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Phytoextraction Efficiency of Lead by Arum (*Colocasia esculenta* L.) Grown in Hydroponics

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Abstract

Lead (Pb) tolerance and phytoextraction efficiency of arum (Pani Kachu; *Colocasia esculenta* L.) were investigated in hydroponics. Plants were grown for 60 days in nutrient solution after addition of Pb at the levels of 0, 50, 200 and 400 µM. The growth of different parts of arum was unaffected at low level of Pb concentration (50 µM Pb) compared with control treatment whereas it decreased gradually with the increase of metal concentration in nutrient solution. Concentration of metal in all parts of arum increased significantly with the levels of Pb in the growth media ($p < 0.05$). In arum shoots, Pb concentration was 1121 mg·kg^{-1}, at its low level in solution. This concentration (50 µM Pb) did not cause any growth retardation which indicated that arum was a Pb hyperaccumulator plant. On an average, translocation of Pb from roots to shoots was 68% of total Pb which indicated that the major portion of Pb was translocated from roots to shoots. Transfer factor (TF) greater than one for this metal as found in the present experiment confirmed the hyperaccumulation characteristics of arum. Lead uptake 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 soil and water contaminated with Pb.

Keywords

Water, Contamination, Metal, Hyperaccumulator, Toxicity

1. Introduction

Environmental pollution by heavy metals, even if it is at low concentrations and the long-term cumulative health

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effects that go with it, are of major health concerns all over the world [1]. Heavy metals occur naturally in the ecosystem with large variations in concentrations. In modern times, anthropogenic sources of heavy metals, *i.e.* pollutions from the activities of humans, have introduced some of these heavy metals into the ecosystem [1]. In Bangladesh, industrial wastes and effluents are commonly discharged onto agricultural lands, into canals and rivers, and along road sides or in the vicinity of industrial operations without any primary or secondary treatment. As a result, they are major pollutants of productive soils, natural water systems and ground water and have created an extremely serious contamination problem in Bangladesh [2].

Among the heavy metals, Pb belongs to those elements that are poorly mobile and rarely available to plants. However, it may create different organic and inorganic compounds that are easily absorbed by plants roots and thus posse's health risk on human through the food chain [3]. Lead concentrations in some industrial sites of Bangladesh are found to range from 17 - 99 $mg \cdot kg^{-1}$ [4] which are above the background level for Pb in soil (12 - 20 $mg \cdot kg^{-1}$) [5]. However, the concentrations of Pb in vegetables grown in Bangladesh are observed in the range of 10 - 26 $mg \cdot kg^{-1}$ dry weight by Ahmad and Gani [6] and 13 - 45 $mg \cdot kg^{-1}$ dry weight by Kashem and Singh [4]. These values exceed the acceptable tolerance level for FAO/WHO standard of 5 $mg \cdot Pb \cdot kg^{-1}$ dry weight [7] and of 10 - 20 $mg \cdot Pb \cdot kg^{-1}$ dry weight recommended by Sauerbeck [8]. There is an urgent need to develop methods to cleanup metals especially Pb in contaminated soils. Most traditional remediation techniques are physical or chemical that involves high cost and has low public acceptance [9]. In recent years, phytoremediation, where hyperaccumulators or accumulators are used to take up large quantities of pollutants, has become a promising remediation technique, for it is both environmentally sound and cost effective [10]. To date, there are more than 400 plant species known to hyperaccumulate heavy metals, of which only few have been considered as Pb hyperaccumulators [11], and most of them have not been widely used because of low biomass production, difficulty in cultivation and slow growth rates [12]. Some metal accumulators such as *Thlaspi caerulescens* [13] [14], *Arabidopsis halleri gemmifera* [2] [15] and *Sedum alfredii hance* [16] have been reported to have substantial potential for phytoextraction at low cost for soil metal remediation. However, these metal accumulators may not always be suitable for large scale remediation efforts because the plants are small, grow slowly and produce very low biomass. In the present investigation, we select a common and locally popular plant species arum (Pani Kachu; *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.* [17] conducted a hydroponics experiment in Japan with a different arum species (*Colocasia antiqourum* L.) and found that this plant had strong tolerance to Cd in the nutrient solution and strong accumulation capability of Cd in its body. Although there are few research on phytoextraction of Pb by some species [18] [19] but till date, there is no research to investigate the Pb tolerance and phytoextraction efficiency of arum species of *C. esculenta* L. neither in hydroponics nor in soil cultures. Hydroponics provides potential to examine metal tolerance and magnitude of metal accumulation in plant species with greater precision than soil studies. In the present paper, Pb tolerance and phytoextraction efficiency of arum grown in nutrient solution containing different levels of Pb were studied.

