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COMPARISON OF ARSENIC ACCUMULATION BY TWO FERNS, *PTERIS VITTATA* AND *NEPHRODIUM MOLLE*

AFROZA HOSSAIN, J.C. JOARDAR AND S.M. IMAMUL HUQ*

Environmental Research Laboratory, Department of Soil, Water & Environment, University of Dhaka , Dhaka-1000, Bangladesh

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Abstract

Two different indigenous ferns *viz. Pteris vittata* and *Nephrodium molle* were grown in pots with spiked As concentrations ranging from 0 to 100 mg/kg to find their abilities as phytoremediators for the element. The results showed that arsenic content in the fronds and rhizoids increased with growth period and increasing As concentration in the soil. At 100 mg As/kg soil treatment and at 75 days after transplantation, arsenic content in the fronds and rhizoids of *P. vittata* reached 1073.52 and 537.35 mg/kg d.w., respectively which is quite different from any normal plants, while in *N. molle*, the values were only 3.11 and 15.6 mg/kg d.w., respectively. In the control treatments, the bioaccumulation coefficients of the fronds and rhizoids of *P. vittata* were as high as 34.18 and 18.2, respectively while the same were 0.82 and 0.74, respectively for *N. molle*. Common with other established phytoremediators, the bioaccumulation coefficients of *P. vittata* as well as *N. molle* showed a decreasing tendency with increasing As in soil. Besides, no significant difference ($p = 0.432$ for *P. vittata*, $p = 0.116$ for *N. molle*) in biomass production was found for different As treatments compared to that of control. *P. vittata* has been found to be extremely tolerant and hyper accumulative to arsenic compared to *N. molle*. The possibility of the ferns *vis-á-vis* their phytoremedial characters have been discussed in the paper.

Introduction

Arsenic is of great environmental concern due to its toxicity and extensive contamination of the environment in many parts of the world including Bangladesh. Ingestion of As contaminated groundwater is the major cause of As poisoning in Bangladesh. Major share of the As contaminated groundwater goes to the irrigation needs thereby causing a slow build-up of the contaminant in the soil.[1] Remediation techniques for soil contamination employed to date include stabilization, soil leaching, and electro kinetics.[2] There is a real need for reliable and cost-effective technologies capable of reducing arsenic in soils. Phytoremediation could be a cost-effective, engineering-economical and environmental friendly technique. Besides, it can also reduce soil erosion, increase soil organic matter and fertility, and thus often

*Author for correspondence. E-mail: imamh@bttb.net.bd

be called "green remediation".[3] For a species to be used for phytoremediation it must possess a character of hyperaccumulation of the particular contaminant. With increased concern on As contamination of soil, attention has been diverted to look for some As hyperaccumulating species. The study by Ma *et al.*[4] was the first to report a fern as an arsenic hyper accumulator, and this species showed considerable promise as a phytoremediator of arsenic contaminated sites. Recently, Imamul Huq *et al.*[5] reported two different plants *viz.* marigold (*Tagetes patula*) and ornamental arum (*Syngonia* sp.) as phytoremediators of arsenic in potted soils of Bangladesh. In the study of Ma *et al.*[4] arsenic contaminated sites were used. In the present study, we investigated this character of As hyper accumulation of indigenous *P. vittata* and compare the property of As hyper accumulation with another commonly grown indigenous fern *N. molle* with the objective to find the accumulating extent with characteristics of the stated ferns and judge the possibility of introducing them as As phytoremediators.

