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Reduction of arsenic entry into rice from arsenic contaminated soil using *Pteris vittata* as trap plant

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ABSTRACT

An experiment was conducted to reduce the entrance of arsenic on rice plant. Experiment consisted three different density of the trap plants viz. P_1: No P. vittata (control); P_2: four P. vittata plant per m^2 and P_3: eight P. vittata plant per m^2. Inter planting of four P. vittata per m^2 reduced 96.24 % and eight P. vittata per m^2 reduced 97.01% arsenic accumulation into rice. Maximum yield was found from P_2 (34.2 g per plant) which was statistically similar with P_3 (32.9 g per plant) while minimum was found from P_1 (30.0 g per plant). Highest amount of arsenic accumulation was found from P_1 in rice grain (1.55 ppm), husk (5.57 ppm) and straw (39.78 ppm). Arsenic accumulation was found in rice grain (0.02 ppm in both P_2 and P_3), husk (0.60 and 0.58 ppm in P_2 and P_3 respectively) and straw (1.05 and 1.00 ppm in both P_2 and P_3 respectively). Concerning both yield of rice and arsenic concentrations in rice plant, it can be recommended to interplant four P. vittata plant per m^2 area as a trap plant to reduce arsenic entrance into rice plant from soil which can keep away of arsenic pollution in food chain.

Key words: *Arsenic, rice, Pteris vittata, inter-planting and trap plant*

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I. Introduction

Arsenic is a toxic metalloid. Its contamination in soil is increasing day by day in some parts of Bangladesh. Arsenic accumulation in food crops is a major concern. Rice is the staple food in Bangladesh. High level of arsenic in irrigated water and soil appears to result in higher concentration of arsenic in rice grain, husk and straw (Abedin *et al.*, 2002; Das *et al.*, 2004). Most of the agricultural soil is contaminated by arsenic due to the irrigation during winter season mostly in southern and western part of Bangladesh. The districts with the highest mean arsenic rice grain levels were all from southwestern Bangladesh, which are Faridpur (boro rice season) 0.51 > Satkhira (boro) 0.38 > Satkhira (aman rice season) 0.36 > Chuadanga (boro) 0.32 > Meherpur (boro) 0.29 μg As g^{-1} (Williams *et al.*, 2006). Dietary intake of rice grain is potentially a major arsenic exposure pathway (Smith *et al.*, 2008) in Bangladesh while husk and straw used as the feed for the domestic birds and animal which

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cause direct effect of animal body. Consuming this animal meat arsenic comes into the human body indirectly. *P. vittata* can survive highly arsenic contaminated soil (4000 ppm) and accumulate up to 27829.7 ppm arsenic (Mayda *et al.*, 2014) and accumulate 23837.2 ppm arsenic from 2000 ppm arsenic contaminated soil (Jamal Uddin *et al.*, 2015) into the plant body. As *P. vittata* can accumulate considerable amount of arsenic from contaminated soil. Thus, it would be potential to reduce the arsenic accumulation into the rice by using *P. vittata* as trap plant. On the hand, *P. vittata* does not require many nutrients to grow. Thus, growing *P. vittata* along with rice may not interfere (and or less interferences) with the growth and yield rice. Considering these points in view, the study was undertaken to reduce the arsenic accumulation into rice plant using *P. vittata* as trap plant.

II. Materials and Methods

Location and duration of the experiment: An experiment was conducted at Department of Horticulture of the Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh and at the Department of Botany of the Jahangirnagar University, Savar, Dhaka-1342, Bangladesh from October 2013 to April 2014.

Arsenic contaminated soil: Arsenic was applied in the form of Arsenic trioxide (As_2O_3) @ 50 ppm at 7 days before transplanting of the rice plant.

Treatments and design: *P. vittata* was inter-planted with rice into pot as trap to mitigate arsenic accumulation into rice plant. Experiment consisted three different density of the trap plants viz. P_1: No *P. vittata* (Control); P_2: four *P. vittata* plant per m^2 and P_3: eight *P. vittata* plant per m^2 following completely randomized design with three replication. Trap plants were planted at the time of transplanting of rice plants.