2. Materials and Methods

The experiment was carried out in the net house of the Department of Soil Science of the University of Chittagong, Bangladesh under natural light conditions. Healthy and uniform size plantlets of arum (*Colocasia esculenta* L.) were collected from the agricultural field near the University of Chittagong Campus. The plantlets were tightly set in the holes in the central position of plastic covers using foam and were placed above the 15-L plastic pots containing 10-L half-strength nutrient solution. The composition of half-strength modified Hoagland-Arnon [20] nutrient solution (standard solution) was: 3.0 mM KNO_3; 2.0 mM $Ca(NO_3)_2$; 0.5 mM $NH_4H_2PO_4$; 1.0 mM $MgSO_4$; 10 μM Fe-EDTA; 1.5 μM H_3BO_3; 0.25 μM $MnSO_4$; 0.1 μM $CuSO_4$; 0.2 μM $ZnSO_4$; and 0.025 μM H_2MoO_4. Two weeks after transplanting, Pb at the levels of 0, 50, 200 and 400 μM as $Pb(NO_3)_2$ (ACS Grade, Sigma-Aldrich Co.) was added to the half-strength nutrient solution according to Tanhan *et al.* [19] and Liu *et al.* [21]. There were three replicates of each treatment and each replicate consisted of one plantlet. The level of the solution was maintained by adding deionized water and renewing once every 7 days. The pH level of the solutions was adjusted to 5.5 daily either with 1 M NaOH or 1 M HCl using a digital pH meter.

Plants were harvested 60 days after addition of Pb in nutrient solution. Plants were then washed with tap water and then deionized water. The fresh weight of plants was measured. Then the plants were separated into dead leaves, normal leaves, stems, rhizomes and roots. All plant parts were oven dried at 68^{0}C for 72 h. After measuring their dry weights, the plant parts were grounded using a stainless steel grinder (Black & Decker BX3600-B5 Blender with Grinder & Chopper-300 Watt). The plants parts were digested with HNO_3-$HClO_4$ (3:1) mixture [22]. Around 0.5 g of plant samples were taken in digestion tube. Almost 20 times HNO_3 was added for each sample and was heated at 100˚C continuously for 10 hours on digestion block. After cooling (overnight 7 hours), additional 5 mL HNO_3 was added and again heated for 11 hours at 140˚C for 7 hours. After cooling (overnight 7 hours), 5 mL $HClO_4$ was added and again heated for 11 hours at 140˚C. Then the digested samples were cooled and were volume in 50 mL volumetric flask and stored in 50 mL acid washed plastic bottle. 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 transfer factor (TF) is the ratio of metal concentration in shoots to those in roots of plant [23]. The TF of Pb was measured by dividing the concentration of this metal in shoots to those in roots of arum grown in nutrient solution. All results are presented on dry weight (DW) basis.

The results obtained were subjected to one way analysis of variance using Minitab Program [24].

3. Results

3.1 Effects of Pb on Plant Growth

There were no visible symptoms of toxicity and growth reduction in any part of arum at low Pb treatment (50 µM), but the growth of the plant was decreased significantly ($p < 0.05$) at the highest Pb treatments (400 µM). The dry weight of arum at 50 µM Pb were 3.2, 34.4, 58, 44.5 and 14.4 g for the dead leaves, normal leaves, stems, rhizomes and roots, respectively, while at 400 µM the corresponding values were 1.6, 21.4, 30.1, 27 and 13.5 g (**Figure 1**).