Materials and Methods

The experiment was conducted to study the accumulation of arsenic into two selected ferns (*viz. Pteris vittata* and *Nephrodium molle*) at different levels of As concentrations and at different days' interval. The experiment was based on arsenic accumulation by the selected indigenous ferns which were collected from different parts of Curzon Hall, University of Dhaka, Bangladesh. The soil samples, belonging to the Sonatola series, were collected from Chornoyadanga village of Manikganj district. The texture was silty loam, pH 6.97 and total As 0.615 mg/kg. The sampling site selected was adjacent to the agricultural land. Samples were collected from a depth of 0 - 15 cm from the surface by composite soil sampling method as suggested by the Soil Survey Staff of the USDA.[6] After collecting the samples, the soils were processed to bring the soil to a favorable condition for better crop growth; the soil samples were air dried and the larger aggregates were gently broken and passed through a 2 mm sieve; the soil thus sieved, was mixed thoroughly and used for pot culture experiments. A portion of this soil further ground and passed through a 0.5 mm sieve for various physical and chemical analyses in laboratory.

Experimental set-up: Three kg size fresh earthen pots having no holes at the bottom were used for the pot experiment. The pots were washed and dried in sunlight. Two kg soils were put in each pot. Arsenic at the rate of 10, 20, 50 and 100 mg per kg soil was applied to the treated pots. A set of control, with no As was also included. Arsenic was applied as solution made from sodium meta arsenite ($NaAsO_2$) with three replications for each treatment. The treatments were applied once at a time. The collected plants were washed with tap water and then with distilled water to remove the extra soils. Three healthy plants were selected and transplanted in

each pot and allowed to grow. Positions of the pots were changed every alternate day to allow equal exposure of each of the pots to sunlight. Plants received watering with tap water daily. Adequate plant protection measures were taken during the growing period. Plants were allowed to grow for 75 days after sowing them in the pots. However, sampling of the plants were done at 30, 45 and 75 days after transplantation (DAT). Plants were harvested manually by uprooting them. Plant roots were washed first with tap water for dislodging the debris then the free space in the roots was cleared by washing in distilled water. Aerial parts of plants were also washed. After harvesting, the plants were cut off into fronds and rhizoids. Fresh weights of the fronds and the rhizoids were noted separately. Plant samples were air dried for a day and then oven dried at 80 ± 5°C for 48 hours. The oven-dried samples were ground by electrical grinder and passed through a 0.2 mm sieve and were used for subsequent analysis. After harvesting, soil samples in each pot were also collected and prepared for analysis as per method mentioned earlier.

Different parameters of the soil and the plant were determined following procedures described in Imamul Huq and Alam.[7] Total arsenic in soil and plant was analyzed by Hydride Generation Atomic Absorption Spectrometer (HG-ASS). Plant samples were extracted with HNO_3 and the soil with aqua regia (HNO_3 : HCl, 1 : 3) solution.[8] Certified reference materials (CRMs) were carried through the digestion and analyzed as part of the quality assurance/quality control protocol. Reagent blanks and internal standards were used where appropriate to ensure accuracy and precision in the analysis of arsenic. Each batch of 20 samples was accompanied with reference standard samples to ensure strict QA/QC procedures.

Results and Discussion

Pteris vittata and *Nephrodium molle* ferns were grown in pots for different time intervals and with different As concentrations. All the plants were transplanted, so they took time to adjust to the new growth media and the adjustment time was up to 30 DAT. The visual symptoms of *P. vittata* and *N. molle* due to phytotoxicity of As were observed during the growing period. Between the two ferns, *N. molle* were healthier and produced much biomass than *P. vittata*. Fresh weights of both the ferns per ten plants at 75 DAT are presented in Table 1 which shows that the weights of *N. molle* was higher than that of *P. vittata* at all the treatments but these did not differ significantly ($p = 0.116$ for *N. molle* and $p = 0.432$ for *P. vittata*) among the treatments relative to the control plants.

Pteris vittata: The experimental results on As content in the fronds and rhizoids of *P. vittata* at different days interval in different treatments are shown in Table 2.

The contents of As in the fronds and rhizoids of *P. vittata* clearly demonstrated that fronds of *P. vittata* accumulate more arsenic compared to that in its rhizoids and which were several times greater in the fronds than in the rhizoids and this tendency increased with increasing As concentration in the environment and with the increasing time of contact with the contaminated soil. The maximum As concentration was found in the fronds at 75 DAT in 100 mg/kg As treated soils. Arsenic accumulation started to increase once the plants settled in their new growth media.

