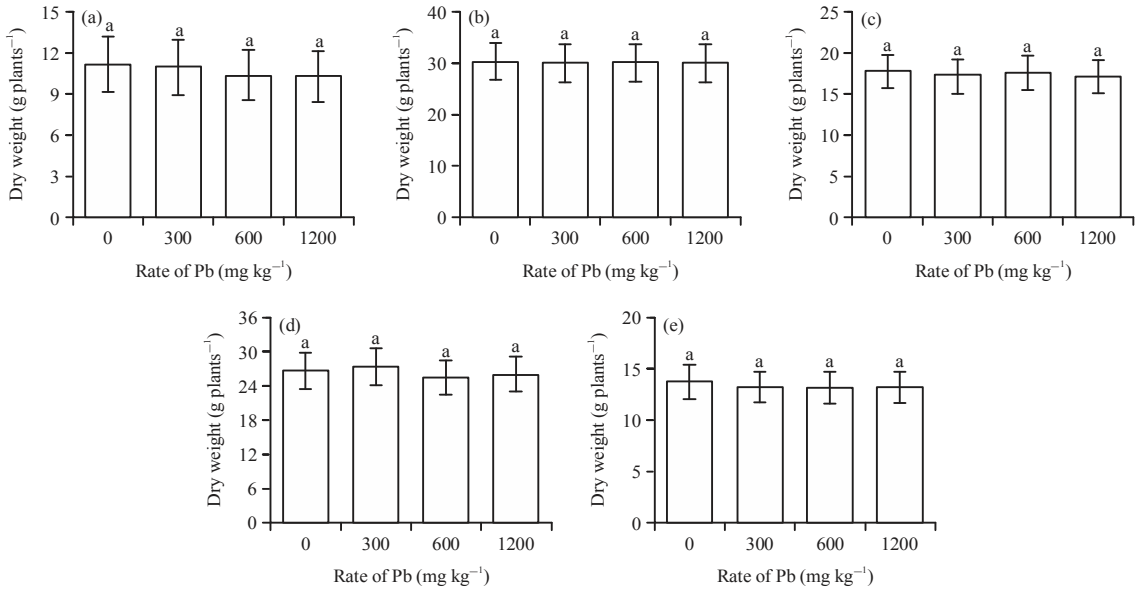

Fig. 1(a-e): Dry weight of arum plant parts (a) Leaves, (b) Stems, (c) Stolons, (d) Rhizomes and (e) Roots. Bars with the same letters within the plant parts are not significantly different from each other at $p<0.05$

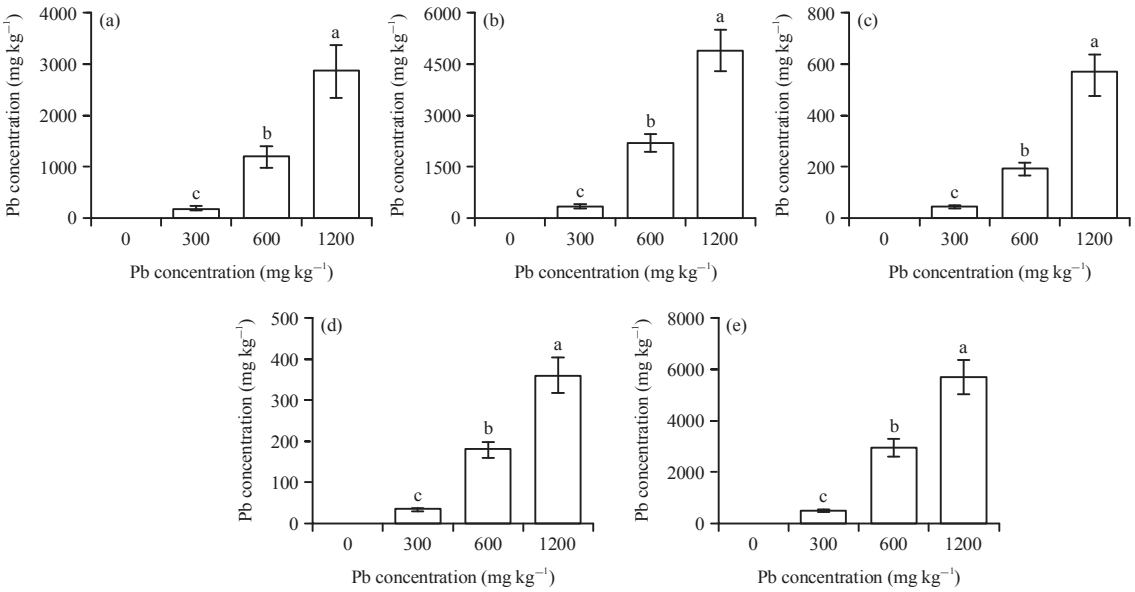

Fig. 2(a-e): Lead concentration in arum plant parts (a) Leaves, (b) Stems, (c) Stolons, (d) Rhizomes and (e) Roots. Bars with the same letters within the plant parts are not significantly different from each other at p<0.05

