

NITROGEN, PHOSPHORUS AND POTASSIUM STATUS OF RICE CO-PLANTED WITH A NOVEL PHYTOREMEDIATOR, *MARSILEA MINUTA* L.

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Abstract

A pot experiment was carried out to assess the effects of arsenic and aquatic fern (*Marsilea minuta* L.), when applied as a phytoremediator, on the nutrient content (nitrogen, phosphorus, and potassium) of rice. Two sets of pot experiments were conducted in the net house on rice (*Oryza sativa* L.) together with aquatic fern (*M. minuta*) and on aquatic fern (*M. minuta*) alone where soils were treated with 1 mg/L As-solution at 80% arsenite and 20% arsenate. No significant difference was found in the nitrogen, phosphorus, and potassium concentrations of rice, in the absence of arsenic, whether grown in the presence of *M. minuta* or not. The uptake of total nitrogen, phosphorus, and potassium was found to be 36%, 23%, and 22% more, respectively in rice plants treated with *M. minuta* and arsenic over the control treatment, although the results were statistically insignificant. However, a significant negative relationship was found between arsenic and root nitrogen (P-value of 0.0017) when grown together with arsenic and *M. minuta*. A significant positive relationship was found between arsenic and shoot phosphorus (P-value of 0.0025) as well as arsenic and shoot and root potassium (P-values were 0.0045 and 0.0115, respectively). The results indicate that *Marsilea minuta* might be used as a phytoremediator of As together with rice plants.

Keywords: Rice, Arsenic, *Marsilea minuta*, Nitrogen, Phosphorus, Potassium

Introduction

The plants of wetland ecosystems have been playing a crucial role in the lives of human beings for thousands of years, serving as food, fodder, and medicine. The medicinal value of pteridophytes has been known to man for more than 2000 years (Chandra, 2000). *Marsilea minutia* L. (Marsileaceae), is a common species of water fern widely found in wet and humid places and is considered a medicinal plant (Tiwari *et al.*, 2010). Recently, aquatic plants have received more attention for their phytoremediation qualities. *Marsilea minuta* can be used as a phytoremediator for chromium, copper, and nickel (Kumar *et al.*, 2012). The bioaccumulation factor of As in *M. minuta* was found to be 2.88-3.88 (Tripathi *et al.*, 2012), demonstrating its potential to be a good phytoremediator for As. Phytoremediation is considered as a low-cost, but efficient method for As reduction (Pilon-Smits, 2005) and could be used to mitigate the wide-spread As

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contamination in paddy fields (Han *et al.*, 2007). Irrigation of agricultural soils with As-contaminated groundwater in Bangladesh, India, China, and other countries in Southeast Asia has caused the accumulation of As in both soils and plants, and elevated the transfer of As in the food chain, which poses long-term risks to human health (Imamul Huq *et al.*, 2006).

Recent studies have shown the accumulation of As in *M. minuta* about 60 mg/kg (Tripathi *et al.*, 2012) and 82.06 mg/kg (Hassi *et al.*, 2017). One of our previous studies have further proved the phytoremediation capacity of *M. minuta*, finding that the bioaccumulation factor of As in *M. minuta* was found to be >5.34 while growing together with rice plants (Hassi *et al.*, 2017). When a plant is used as a phytoremediator for any metal, the main concern is then how much metal could be remediated by the phytoremediators. It is also necessary to assess the nutrient competition between the host plants and the phytoremediator, especially when it is applied in a grain field. However, there is no report on the nutrient status of rice when *M. minuta* is applied as a phytoremediator of As. In the present study, two pot experiments were conducted to investigate the effects of As on nitrogen, phosphorus and potassium concentrations in rice and *M. minuta*.