Table 1. Biomass production (average value) of *P. vittata* and *N. molle* at 75 DAT.

Treatment (mg/kg)	*P. vittata* F.W. (g/10 plants)	*N. molle* F.W. (g/10 plants)
0	22.67	26.37
10	23.43	28.50
20	20.93	26.40
50	20.67	28.13
100	21.00	27.73

Table 2. Arsenic contents in the fronds and rhizoids of *P. vittata*.

	30 DAT		45 DAT		60 DAT		75 DAT	
	Rhizoid	Frond	Rhizoid	Frond	Rhizoid	Frond	Rhizoid	Frond
0	1.42	1.14	3.17	5.70	4.19	9.32	11.19	21.02
10	4.46	1.39	7.79	10.94	13.33	10.14	12.12	43.14
20	4.22	3.82	10.05	29.80	29.58	74.33	41.62	100.52
50	4.93	4.58	19.04	35.35	35.91	78.37	233.45	541.99
100	6.82	4.70	25.69	50.05	74.33	248.87	537.35	1073.52

For rhizoids, the p values are 0.006 for treatment and 0.013 for growth period and the LSD at 5% level is 20.1. For fronds, the p values are 0.202 for treatment and 0.078 for growth period and the LSD at 5 % level is 328.47.

After their settlement, the plants started to translocate As at an accelerated rate into their aerial parts. Before transplantation, the arsenic content in the fronds and in the rhizoids was < 0.002 mg/kg which however, increased with increasing As treatment and with increasing growth period and after 75 days, the values in the two organs reached as high as 1073.52 and 537.35 mg/kg, respectively at 100 mg As/kg spiked soil. Since the As content in the ferns increased with increasing As concentration and with increasing time of growth, we thus considered the highest treatment (100 mg As/kg soil) and maximum growth period (75 DAT) for analyzing the outcome of the experiment. The discussion centers on 100 mg/kg application at 75 DAT if not otherwise mentioned. Up to 75 days of growth the As content in the fronds and rhizoids of *P. vittata* was constantly increasing with increasing As (R^2 = 0.99, P =

0.001 for fronds and R^2 = 0.98, P = 0.001 for rhizoids) (Fig. 1a). The time effect was also similar; arsenic content in the fronds and rhizoids of *P. vittata* was found constantly increasing with the contact time (R^2 = 0.78, P = 0.116 for fronds and R^2 = 0.70, P = 0.161 for rhizoids) (Fig. 2a). The findings indicate therefore, that purposeful cultivation of the fern could eventually remove a substantial amount of As from soil and thus could be successfully used for phytoremediation of arsenic contaminated soils. In the control treatment with no added arsenic, fronds and rhizoids of *P. vittata* (Table 2) showed a bioaccumulation coefficient of 34.18 and 18.2 at 75 DAT while this

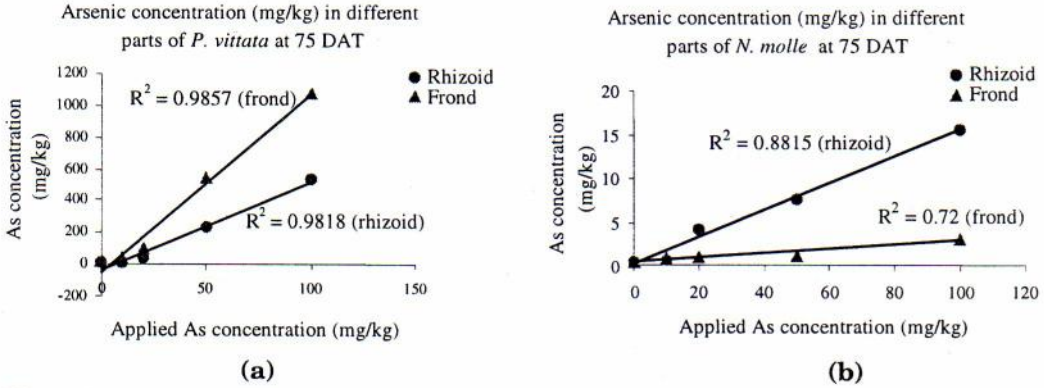

Fig. 1. Arsenic concentration (mg/kg) in fronds and rhizoids of (a) *P. vittata* and (b) *N. molle* after 75 days of growth at different spiked As concentrations.

