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Distribution of Arsenic in Kangkong (*Ipomoea reptans*)

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ABSTRACT: The distribution of arsenic in roots and edible parts of *Ipomoea reptans* in presence of phosphate fertilizer was assessed in a pot experiment in Khulna, Bangladesh. There were 9 treatments with the combination of 3 levels of arsenic (0, 2 and 30 mg kg^{-1} soil) and 3 levels of phosphorus (0, 40 and 80 mg kg^{-1} soil). The study was carried out in a 3x3 factorial experiment with three replicates using completely randomized design. Arsenic application in soils significantly increased its accumulation in plants, and arsenic content in roots was significantly higher than in edible parts. Phosphorus fertilizers enhanced arsenic accumulation in plants. The maximum accumulations of arsenic in roots and edible parts at 30 days of plant's age were 12.1 mg kg^{-1} and 7.7 mg kg^{-1} dry matter, respectively.

KEYWORDS: Arsenic, phosphorus, arsenic accumulation, plants.

INTRODUCTION

Arsenic is a toxic element. Natural arsenic concentrations in some areas are high enough to affect human health[1]. It can be toxic to plants or may accumulate in plants and thereby enter the animal and human food chain. Edible vegetables arum, gourd, and kankong (*Ipomoea*) may have high contents of water-soluble arsenic[2]. At present arsenic in the ground water is considered a major health concern in many countries of the world and it could represent a severe problem in the 21st century if not controlled. In Bangladesh, it is a matter of great concern that the environmental impact of arsenic has been causing widespread disquiet. According to World Health Organization about 80 million people in Bangladesh are threatened by arsenic poisoning. Long-time consumption of arsenic-contaminated food and water may damage kidneys, liver, lungs, bladder and other major organs of human body. The most affected areas in Bangladesh have more than 1 mg l^{-1} arsenic contamination in ground water[3]. Arsenic was found 1.16 mg l^{-1} in tube-well water of Samta village in Jessore district[4], whereas the permissible level of arsenic in drinking water is 0.05 mg l^{-1} for Bangladesh.

Use of arsenic containing groundwater in agriculture together with phosphate fertilizer may result in accumulation of arsenic in soil. According to BARC[5] the concentration of phosphate (P) in soil in Bangladesh varies between a few milligrams to 1000 mg kg^{-1}. The arsenic content of alluvial sediments in Bangladesh is in the range 2-10 mg kg^{-1} [6]. Therefore, the relatively high amount of P in soil would compete with arsenic for sorption sites in the soil and thereby would favor high arsenic concentration being available in soil solution, which further may leach down from agricultural soil or result in high uptake by plants[7]. Consequently, widespread use of arsenic contaminated groundwater for agricultural irrigation could be a major source of arsenic in the food chain. Arsenic accumulation in plants, phyto-toxicity due to increased arsenic in soil and water, and the long-term impacts on agricultural yield and subsequent effects on human health are areas of concern and further study. It is therefore necessary to quantify food crop uptake of arsenic from soil and to investigate the effect of P fertilizer on its accumulation. This study was carried out to determine the distribution of arsenic between roots and edible parts of a crop plant, and to quantify the effects of P on arsenic uptake by the plant. Kangkong is an important market vegetable in Thailand, Indonesia, Taiwan and some regions of China where it is intensively grown and frequently eaten. Kangkong is an excellent source of vitamin A, and fairly rich in vitamin C, calcium, iron, potassium and phosphorus, and in South and South-East Asia it is an important leafy vegetable for human consumption. Therefore, kangkong (*Ipomoea reptans*) was selected as a test crop in the study.

MATERIALS AND METHODS

Experimental Set Up

A pot experiment with combinations of 3 levels of arsenic (0, 2 and 30 mg kg^{-1}) and 3 levels of phosphorus

(0, 40 and 80 mg kg^{-1}) was carried out on the premises of Regional Laboratory, Soil Resource Development Institute (SRDI), Daulatpur, Khulna, Bangladesh during May 2000 to July 2000. The average arsenic concentration in alluvial soils of Bangladesh is in the range 2-4 mg kg^{-1} [6], and it can be up to 57 mg kg^{-1} [8]. Therefore, arsenic was applied at 2 and 30 mg kg^{-1} to the soil in pots to assess the effect on its accumulation between lower and higher doses. The phosphate fertilizer was applied considering optimum and higher levels of P in soil (40 and 80 mg kg^{-1}). The amount of soil in each pot was 13 kg, thus amounts of arsenic and phosphorus for individual pot were calculated. Arsenic was applied to pot soil as As_2O_3 after dissolving in water with addition of few drops of NaOH solution. The study was conducted in a 3x3 factorial experiment with three replicates in completely randomised design. In each pot, eight seeds of kangkong were sown and after germination and seedling establishment were thinned to only four seedlings. The size of the individual plastic pot was 20x30 cm and they were almost cylindrical in shape.