Pot size: The pot size was 1.5 m in length, 0.5 m in width and 0.5 m in depth.

Data collection: Data were collected on plant height, number of tillers per plant, number of effective tillers per plant, panicle length, total number of grains per panicle, number of filled grains per panicle, number of unfilled grains per panicle, percentage of unfilled grains, 1000-grains weight, yield per plant, arsenic accumulation by rice grain, husk and straw, arsenic accumulation by *P. vittata* and reduction of arsenic accumulation in rice by *P. vittata* over control.

Chemical analysis for arsenic: Plant biomass was measured by using precision balance after drying. After growing, plants were collected and dried. After drying, samples were smashed by mortar and pastel machine. The arsenic analysis for was performed by using "Atomic Absorption Spectrometer", where use of argon for carrier gas and arsenic was melted by 925ºC; was approved by ISO organization in Bangladesh Council of Scientific Research Institute (BCSIR), Dhaka, Bangladesh.

50 times dilution: 05 ml concentrated HCl was taken at 50 ml volumetric flasks for transferring arsenic into arsenic trioxide and a little bit of distilled water was added. Then 1ml solution was taken very carefully from each volumetric flask to avoid bubble and KI (01 gm) wash added in solution with 150 ml distilled water. After that 0.5 gm sample was taken in volumetric flask and mixed distilled water up to 50 ml and solution turned into yellow color. Another volumetric flask made blank solution, where contain only HCl, KI and distilled water for arsenic analysis.

1000 time dilution: 02 ml solution was taken into 500 volumetric flasks, mixed with distilled water up to 500 ml and shaken very carefully. Then, 05 ml solution was taken into 250 ml volumetric flask and mixed with distilled water up to 250 ml. Again, 05 ml solution from 250 ml solution was taken into 25 ml volumetric flask then 2.5 ml HCl and 2.5 ml KI was added and mixed distilled water, shaking was done very smoothly until turn it into yellow color. Standard solution arsenic was added with HCl 2, 5, 10, 15, 20 ppb respectively.

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5000 time dilution: Similar to the 1000 time dilution. But 04 ml solution was taken in case of 2 ml. 05 ml HCl and KI was added into 5000 ml volumetric flask, solution was made into 25 ml and 05 ml was taken in flask.

Statistical analysis: Data were statistically analyzed using MSTAT-C computer package programme. Difference between treatments was assessed by Least Significance Difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

III. Results and Discussion

Plant height: Plant height of rice showed significant variation among the treatments viz. P_1, P_2 and P_3. Tallest plant was found from P_1 (87.1 cm) whereas shortest from P_3 (80.8 cm) at harvest (Figure 1). Tallest (96.64 ± 0.73 cm) and shortest (82.66 ± 7.6 cm) plant were found in 0.5 and 4.0 mg/L arsenic amended plots (Azad *et al.*, 2013). Abedin *et al.* (2002) also found that arsenic contaminated irrigation water significantly reduced the plant height.