Materials and Methods

A pot experiment was conducted in the net house of Department of Soil, Water and Environment, University of Dhaka. The experiment was conducted in As-contaminated soils (non-calcareous alluvium) collected from Manikganj district, Bangladesh (23°52'60" N and 90°02'12" E). Soils were collected from a depth of 0 – 15 cm, as recommended by the Soil Survey Staff of the USDA (1951). Rice seeds of BRRI dhan 28 were collected from authorized seed market, and *M. minuta* was collected from the Botanical Garden of the University of Dhaka. *Marsilea minuta* was allowed to grow in the water medium for seven days before transplantation. For optimum growth of plants, recommended doses of N, P, and K (N = 230.8 kg urea/ha, P = 78.05 kg TSP/ha, and K = 128 kg MoP/ha soil) was applied following a fertilizer recommendation guide (BARC, 2012). In the first phase, *M. minuta* was cultivated into 1.5 kg of soil, in the presence and absence of As, representing as MX and MA. In the next phase, *M. minuta* was allowed to grow together with rice in the presence and absence of As into 5 kg of soil which are represented as RAM (rice with *M. minuta* and As), RXM (rice with *M. minuta* and without As), RAX (rice with As and without *M. minuta*), and RXX (rice without As and *M. minuta*). Three replications were conducted for each treatment. Throughout the growth period, 100 mL and 250 mL of As-treated water (1 mg/L As) was applied to the plants grown in 1.5 kg and 5 kg of soil, respectively. Arsenic stock solution was made from 80% arsenite (NaAsO₂) combined with 20% arsenate (Na₂HAsO₄.H₂O) (Ali *et al.*, 2016). Three replications were conducted for each treatment. Rice plants were harvested at 90 days after transplantation, and *M. minuta* plants were harvested at 70 days after transplantation. After harvesting and washing, the whole rice plants were

separated into roots, shoots, and unhusked grain and *M. minuta* were separated into roots and shoots. Plant samples were first air-dried, then oven dried at $85^{\circ} \pm 5^{\circ}\text{C}$ for 48 hours, then ground with mortar and pestle for chemical analysis.

Soil samples were sieved through a 0.5 mm sieve for chemical analysis. Particle size analysis of soil was conducted following the USDA (1951) methods. Total organic C was determined following the methods of Walkley and Black (1949), and available N (%) and P (%) were estimated following the procedures described in Carter and Gregorich (2007). Moisture content was determined by using the gravimetric method. Total N of the plant samples was determined by Kjeldahl's method following concentrated sulphuric acid (H_2SO_4) digestion as described by Imamul Huq and Alam (2005). Total P of the plant samples was determined by Vanadomolybdophosphoric Yellow Color method using a spectrophotometer wavelength ranging from 400 to 490 nm (according to the sensitivity needed) (Imamul Huq and Alam, 2005). The amount of potassium was estimated using flame emission spectrophotometer, wavelength settings at 766.5 (Imamul Huq and Alam, 2005). Total soil arsenic (As) was extracted by aqua-regia (concentrated HCl: concentrated $\text{HNO}_3 = 3:1$) digestion, and As from plant samples was extracted by concentrated HNO_3 digestion with a 24-hour pre-digestion, determined by a Hydride Vapor Generator (HVG) with 20 % KI in acid medium. The hydride was generated using 5M HCl, 0.4% NaBH_4 , and 0.5% NaOH in deionized water (Hassi *et al.*, 2017; Ganje and Rains, 1982).

Data were analyzed by one-way Analysis of Variance (ANOVA), with significance values calculated using the least-significant-difference (LSD) test, using MINITAB-18.

Results and Discussion

The collected soil samples and fern (*M. minuta*) were analyzed before setting up the experiment to observe the nutrient status as well as the concentration of As (Tables 1 and 2).

Table 1. Background analysis of soil

pH	Moisture (%)	Sand (%)	Silt (%)	Clay (%)	Textural Class	Org. C (%)	Total N (%)	Available P (%)	Available K (%)	Total As (mg/kg)
6.52	21.45	13.4	74.6	12.0	Silt loam	0.87	0.09	0.0033	0.0023	15.52

Table 2. Background analysis of *M. minuta*

N (%)		P (%)		K (%)		As (mg/kg dry wt.)	
Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
2.06	3.63	0.91	1.37	BDL	2.00	0.23	1.70