N, P and K are the three major fertilizer elements used for crop production in Bangladesh. N and K were applied as a basic fertilizer to the pots in doses recommended by BARI[9]. The recommended doses of N and K for kangkong were 150 and 25 kg ha^{-1} respectively. Average weight of soil ha^{-1} is $2x10^6$ kg, thus the amount of N as urea and K as muriate of potash (MP) was applied to each pot (13 kg soil) were 0.98 g and 0.16 g, respectively. Half of the urea (0.49 g) and the full dose of MP were applied during preparation of soil for pot. The rest of the urea was applied to pots at 20 days after sowing (DAS). The arsenic contents of urea and MP were negligible but in triple super phosphate (TSP) it was 3.6 mg As kg^{-1} fertilizer. Charter *et al.*[10] found that the range of arsenic content in TSP was 2.4-18.5 mg kg^{-1}. The amount of TSP fertilizer applied in each pot was rather small, only 0.52 and 1.04 g pot^{-1}; therefore, the amount of arsenic added to soil from TSP was also considered negligible. Arsenic free tap water was applied to the pots as and when necessary avoiding leaching. It should be mentioned that no rainwater was allowed to fall on the pots. A polyethylene sheet was placed over the experimental pots during rain.

Sampling

The plant samples were collected at 30 days after sowing. The plants were obtained with complete root systems. The plant samples were washed and cleaned thoroughly with arsenic free water. Then plant roots and edible parts were separated for analysis of arsenic. After storage for two days in well-aerated room, samples were placed in an oven at 70^0C for 24 hours. After cooling in a desiccator, samples were ground in a mortar and placed in plastic pots separately with labelling for analysis. The soil used in the pots was also analyzed to establish the background concentration of arsenic and phosphorus.

Chemical Analysis

For digestion, 1.0 g soil or 0.50 g plant materials were taken in a digestion tube and 5 ml 68% HNO_3 added, mixed and kept overnight. The samples were the digested at 125^0C for 4 hours. After cooling, the digestion mixture was carefully transferred to 100 ml volumetric flask; digestion tubes were rinsed with distilled water 2-3 times and added to the flask. The flasks were filled up to the mark with distilled water and filtered with Whatman No. 42 filter paper. Perkin-Elmer (Model 3110) Atomic Absorption Spectrophotometry (AAS) method[11] equipped with MHS-10 hydride generator assembly, EDL power supply and electrodeless discharge arsenic lamp was used for analysis of arsenic in plant as well as soil and water samples. The analyses were done in the Regional Laboratory, SRDI, Khulna, Bangladesh. Arsenic detection limits of the method are 0-4 μg l^{-1}in water, 0-4 mg kg^{-1} in soil and 0-8 mg kg^{-1} in plant samples.

Quality Assurance/Quality Control of the Analysis

The equipments used for arsenic analysis were cleaned thoroughly. All glassware and plastic ware were washed in washing solution (2% HNO_3), rinsed with deionised water and then dried in oven at 105^0C for overnight before using for analysis. The regression analysis between concentrations of arsenic standard solutions and their absorbance offered an r^2 value of 0.98. Duplicate samples were analysed for all edible parts and root samples, and the mean values were used for the study. After doing analysis of each 10 samples, readings for standard solutions were taken to check the instrument. To check the accuracy of the data a few samples were also analysed in another laboratory of SRDI, Bangladesh.

Statistical Analysis

The experimental data were analysed by two-way analysis of variance (ANOVA) and t-test using SPSS (Version 9.0) statistical software package (SPSS Inc., Chicago, USA). Independent sample t-test was performed for different samples (roots and edible parts). Differences were considered significant at an alpha level of 0.05.

Results and Discussion

Characteristics of Soil Used in Pots

The soil used in the pot experiment was collected

Table 1. Characteristics of soil used in pots.

pH	EC (ds/m)	OM (%)	Total-N (%)	Available P (µg/g)	Available K (meq/100g)	Available S (µg/g)	Total As (µg/g)
7.5	7.3	3.5	0.41	10.6	0.29	25.4	10.4

from a field that was under an agro-ecological zone of Ganges Tidal Floodplain. The soil was loamy, and land type was medium-high, which is normally flooded up to about 90 cm depth during the rainy season for more than 2 weeks to about 1 month continuously. During collection of soil the field was dried and well aerated. The state of As in soils depends on the pH and redox potential. Under aerobic condition arsenic in soil presents as arsenate, while in flooded soil arsenite predominates[12]. Arsenite converts readily to arsenate under oxidizing condition. Under oxidizing condition where pH <6.9, $H_2AsO_4^-$ is the major species, and at pH>6.9, $HAsO_4^{2-}$ becomes dominant[13]. The environment of the pot soil was well aerated, and pH 7.5, therefore, arsenic in pot soils should be in the $HAsO_4^{2-}$ form. Properties of that particular soil were analyzed and presented in Table 1. The results were interpreted based on the soil test interpretation class given by BARC[5].