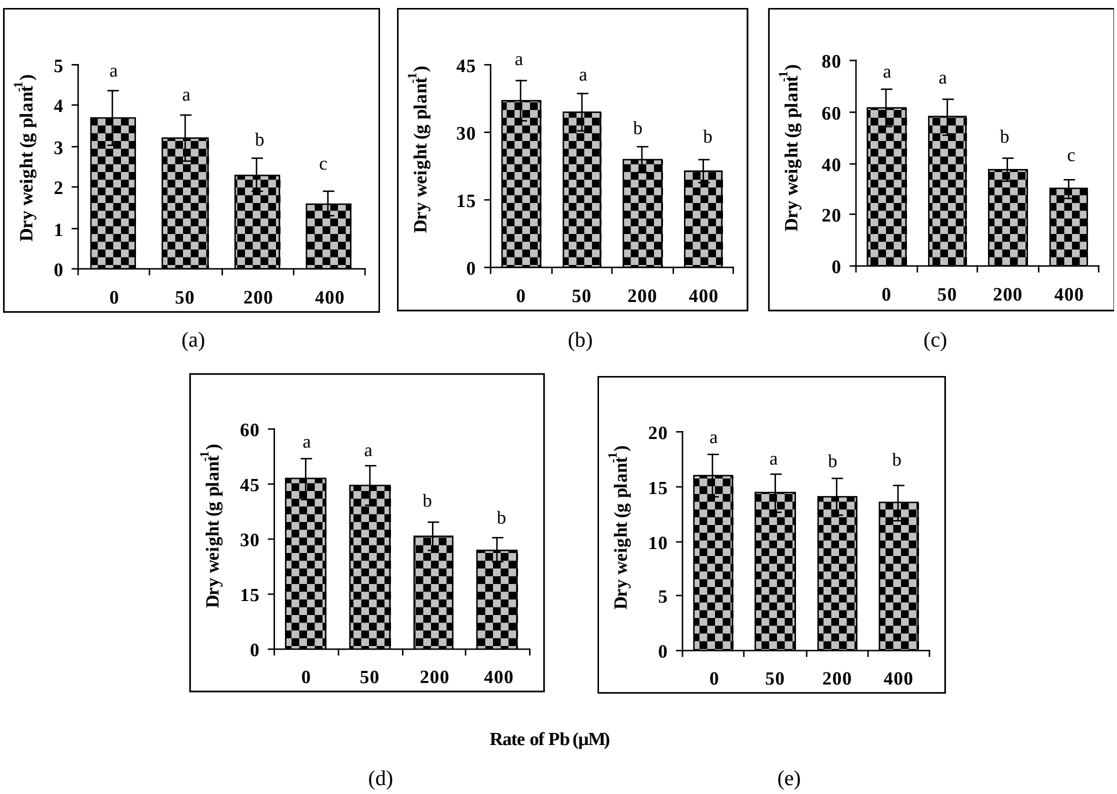

Figure 1. Dry weight of arum plant parts (a) Dead Leaves; (b) Normal Leaves; (c) Stems; (d) Rhizomes; (e) Roots. Bars with the same letters within the plant parts are not significantly different from each other at $p < 0.05$.

3.2. Lead Concentrations and Its Accumulations in Arum Plant

The concentrations of Pb in different parts of arum increased significantly ($p < 0.05$) with increasing its levels in the nutrient solution. In control treatment, concentrations of Pb could not be detected in plant parts and were not shown in table and figures. Lead concentrations increased from 406 to 8266 mg·kg^{-1} in the dead leaves, 273 to 3999 mg·kg^{-1} in the normal leaves, 442 to 7004 mg·kg^{-1} in the stems, 49 to 807 mg·kg^{-1} in the rhizomes and 1062 to 9014 mg·kg^{-1} in the roots when Pb concentration in the nutrient solution increased from 50 to 400 µM Pb. In the whole plant, Pb concentration was 348, 2222 and 4839 mg·kg^{-1}, respectively at solution Pb levels of 50, 200 and 400 µM (**Figure 2**).

Similar to Pb concentrations, the accumulations of Pb based on concentration and dry weight of plant significantly ($p < 0.05$) increased in different parts of plant with the Pb application rates applied in nutrient solution. At the lowest rate of Pb application (50 µM), Pb accumulations were 1, 9, 26, 2 and 15 mg·plant^{-1} in the dead leaves, normal leaves, stems, rhizomes and roots, respectively and the corresponding values at the highest Pb application rate (400 µM) were 13, 86, 211, 22 and 122 mg·plant^{-1} in the dead leaves, normal leaves, stems, rhizomes and roots, respectively. However, accumulation of Pb in the whole plant was 53, 242 and 454 mg·plant^{-1} at Pb levels in the solution were 50, 200 and 400 µM, respectively. Lead accumulations in different parts of plant decreased in the order: stems > roots > normal leaves > rhizomes > dead leaves (**Figure 3**).

3.3. Distribution and TF of Pb in Arum Plant

Lead distribution (percent of the total uptake) of arum did not very significantly in the plant parts with Pb application rates except in the rhizomes, where it increased from 4% to 5% when the Pb application rates increased from 50 to 400 µM. On an average, about 68% and 28% of the total Pb were found in the shoots (leaves plus stems) and in the roots of arum which indicated that the major portions of the metal were translocated from roots to shoots irrespective of its application rates in the nutrient solution (**Table 1**).