coefficient decreased to 10.67 and 5.34 at 100 mg As/kg soil for the fronds and the rhizoids, respectively. This phenomenon indicated that although bioaccumulation coefficient is an important factor for identification of hyper accumulators, it will be met with limits during application because of the influence of total contents in soil. The arsenic bioaccumulation coefficients for the fronds are greater than 1 in 60 DAT and 75 DAT samples at all the treatments as the plants were grown directly on highly arsenic contaminated soil. Usually, As accumulates more in the underground parts than that in the above ground parts for most plants.[9] Many investigators have reported the same, in rice by Odanka *et al.*[10] in alfalfa, lettuce and wheat by Kapustka *et al.*[11] On the other hand, plants showing phytoremedial characteristics show the reverse phenomenon.[12] In the present case, *P. vittata* exhibited similar phenomenon except at 30 DAT. This could be due to the fact that the newly transplanted plants needed time to adjust to the new environment and/or could not get enough time to translocate the As to the above ground parts. It has been demonstrated that brake fern growing in lumber antiseptic CCA contaminated soil (arsenic content is 97 mg/kg) could accumulate 1442 - 7526 mg/kg arsenic in its leaves on a dry matter basis, and bioaccumulation coefficients were 14.9 - 77.6.[4] Similar observations have also been made by Chen *et al.*[13] Our findings also show

that *P. vittata* can grow in soils of high arsenic contents without any apparent toxic effect, thus demonstrating greater tolerance to arsenic.

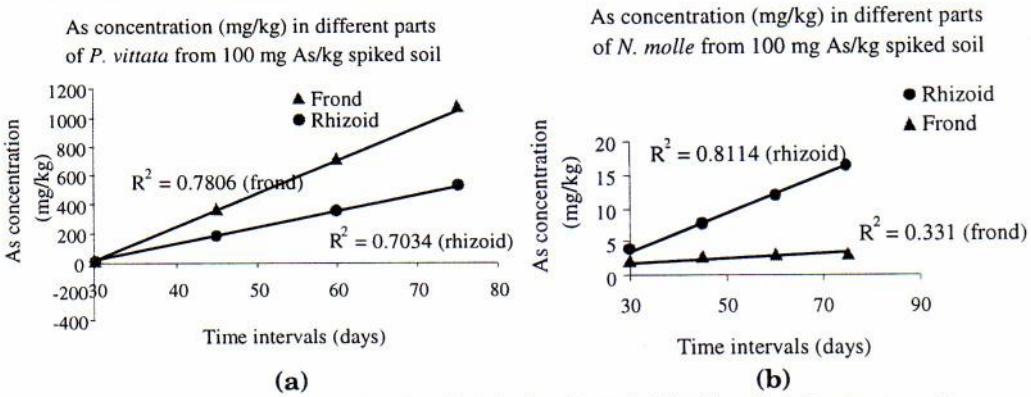

Fig. 2 Arsenic concentration (mg/kg) in fronds and rhizoids of (a) *P. vittata* and (b) *N. molle* at 100 mg As/kg soil after different days of growth.

The above ground biomass, speedy growth, bioaccumulation coefficients and the tolerance to soil metals are factors determining the remediation effectiveness of hyper accumulators.[14] In our study, *P. vittata* was found to accumulate large amounts of arsenic in fronds while growing rapidly with great biomass production. Furthermore, the bioaccumulation coefficients of *P. vittata*, even at no application of As showed a very high value indicating its affinity to hyper accumulate the toxicant. This indicates that, *P. vittata* could be effectively used for the remediation of arsenic contaminated soil. Moreover, As content in normal plants is lower than 3 mg/kg[15] whereas, the arsenic in *P. vittata* has been found to be several times greater than this limit.