The pH and Electrical Conductivity (EC) indicated that the soil was slightly alkaline and saline respectively. Organic matter content was high, N was also high, P content was low, and K was optimum and S medium. Arsenic level in the pot soil was 10.4 µg g^{-1} soil, which is the background level prior to apply arsenic in soils of pots. This is at least two times higher than typical arsenic level in agricultural soils. The typical concentration of As in alluvial soils of Bangladesh is 2-4 mg kg^{-1} [6].

The Effects of Arsenic Application on Its Accumulation and Distribution in Kangkong

Arsenic levels in edible parts and roots of kangkong

Arsenic accumulation in edible parts at different levels of As and P application varied from 4.87 mg kg^{-1} to 7.71 mg kg^{-1} of dry matter (Fig. 1). But in root samples it ranged from 6.46 mg kg^{-1} to 11.57 mg kg^{-1} of dry matter (Fig. 1). In edible parts, 24.85% and 46.20% higher arsenic accumulation resulted from As_2 and As_{30}, respectively compared to As_0 (control). But in root sample, there was a 19.35% and 79.10% increase in arsenic accumulations observed from As_2 and As_{30}, respectively compared to the control. In the control, arsenic accumulations were 4.87 mg kg^{-1} in edible parts and 6.46 mg kg^{-1} in root samples. Arsenic accumulation in control was derived mainly from background concentration of As in soil, and in a less extent from the impurities of fertilizer added to the experimental pots.

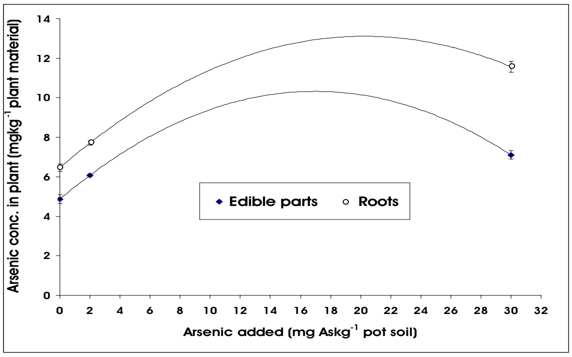

Fig 1. Effects of arsenic application on its uptake (mean) by kangkong.

It was observed that the mean differences of accumulated arsenic in edible parts and roots were significantly higher at higher amount of arsenic application (ρ<0.01) (Fig. 1 and Table 2). Creger and Peryea[7] and Martin *et al.*[14] also found that arsenic concentration in shoots and roots increased upon increased application. Abedin *et al.*[15] found that arsenic concentration in roots, straw and rice husk increased with the increased application of arsenic in rice production, which also support findings in the current study.

Table 2. ANOVA for As concentrations in edible parts and root samples.

Source	df	Edible parts				Roots			
		SS	MS	F	Sig.	SS	MS	F	Sig.
As	2	22.81	11.404	105.988	0.000	127.441	63.721	420.444	0.000
P	2	2.556	1.278	11.878	0.001	2.330	1.165	7.687	0.004
As* P	4	1.122	0.281	2.608	0.070	0.103	0.026	0.170	0.951
Error	18	1.973	0.108			2.728	0.152		

Comparison of accumulated As between edible parts and roots

Arsenic accumulations in roots were significantly higher ($\rho<0.01$) than arsenic accumulation in edible parts (Table 3). Abedin *et al.*[15] carried out a green house experiment with rice in Aberdeen, UK and observed that roots contained higher amount of arsenic than any other part of the rice plant. Martin *et al.*[14] in rice, Sachs and Michael[16] in bean also found that roots tend to accumulate more arsenic than any other parts of the plant.

The Effects of Phosphate Fertilizer Application on Arsenic Accumulation in Kangkong

Arsenic accumulation in edible parts and roots of kangkong

The arsenic accumulation in edible parts increased generally with increasing P application (Fig. 2). In edible parts, the arsenic accumulation did not increase significantly with increased P application from 0 to 40 mg kg^{-1} ($\rho>0.05$). However, it increased significantly when P application increased further to 80 mg kg^{-1} ($\rho<0.01$) (Fig. 2 and Table 2). The similar patterns of arsenic accumulation in roots were also observed.

In edible parts there were 4.93% and 13.03% increase in arsenic accumulations observed in P_{40} and P_{80}, respectively, compared to control (P_0). But in the roots there were 1.93% and 8.19% increase in arsenic accumulations observed in P_{40} and P_{80}, respectively, compared to control.