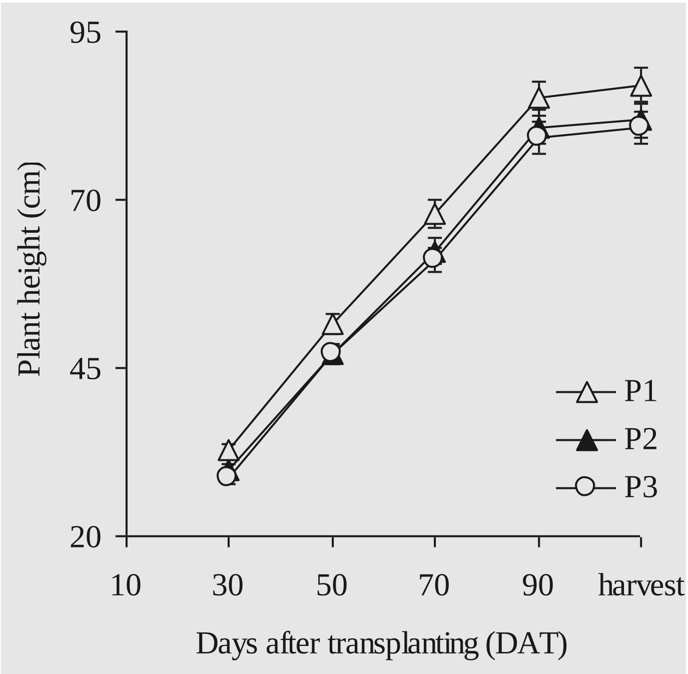

Figure 01. Response of rice to different density of trap plant (*P. vittata*) for arsenic on plant height

Number of tillers: Number of tillers showed a significant variation among the treatments. Maximum number of tiller was found from P_1 (12.3 per plant) while minimum from P_3 (11.7 per plant) which was statistically identical with P_2 (11.9 per plant) at harvest (Table 01). When plants are exposed to excess arsenic either in soil or in solution culture, they exhibit toxicity symptoms like decrease in plant height (Abedin *et al.*, 2002).

Number of effective tillers: Maximum number of effective tillers was found from P_2 (11.7 per plant) which was statistically identical with P_3 (11.4 per plant) whereas minimum from P_1 (11.0 per plant) (Table 01). BRRI-29 showed more number of effective tillers per plant than control in 15 ppm arsenic concentration (Huda *et al.*, 2009) while Abedin *et al.* (2002) observed that tiller number was reduced significantly due to arsenic concentration in irrigation water.

Panicle length: Panicle length of rice was varied significantly among the treatments. Longest panicle was found from P_1 (24.1 cm) which was statistically similar with P_2 (23.8 cm) whereas shortest from

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P_3 (23.4 cm) (Table 01). This result indicated that without *Pteris vittata* BRRI-29 uptake arsenic showed longest panicle length. Longest panicle (24.20 ± 0.69 cm) and shortest panicle (21.83 ± 0.84 cm) were found in control and 4.0 mg/L arsenic treated plot (Azad *et al.*, 2012).

Total number of grains: There was no significant variation was observed among the treatments in terms of total number of grains. However, the total number of grains was 142.7 per plant, 143.0 per plant and 142.9 per plant in P_1, P_2 and P_3 respectively (Table 01). Increasing the concentration of arsenate in irrigation water significantly decreased the number of grains (Abedin *et al.*, 2002).

Table 01. Response of rice to different density of trap plant (*P. vittata*) for arsenic on some yield related attributes [x]

Treatments	Number of tillers/plant at harvest	Number of effective tillers/plant	Panicle length (cm)	Total number of grains/panicle
P_1	12.3 a	11.0 b	24.1 a	142.7 a
P_2	11.9 b	11.7 a	23.8 ab	143.0 a
P_3	11.7 b	11.4 a	23.4 b	142.9 a
LSD 0.05	*0.5*	*0.7*	*1.5*	*1.9*
CV%	*2.8*	*2.7*	*1.4*	*0.6*

[x]In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.01 level of probability

Number of filled grains: Number of filled grains varied significantly among the treatments. Maximum number of filled grains was found from P_2 (135.0 per panicle) which was statistically identical with P_3 (134.4 per panicle) whereas minimum P_1 (127.0 per panicle) (Table 02). Number of panicle was found to be decreased significantly with the increase of soil arsenic concentrations (Rahman *et al.*, 2004). Rice plant grown in arsenic contaminated soil may cause the reduction of number of filled grain per panicle along with other factors.