Nephrodium molle: The As concentration in *N. molle* at different As treatments and at different days of growth are shown in Table 3.

Table 3. Arsenic contents in the fronds and rhizoids of *N. molle*.

	30 days		45 days		60 days		75 days	
	Rhizoid	Frond	Rhizoid	Frond	Rhizoid	Frond	Rhizoid	Frond
0	0.39	0.36	0.38	0.17	0.06	0.35	0.46	0.50
10	3.73	3.53	1.08	0.31	1.04	1.14	0.68	0.83
20	2.53	0.73	3.04	0.71	2.18	1.03	4.11	0.98
50	3.23	0.61	6.32	4.13	2.69	2.88	7.56	1.15
100	4.01	2.26	2.11	1.01	10.14	3.76	15.60	3.11

For rhizoids, the p values are 0.027 for treatment and 0.367 for growth period and the LSD at 5% level is 4.6. For fronds, the p values are 0.121 for treatment and 0.875 for growth period and the LSD at 5% level is 1.85.

Although *N. molle* produced greater amount of biomass than that of *P. vittata,* the As accumulation in *N. molle* however, was not as high as that in *P. vittata*. *N. molle*, somewhat showed a tendency to accumulate As from the growth medium. Arsenic accumulation by *N. molle* increased with increasing As concentration and with days of growth but there was no uniformity in the As uptake with days of growth and treatments like *P. vitatta*. Besides, the As uptake by *N. molle* rhizoids was higher than its fronds which was inverse to that of *P. vittata*. After 75 days of cultivation the As content in the fronds and rhizoids of *N. molle* was found to be increasing with increasing As (R^2 = 0.92, P = 0.01 for fronds and R^2 = 0.99, P = 0.001 for rhizoids) (figure 1b). At the highest treatment, arsenic content in the fronds and rhizoids of *N. molle* increased with the contact time (R^2 = 0.33, P = 0.424 for fronds and R^2 = 0.81, P = 0.099 for rhizoids) (Fig. 2b). Although the criteria for phytoremediator[14] have been shown by *N. molle*, yet the hyper accumulating capacity is absent. Furthermore, the bioaccumulation coefficients of *N. molle* was not as high as that of *P. vittata* meaning that it cannot translocate the accumulated As to its upper parts. *N. molle* does not qualify to be a phytoremediator for As. Comparing the two ferns, it could be seen that about 95.06 % As was removed by *P. vittata* as against 68.95 % by *N. molle* from the contaminated soil (Table 4). This again underlines the better phytoremedial character of *P. vittata*. Similar observations have been made with other plant species. [16]

Table 4. Arsenic removed from soil by *P. vittata* and *N. molle* under each treatment at 75 DAT (the initial soil As was 0.615 mg/kg).

Soil	*Pteris vittata*		*Nephrodium molle*	
Applying As in soil (mg/kg)	As present in 75 DAT soil (mg/kg)	% of applied As removed	As present in 75 DAT soil (mg/kg)	% of applied As removed
10	3.22	69.67	5.21	50.92
20	1.80	91.27	8.18	60.32
50	2.73	94.61	16.92	66.57
100	4.97	95.06	31.24	68.95

Conclusion

It has been observed in this experiment that ferns in general are tolerant to high soil As. Of the two indigenous ferns, *P. vittata* was found to be a hyper accumulator which character could be efficiently used for phytoremediating As contaminated soil. Our results further prove the hyper accumulation of As by *P. vittata* as observed by others. *N. molle*, though can tolerate high soil As, however, cannot be used as a phytoremediating plant. The wide distribution with easy adaptation to different conditions, *P. vittata* demonstrated a favorable prospect for its application in the phytoremediation of arsenic contaminated soils. Therefore, commercial cultivation of

P. vittata particularly along agricultural fields receiving As contaminated ground water irrigation could be a prospective remedial measure.

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