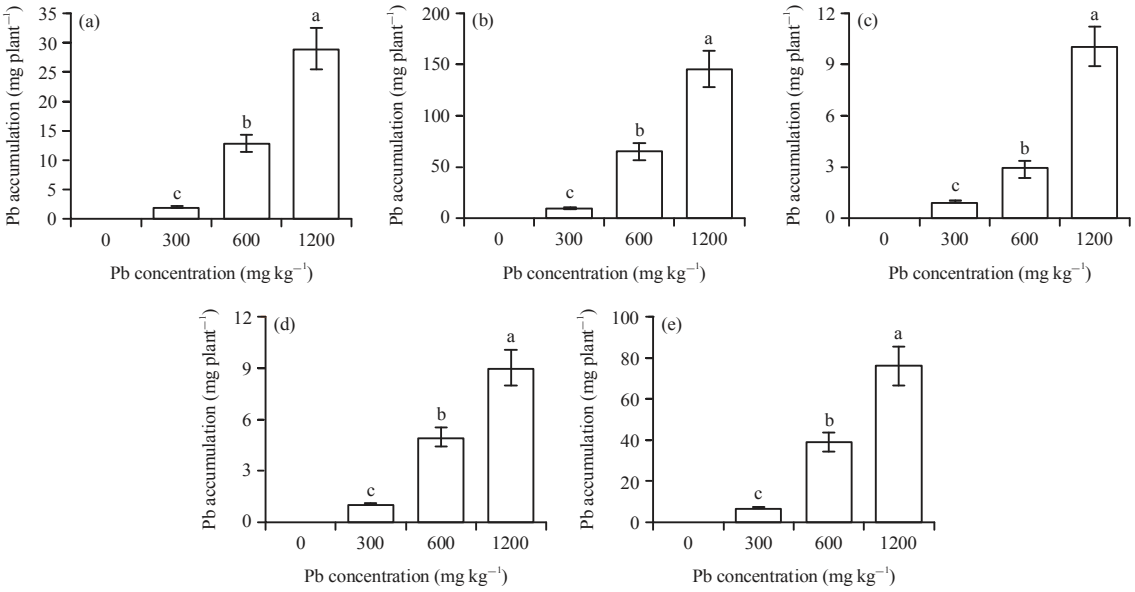

Fig. 3(a-e): Lead accumulation in arum plant parts (a) Leaves, (b) Stems, (c) Stolons, (d) Rhizomes and (e) Roots. Bars with the same letters within the plant parts are not significantly different from each other at p<0.05

Pb in the whole plant were 21, 126 and 270 mg plant^{-1} at Pb levels in the soil were of 300, 600 and 1200 mg kg^{-1}, respectively. Lead in different parts of arum decreased in the order: Stems>roots>leaves>rhizomes>stolons (Fig. 3).

Distribution of Pb in arum plant: Distribution of Pb (percent of the total uptake) in plant parts varied significantly (p<0.05) with Pb application rates in soil. The distribution of Pb increased in leaves and stems while it decreased in roots of the plant with the increase of its application rates in soil. However, the distribution of Pb in stolons and rhizomes remained almost similar among the application rates. At the highest rate of Pb application (1200 mg kg^{-1}) Pb distribution were 11, 54, 3.6, 3.5 and 28% in the leaves, stems, stolons, rhizomes and roots. However, translocations of Pb in arum parts at this high level were of 62% of total Pb, respectively which indicated that the major portions of Pb were translocated from roots to shoots (Table 1).

Table 1: Effect of Pb application on the Pb distribution and TF of arum plant grown in soil

Treatment	Pb distribution (%)					
Pb (mg kg^{-1})	Leaves	Stems	Stolons	Rhizomes	Roots	TF
0	nd	nd	nd	nd	nd	0
300	9.6±0.23^{b}	49.0±0.73^{b}	3.6±0.12^{a}	4.5±0.20^{a}	33.3±0.49^{a}	1.0
600	10.0±0.11^{b}	52.7±0.81^{a}	2.7±0.06^{b}	3.6±0.05^{b}	30.9±0.78^{b}	1.2
1200	10.9±0.29^{a}	54.0±0.47^{a}	3.6±0.04^{a}	3.5±0.04^{b}	28.0±0.28^{c}	1.4

Means with the same letter in each column within the parameter are not significantly different at p<0.05, nd: Not determinant, TF: Transfer factor

The TF values for Pb increased with its application rates in soil. The TF values of Pb ranged from 1.1-1.4 (Table 1).