Otte *et al.*[17] found that *Urtica dioica* uptake more arsenic with the increasing addition of phosphate to soil. Abedin *et al.*[15] found no differences in As concentrations in rice roots, straw, and grain due to application of phosphate fertilizer. But they found that As concentration in rice husk increased significantly with increased application of phosphate fertilizer. In general, increased amount of phosphorus in soil solution is known to decrease plant affinity for arsenic. The amount of phosphate sorbed is greater than that of arsenic in soil. Phosphate substantially suppressed arsenic adsorption by soil and the extent of suppression varied from sample to sample[18]. Phosphate displaces sorbed arsenic from soil[19]. After addition of phosphate at high doses to As contaminated soil, about 80% of the total As becomes available in soil solution, which is subject to leaching or readsorbing in a lower soil profile[20]. The current study was done in pots and no leaching was allowed from the pots. Furthermore, the root system of kangkong was well distributed in the pots, which might be the cause of higher As uptake by the crop. Therefore, the accumulation of arsenic was found positive to the amount of added phosphate fertilizer. The pot experiment does not reflect field experiment, where As would be mobile.

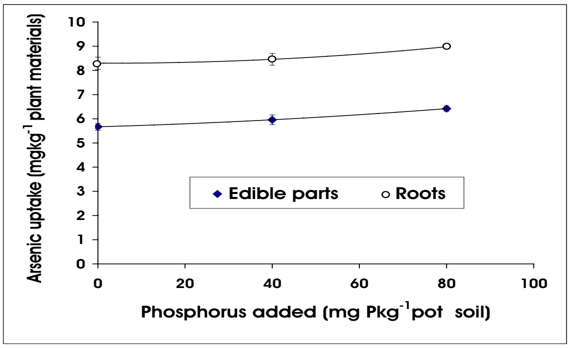

Fig 2. Effects of phosphate fertilizer application on arsenic uptake (mean) by kangkong

Interaction of arsenic and phosphorus

There was no statistically significant interaction-effect that could be detected between arsenic and phosphorus for increasing arsenic accumulation in plant ($p>0.05$). Perya[21] found that arsenic release was positively related to added phosphate because arsenic solubility increased through the competitive PO_4-AsO_4 exchange. Marschner[22] and Asher and Reay[23] reported that plant uptakes P by phosphate carriers located in the root plasma membranes. Arsenic in pot soil was considered in the arsenate form. Arsenate and phosphate in soil exhibit similar behavior[24], and As is also taken by plants by the same carrier system. In the current study application of phosphorus up to 80 mg kg^{-1} did not show suppression of arsenic uptake by plants. Arsenic continued to increase in the plants regardless of the variation in P application. Therefore, a further study the interaction of As and P needs careful attention.

Table 3. Comparison of accumulated arsenic in mg kg^{-1} (mean± standard deviation) between edible parts and roots at different levels of arsenic application

As_0 (mg kg^{-1})		As_2 (mg kg^{-1})		As_{30} (mg kg^{-1})	
Edible parts	Roots	Edible parts	Roots	Edible parts	Roots
4.87 ± 0.17 ^{a}	6.46 ± 0.20 ^{b}	6.08 ± 0.05 ^{a}	7.71 ± 0.11 ^{b}	7.12 ± 0.22 ^{a}	11.57 ± 0.21 ^{b}

^{a} and ^{b} means that mean arsenic accumulation are significantly varied to each other for each level of arsenic accumulation between edible parts and roots.

Conclusions

Arsenic accumulation in kangkong increased significantly with increased amount of arsenic application. Application of phosphate fertilizer enhanced arsenic accumulation in plant. Plant roots accumulated more arsenic than edible parts. Arsenic accumulation in roots was around two times higher than in edible parts. The safe limit of arsenic in plants for human consumption without affecting health is only 1.0 mg kg^{-1} dry matter[25]. It was found from this study that the minimum amount of arsenic accumulation in kangkong was 4.61 mg kg^{-1} dry matter in edible parts from the control.

A research program should be launched to consider the potential hazards of arsenic, especially the risk to human health and other animals, due to consumption of contaminated food crops and drinking water. More research is needed to study the nature of arsenic mobility and phytoavailability in the soil solution with the interaction of P, Fe, Mn and Al. To transfer our results in practice for any As contaminated soils, recommendation of P fertilizer to be applied for crops need to be carefully determined to avoid stimulating a release of indigenous arsenic from the soil. Arsenic in soil solution may cause higher As uptake by plants or can leach down from top soil, which may further contaminate underground water. Close monitoring of soil and food crops in As contaminated areas is recommended.

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