The mean concentration of nitrogen, phosphorus, potassium, and arsenic in the shoots and roots of *M. minuta*, grown without rice and with rice, are shown in Table 3 and Table 4, respectively. There was an increase of nitrogen concentration in shoots of *M. minuta*, whereas the nitrogen concentration was decreased in roots for all treatments. The nitrogen concentrations of shoots and roots represented a significant difference between the control and As-treated ferns when grown alone (the value of P was 0.005 and 0.00, respectively), but the difference is not significant when grown together with rice (the value of P was >0.05). Phosphorus concentration also decreased for all treatments, although it was statistically insignificant with a P-value of >0.05 for shoots and roots. The potassium concentration increased in shoots of *M. minuta* when grown with rice compared to ferns grown alone. The ANOVA test shows a P-value of 0.017 indicating a significant difference among treatments. The mean uptake values (Table 4) of nitrogen, phosphorus, and potassium in *M. minuta* growing with rice plants showed that different parts of *M. minuta* uptake less nutrients compared to those plants (Table 3) grown without rice. These results imply that nutrient competition occurred between rice plants and *M. minuta* and most of the nutrients were uptaken by rice plants rather than the ferns.

Table 3. Concentration and uptake of N, P, and K in *M. minuta* grown without rice

Treatment	N (%)		P (%)		K (%)		N uptake (mg/kg soil)		P uptake (mg/kg soil)		K uptake (mg/kg soil)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
MX	2.06	1.40	0.45	0.41	2.46	1.68	52.68	30.05	11.49	8.80	62.81	36.06
MA	3.13	3.15	0.41	0.62	1.41	0.32	75.54	103.74	9.89	20.42	34.03	10.54

Table 4. Concentration and uptake of N, P, and K in *M. minuta* grown with rice

Treatment	N (%)		P (%)		K (%)		N uptake (mg/kg soil)		P uptake (mg/kg soil)		K uptake (mg/kg soil)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
RXM	2.94	3.13	0.72	0.96	4.81	BDL	11.94	5.57	2.92	1.71	19.53	BDL
RAM	3.06	1.49	0.71	0.72	3.13	BDL	16.22	3.96	3.76	1.92	16.59	BDL

Effect of As and *M. minuta* on Nitrogen Status of Rice

The total concentration of nitrogen, phosphorus, potassium and their uptake in rice plants were calculated, in the presence and absence of As, showing in Fig. 1. It has also been reported that the nitrogen concentrations in grain and straw increased with increasing addition of As (Begum *et al.*, 2008). In this experiment, the nitrogen concentration was increased with As treatments and the highest concentrations of nitrogen in grain and shoot were observed in rice plants treated

with As and *M. minuta*, which indicates that the presence of *M. minuta* did not affect the uptake of nitrogen in rice plants. However, the relationship between arsenic and nitrogen in the roots of rice was found to be negative, but significant with a P-value of 0.0017. In the case of unhusked grain and shoots, a positive correlation was observed although ANOVA tests resulted in P-values of >0.05 for unhusked grain and shoots. The highest uptake of nitrogen for unhusked grain and shoots was found for rice plants growing with *M. minuta* and As (113.6553 mg/kg soil and 124.6548 mg/kg soil, respectively). Total nitrogen uptake was found to be 36% more in *M. minuta* and As-treated rice plants than the control plants, however it is not statistically significant.