The TF values of Pb increased with its application rates in solution. The TF values for Pb ranged from 1.1 to 2.1 in the metal added treatments (**Table 1**).

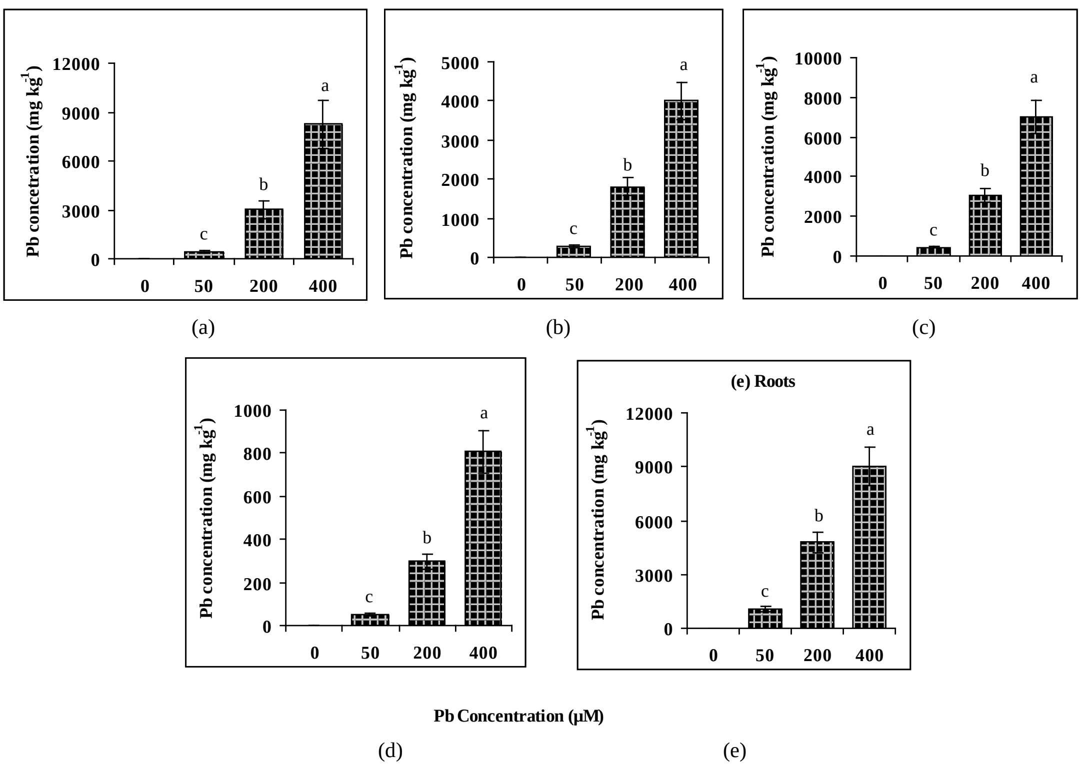

Figure 2. Lead concentration in arum plant parts (a) Dead Leaves; (b) Normal Leaves; (c) Stems; (d) Rhizomes; (e) Roots. Bars with the same letters within the plant parts are not significantly different from each other at $p < 0.05$.