Number and percentage of unfilled grains: Maximum number of unfilled grains was found from P_1 (15.7 per panicle) while minimum from P_2 (8.0 per panicle) which was statistically identical with P_3 (8.6 per panicle) (Table 02). This result indicated that arsenic contamination in soil increase the number of unfilled grain but *P. vittata* can help to reduce arsenic accumulation into rice. Plant might be reduced the number of unfilled grains. *P. vittata* uptake high amount of arsenic from soil and accumulate arsenic into their fronds. Most of the arsenic was accumulated in the fronds (i.e., a frond is a large, divided leaf of fern) of *P. vittata* (89–93%), so metal uptake by *P. vittata* can be used as a cost-effective amendment for phytoremediation of arsenic and metal polluted soils (Ma *et al.*, 2001). Maximum unfilled grains were found from P_1 (11.0%) while minimum from P_2 (5.6%) which was statistically identical with P_3 (6.0%) (Table 02). Addition of arsenic significantly reduced tillering (Khan *et al.*, 2010) and increasing the concentration of arsenate in irrigation water significantly decreased the number of filled grains (Abedin *et al.*, 2002).

1000-grains weight: There was no significant variation was observed among the treatments in terms of 1000-grains weight. However, maximum 1000-grains weight was found from P_1 (21.5 g) whereas minimum from P_2 and P_3 (21.3 g) (Table 02). Presence of arsenic arsenate at a higher concentration in irrigation water significantly reduced 1000-grain weight (Abedin *et al.*, 2002). 1000-grain weights of rice were decreased with increasing of arsenic in irrigation water but the differences were not statistically significant (Azad *et al.*, 2012).

Yield: Yield of each plant was varied significantly among the treatments. Maximum yield was found from P_2 (34.2 g/plant) which was statistically identical with P_3 (32.9 g/plant) while minimum from P_1 (30.0 per plant) (Table 02). Grain yield of rice was decreased as the level of arsenic addition was increased, and the yield was reduced drastically with the 30 mg As/kg addition (Hossain *et al.*, 2009). From the experiment, it can be stated that using *P. vittata* reduces the arsenic toxicity from the soil and increase the yield of each plant. *P. vittata* uptake huge amount of arsenic from soil and accumulate

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arsenic into their fronds. Contaminated site with 38.9 mg/kg of arsenic in the soil, the fern's fronds had 7526.0 mg/kg of arsenic and under experimental conditions where soil was loaded with arsenic; fern accumulated 22630.0 mg/kg (2.3%) of the heavy metal (Singh and Ma, 2006).

Table 02. Response of rice to different density of trap plant (*P. vittata*) for arsenic on some yield related attributes and yield [x]

Treatments	Number of filled grains/panicle	Number of unfilled grains/panicle	Unfilled grains (%)	1000-grains weight (g)	Yield (g)/plant
P_1	127.0 b	15.7 a	11.0 a	21.5 a	30.0 b
P_2	135.0 a	8.0 b	5.6 b	21.3 a	34.2 a
P_3	134.4 a	8.6 b	6.0 b	21.3 a	32.9 a
LSD 0.05	*2.2*	*2.7*	*1.8*	*0.5*	*1.4*
CV%	*0.8*	*11.2*	*10.5*	*1.1*	*1.3*

[x]In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.01 level of probability