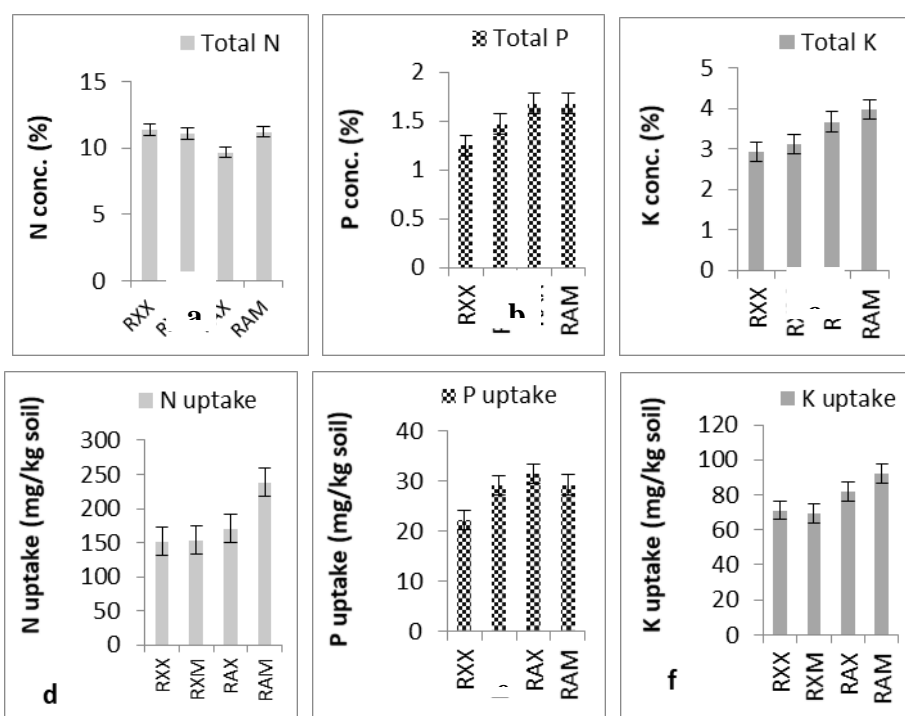


Fig. 1. Total concentration and uptake of N, P and K in rice plant.

Effect of As and *M. minuta* on Phosphorus Status of Rice

The competition between phosphorus and As under flooded conditions is more complicated. It has been found that the addition of phosphorus fertilizer to flooded paddy soil increased arsenic accumulation in rice (Hossain *et al.*, 2009). In this experiment, the highest concentration of phosphorus was found in roots of rice grown together with As and *M. minuta* (the value is 0.85 %). Nevertheless, the highest uptake of phosphorus in unhusked grain was found for rice plants growing with *M. minuta* without As (17.12 mg/kg soil) followed by plants growing with As alone (16.87 mg/kg soil). In case of shoots and roots of rice, the

highest uptake value was also found for As treatments alone (the value was 12.51 mg/kg soil and 2.11 mg/kg soil, respectively). ANOVA tests resulted in P-values of 0.0025 for shoots indicating a significant difference in phosphorus of shoots but >0.05 for grain and root indicating a non-significant difference in shoots and roots among the experiments. In this experiment, 23% greater phosphorus uptake was found in *M. minuta* and As-treated rice plants than the control plants, although the result is statistically insignificant. A positive correlation was observed between phosphorus and As which is evidenced by the report of Islam and Jahiruddin (2010) that the phosphorus concentration of rice grain increased with As doses, although in the present experiment, the application rate of As was only 1 mg As/L.

Effect of As and *M. Minuta* on Potassium Status of Rice

A wide variation of potassium concentrations in unhusked grain was observed and the presence of husks may have caused this. The highest concentration and uptake of potassium were found in the shoots of rice growing together with As and *M. minuta*, the values were 2.96% and 86.43 mg/kg soil, respectively. On the other hand, in unhusked grain and roots of rice, the highest uptake value was found for plants growing with As alone (4.74 mg/kg soil and 2.66mg/kg soil). About 22% more potassium uptake occurred in *M. minuta* and As-treated rice plants than the control treatments. A positive relationship was found for shoot and root phosphorus and As concentrations and ANOVA tests resulted in P-values of 0.0045 and 0.0115 for shoots and roots indicating a significant difference in potassium concentration in shoots and roots among the experiments.

Abedin *et al.*, (2002) reported that As accumulation in rice follows the order of root> stem>grain. However, the application of *M. minuta* as a phytoremediator could reduce the uptake of As up to 58.67% for roots and 21.72% for shoots of rice (Hassi *et al.*, 2017). It has also been observed that the nutrient necessities and distributions within the *Pteris* species were altered distinctly when the plants were exposed to As (Srivastava *et al.*, 2006). Arsenic significantly decreases nitrogen, phosphorus, and potassium concentrations in both shoots and roots of winter wheat (Lui *et al.*, 2008).

Conclusion

It could be concluded from the present study that nitrogen, phosphorus, and potassium concentrations of rice was not affected due to the application of *M. minuta* in rice cultivation. Moreover, the concentration of these three nutrients in different parts of *M. minuta* was not affected due to the application of As. Additional studies are needed before introducing *M. minuta* to an As-contaminated rice field.

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