DISCUSSION

Lead is a non essential and toxic element for plants. Growth inhibition is known as a result of Pb toxicity. In the present study, there were no visible symptoms and growth retardation of arum at any levels of Pb application (0-1200 mg kg^{-1}) in soil culture. Among the plant parts, 62% of total Pb was accumulated in the shoots (Leaves plus stems) of arum, which indicated that major portion of Pb was translocated from sroots to shoots. There are three indicators to define a plant as Pb hyperaccumulator: (1) The concentrations of Pb in plant shoots >1000 mg kg^{-1} [30], (2) The concentration of Pb in shoots is 10-500 times more than that in plants from non-polluted areas (Pb 5 mg kg^{-1})[31] and (3) The TF>1[28,30]. In the present study *C. esculenta* L. could be considered as a Pb hyperaccumulator according to all three indicators. It showed that this plant had high Pb concentration in its shoots (7748 mg kg^{-1}) and a TF>1. A higher TF in plant is important in practical phytoremediation of heavy metal contaminated soil because it enables phytoremediation by harvesting only the above ground parts of plants[32]. The TF in all confirmed hyperaccumulator are therefore above one, whereas they are invariably below unity in nonacccumulators[33]. Several studies have demonstrated the metal tolerance and phytoextraction efficiency of arum species (*C. esculenta*, C. *antiqourum*) to various heavy metals such as Pb, Cd and Zn[13-15,21]. In our previous hydroponics experiment, *C. esculenta* L. was exposed to 0, 50, 200 and 400 μM of Pb for 60 days. Lead concentration in shoots of this plant found 1121 mg kg^{-1} with TF>1 at 50 μM of Pb in nutrient solution. This concentration (50 μM Pb) did not cause any growth retardation which indicated that arum was a Pb hyperaccumulator plant[21]. Kashem *et al.*[13,14] investigated Cd tolerance and phytoextraction efficiency of a different arum species (*Colocasia antiqourum*) grown in hydroponics and soil culture and found that this plant could accumulate >100 mg kg^{-1} of Cd in its shoots in both the media. They mentioned arum as a Cd hyperaccumulator as the value >100 mg kg^{-1} is considered to be the accepted threshold value for Cd hyperaccumulator[30]. Moreover, Chayapan *et al.*[15] demonstrated *C. esculenta* as Cd and Zn hyperaccumulators according to their aboveground levels attaining C>100 mg kg^{-1} of Cd and Z>10,000 mg kg^{-1} of Zn[34].

Scientists are always searching new plants for the purpose of phytoremediation. There are several studied reported on Pb hyperaccumulation by various plant species. It showed that *Sesbania drummondii* could accumulate >4000 mg kg^{-1} of Pb in its shoots when treated with 1000 mg L^{-1} Pb[33], *A. porrum* 'Starozagorski Kamuš' could accumulate 1427 and 4888 mg kg^{-1} of Pb at 0.05 and 0.25 mM Pb[18] and *Euphorbia macroclada* and *Euphorbia platyloba* could accumulate 1138 and 10126 mg kg^{-1} of Pb, respectively from heavily polluted mining sites[16,17]. In comparison to these, *C. esculenta* L. can be classified as a good hyperaccumulator as this plant could accumulate 7748 mg kg^{-1} of Pb without growth retardation when treated with 1200 mg kg^{-1} of Pb.

It is important to select a phytoextraction plant with high metal accuumulation capability and is also compatible with mechanized cultivation practice and local weather conditions. However, it is unfortunate that some of the best hyperaccumulator are relatively small in size, grow very slowly and making it difficult to harvest them mechanically and limiting the metal extraction that can be achieved[35]. In the present study *C. esculenta* L. with many roots can absorb and accumulate substantial amounts of Pb and it is possible to harvest the entire plant including roots. It is fast growing, easily propagated, easy to manage and capable of growing in both dry and marshy conditions. This plant appears to possess the potential to provide a novel technique for the remediation of Pb in contaminated soil and water.

CONCLUSION

The results of this study indicate that the growth of arum is unaffected by application of any levels of Pb in soil. The concentrations of Pb found in shoot tissue and the TF of Pb in arum plant indicates that this plant has an excellent potential for Pb phytoextraction. This implies that arum is a potential candidate for the reclamation of Pb contaminated soil and water.

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