Arsenic accumulation: Arsenic accumulation was varied significantly among the treatments by rice grain, husk and straw. Maximum arsenic accumulation was found from P_1 (1.55 ppm in grain, 5.57 ppm in husk and 39.78 ppm in straw) whereas minimum from P_3 (0.02 ppm in grain, 0.58 ppm in husk and 1.00 ppm in straw) which was statistically identical with P_2 (0.02 ppm in grain, 0.60 ppm in husk and 1.05 ppm in straw) (Table 03). On the contrary, *Pteris vittata* used as trap to arsenic was accumulated arsenic 45.13 ppm by P_2 and 45.49 ppm by P_3 (Table 3). P_1 and P_2 were reduced 96.24% and 97.01% arsenic accumulation into rice over P_1 (control) (Table 03). The arsenic concentrations in rice grain were varied widely depending on the cultivars, as status of soil and irrigation water (Abedin *et al.* 2002, Norton *et al.* 2009a and 2009b). *Pteris vittata* uptake high amount of arsenic. It revealed that P_1 and P_2 treated BRRI-29 rice grain accumulate only low amount of arsenic and reduction of arsenic accumulation into rice is 96.24% and 97.01%. Arsenic content in rice grain ranged from 0.80 to 1.18 mg/kg in unplanted control where as it was 0.59 to 0.81 mg/kg after phytoextraction by *Pteris vittata* in one growing cycle and 0.35 to 0.61 mg/kg after phytoextraction with two successive growing cycles (Mandal *et al.*, 2012). Ferns grew well and took up arsenic from soils. Fern biomass ranged from 24.8-33.5 g/plant after 4 months of growth but was reduced in the subsequent harvests and frond arsenic concentrations ranged from 66.0-6151.0 mg/kg, 110.0- 3056.0 mg/kg and 162.0-2139.0 mg/kg from the first, second and third harvest respectively; subsequently *P. vittata* reduced soil arsenic by 6.4-13.0% after three harvests (Ma *et al.*, 2008). Straw yield was decreased significantly with arsenic addition in irrespective of season, year, method and level of arsenic application (Khan *et al.*, 2010). The highest straw yield (39.07 ± 4.08 g) and lowest straw yield (27.01 ± 6.74 g) were found in 0.5 mg/L and 4.0 mg/L arsenic treatment (Azad *et al.*, 2012). *Pteris vittata* uptakes high amounts of arsenic in their fronds (Mandal *et al.*, 2012). It was observed that arsenic uptake by rice straw decreased significantly in arsenic ameliorated soil by two harvests of *Pteris vittata*. The arsenic uptake of rice root followed a similar trend as that of straw uptake. These findings differ from the results of studies using other ferns. Arsenic concentrations in the ladder brake fern fronds increased as more water soluble arsenic became available to the plant (Tu and Ma, 2002) and concentrations of arsenic in both fronds and roots of *P. vittata*, *P. cretica*, *P. longfoila* and *P. umbrosa* increased linearly with increasing additions of substrate arsenic concentrations (Zhao, 2002). Therefore, the significant accumulation of arsenic into the roots of the marsh fern and the non-significant arsenic accumulation in fronds are maybe the result of small amounts of arsenic becoming stored in vacuoles of the root cells and later being released from the vacuoles of back into the back into the plant (Ponyton *et al.*, 2004). From the study, it was found that *P. vittata* had significant effect on growth and yield of rice on arsenic contaminated soil. Bioaccumulation factors for *P. vittata* after exposure to soil arsenic levels less than 400 mg/kg. High bioaccumulation factors can be an indication of strong phytoremediation potential (Wei *et al.*, 2006). Arsenic had a significant effect upon arsenic accumulation in rice but trapping arsenic through *P. vittata*.

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Table 03. Response of rice to different number *P. vittata* as trap for arsenic on arsenic accumulation [x]

Treatments	Arsenic accumulation (ppm) by							Reduction (%) of arsenic accumulation into rice
	rice grain		rice husk		rice straw		*P. vittata*	
P_1	1.55	a	5.57	a	39.78	a	-	-
P_2	0.02	b	0.60	b	1.05	b	45.13	96.24
P_3	0.02	b	0.58	b	1.00	b	45.49	97.01
LSD 0.05	*0.07*		*0.16*		*1.12*			
CV%	*6.44*		*3.14*		*3.53*			

[x]In a column means having similar letter (s) are statistically identical and those having dissimilar letter (s) differ significantly as per 0.01 level of probability

IV. Conclusion

It was found that rice plant grown with *P. vittata* accumulate only 0.02 ppm arsenic in grain while 1.55 ppm arsenic accumulation was found in grain without *P. vittata*. So, it can be stated that *P. vittata* might be acted as the trap plant and reduce the arsenic accumulation into rice about 96.24-97.01%. Further experiment should be conducted on various arsenic contaminated areas with different intercrop density to clarify and strengthen the findings of the study.

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