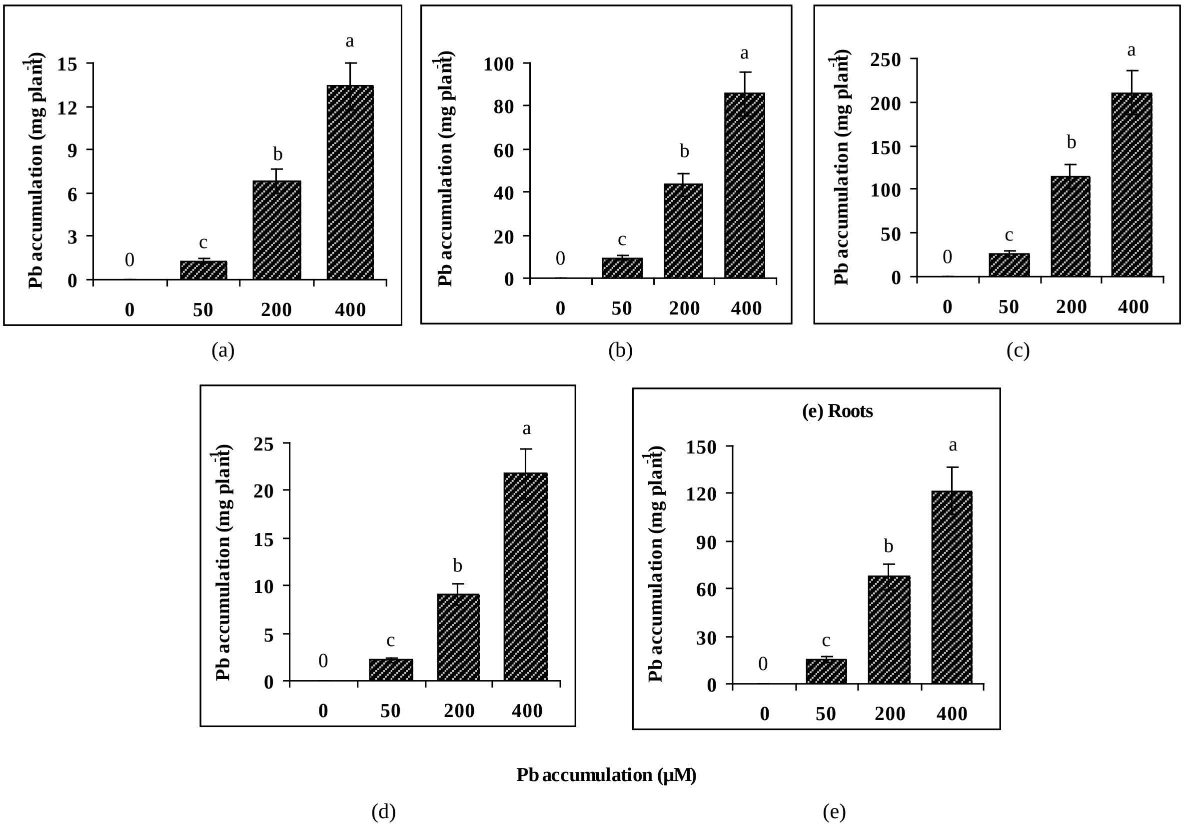

Figure 3. Lead accumulation in arum plant parts (a) Dead Leaves; (b) Normal Leaves; (c) Stems; (d) Rhizomes; (e) Roots. Bars with the same letters within the plant parts are not significantly different from each other at $p < 0.05$.

Table 1. Effect of Pb application on the Pb distribution and TF of arum plant grown in hydroponics.

Treatment Pb (µM)	Pb distribution (%)					TF
	Dead Leaves	Normal Leaves	Stems	Rhizomes	Roots	
0	nd	nd	nd	nd	nd	0
50	2.4 ± 0.03a	17.5 ± 0.32a	47.6 ± 0.35a	4.1 ± 0.12b	28.4 ± 0.71a	1.1
200	2.8 ± 0.05a	18.0 ± 0.46a	47.4 ± 0.63a	3.8 ± 0.11b	28.0 ± 0.71a	1.6
400	3.0 ± 0.41a	18.9 ± 0.39a	46.5 ± 0.41a	4.8 ± 0.22a	26.8 ± 0.47a	2.1

Means with the same letter in each column within the parameter are not significantly different at $p < 0.05$.

4. Discussion

There are three indicators to define a plant as Pb hyperaccumulator: (1) the concentrations of Pb in plant shoots > 1000 mg·kg^{-1} [25]; (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}) [26] and (3) The TF>1 [23] [25]. 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 (1121 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 [27]. The TF in all confirmed hyperaccumulator are therefore above one, whereas they are invariably below unity in nonacccumulators [19].

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 *Brassica pekinesis* accumulate 3688 and 10028 mg·kg^{-1} of Pb in its shoots at 500 and 1000 µg·ml^{-1} of Pb, respectively [18]. *Sesbania drummondii* could also accumulate >4000 $mg \cdot kg^{-1}$ of Pb in its shoots when treated with 1000 $mg \cdot L^{-1}$ Pb [19]. In comparison to these, *C esculenta* L. can be classified as a good hyperaccumulator as this plant could accumulate 1121 $mg \cdot kg^{-1}$ of Pb without growth retardation when treated with as low as 50 μM Pb (10.4 $mg \cdot L^{-1}$ 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 [28]. 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.

5. Conclusion

The results of this study indicate that the growth of arum is unaffected by application of low level of Pb in nutrient solution. The concentrations of this metal found in shoot tissue and the TF of this metal in arum plant indicate that this plant has an excellent potential for Pb phytoextraction. If plant uptake under field soil conditions is similar to that observed in this experiment, then this plant can be used to decontaminate soil moderately contaminated with Pb. Future studies need to be conducted with different types of arum species in both hydroponics and soil media.

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