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Bioremediation of arsenic toxicity by algae in rice culture

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Abstract

A rice variety (BRRI Dhan-28) receiving arsenic-contaminated irrigation water ranging from 0 to 2.0 mg As/L, and grown with and without algae, showed that the presence of algae in the growth medium reduces As accumulation in rice. The pot experiment showed that algae could reduce accumulation of arsenic in rice plants by as much as 71%. Algal growth was also found to depress arsenic accumulation in soil.

Key words: algae, arsenic, bioremediation, rice

INTRODUCTION

Groundwater contamination by As in Bangladesh has been called one of the worst calamities of the last 100 years. Only a small portion of the extracted groundwater is used for drinking. The majority (~85%) is used for irrigation (BADC 2002). About 40% of the net cultivable area of the country is under irrigation, and the major recipient (60%) of the irrigation water is Boro (dry season) rice, along with wheat and some vegetable crops (Imamul Huq *et al*. 2005).

The use of As-contaminated irrigation water in Bangladesh has been found to cause accumulation of As in rice and rice plants (Rahman *et al*. 2005; Williams *et al*. 2005). Estimates showed that As in rice and other food sources could contribute to about 30% of the total arsenic ingestion (Correll *et al*. 2006). Since rice is the staple food in Bangladesh, any adverse effect on the nutrient content of rice due to As-contaminated irrigation water would exacerbate the malnutrition problem; however, As uptake and accumulation in rice plants from irrigation water may differ depending on the cultivars used (Abedin *et al*. 2002).

The ability of algae to absorb metals has been recognized for many years (Megharaja *et al*. 2003). In natural environments, algae play a major role in controlling metal concentration in lakes and oceans (Sigg 1985, 1987). Algae possess the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water (Megharaja *et al*. 2003; Shamsuddoha *et al*. 2006). Bioaccumulation studies reveal the accumulation of the contaminant in the organism via uptake of food or water containing the contaminant. Historically, the growth of algae in rice fields has been considered a natural fertilization process, as decomposition of algae adds nitrogen and other nutrients to the soil. The algae growing in rice fields are thought to take up, *inter alia*, the As present in the water. In recent studies, it has been observed that algae can hyperaccumulate As from water (Imamul Huq *et al*. 2005; Shamsuddoha *et al*. 2006). This hyperaccumulation characteristic of algae could be used to remediate the rice grown with As-contaminated irrigation water.

The present study is an attempt to examine the possible extent of bioremediation by algae of As in rice culture, by growing Boro rice with and without the presence of algae.

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MATERIALS AND METHODS

The present experiment was carried out to observe simultaneous uptake of As by algae and rice plants. The experiment consisted of two phases; the first phase included *in vitro* algae cultivation, and the second phase included bioremediation of As in rice culture by these algae. Two sets of experiments were carried out as follows: rice was grown and treated with different levels of As with and without algae in the growth media. Four treatments of As at 0, 0.5, 1.0 and 2.0 mg As/L in the irrigation water were applied.

CULTIVATION OF ALGAE

In the first phase, algae were cultured following the prescribed medium for cyanobacteria (Atlas and Parks 1993). The algae were cultured in the laboratory of the Bangladesh–Australia Centre for Environmental Research, Department of Soil, Water and Environment, University of Dhaka. The algae were isolated from garden soil taken from the botanical garden at the University of Dhaka.

A suspension of soil was made by mixing the garden soil with sterilized water, and 1 mL of soil suspension was inoculated into the medium in the conical flasks. The flasks were plugged with cotton and were shaken on a rotary shaker for two weeks. Once algal growth became noticeable, the flasks were put in a place where they received sunlight at room temperature (~ 28°C). The growing algae were observed under a light microscope, blue-green and green algae being identified in the growth medium. As many as seven different algae were identified (Table 1). The green and blue-green algae thus identified were sub-cultured using the same medium. The sub-cultured algae were further identified to verify any possible contamination.

When the growth was found to be uncontaminated, a mass culture of the sub-cultured algae (the seven identified) was made in plastic bowls at room temperature with exposure to sunlight. The mass culture was continued for three months.

At the end of three months, the algae were harvested carefully from the container by filtration, and a portion was allowed to dry in the air. After drying, the algae were pulverized in a mortar, and the pulverized algae were preserved in closed plastic pots for subsequent analysis. A portion of fresh algae was also digested for analysis. The rest of the fresh algae was used for application to rice plants.

Table 1. Algae identified in the cultured medium

Name	Algae
Gloeotrichia	Blue-green algae
Oscillatoria	Blue-green algae
Lyngbya	Blue-green algae
Chlamydomonas	Green algae
Chlorella	Green algae
Nitzschia	Diatom (Bacillariophyceae)
Navicula	Diatom (Bacillariophyceae)

RICE CULTIVATION

A pot experiment was carried out in the net house with a soil (Typic Endoaquept) collected from Dhamrai, Bangladesh. The soils were dried, crushed, and then stored in a plastic bag for further analysis. Various physical, chemical and physicochemical properties were determined (Table 2) in order to assess the nutritional and As status of the soils, following the procedures described by Imamul Huq and Alam (2005).

Table 2. Some properties of the soil

Properties	Values
pH (soil:water = 1:2.5)	6.36
Electrical conductivity	0.07 μS
Sand	7%
Silt	49%
Clay	44%
Texture	Silty clay
Organic matter	1.4%
Total nitrogen	0.15%
Total phosphorus	0.05%
Total potassium	0.32%
Total sulphur	0.21%
Arsenic	1.65 mg/kg
Zinc	0.4 mg/kg

The soil used for the pot experiment was sterilized in an autoclave (at 15 psi, 121°C for 15 minutes) in batches of 5 kg. The pots were separated into two groups: one group received algal inoculation, while the other group was not inoculated. The whole of the P and K and one-third of the N doses as estimated for rice cultivation were mixed with the soil (BARC 2005). The sources of N, P and K were urea, TSP and MP respectively. Arsenic treatments were applied with irrigation water. Each pot was filled with 5 kg of soil. BRRI Dhan-28 (BR-28) seeds were collected from the Bangladesh Rice Research Institute (BRRI), sown in pots, and allowed to grow. The pots were thinned to three plants after the seedlings were established. Another one-third of the dose of urea was applied after 35 days, and the final one-third was applied during the panicle initiation stage of the rice plants. Intercultural operations were carried out whenever necessary. Weeds were removed manually.

Ninety-day-old algal samples were inoculated into each pot in equal amounts (15 mL). Ten days after inoculation, As at 0, 0.5, 1.0 and 2.0 mg/L was applied to all the pots (with and without algae) with the irrigation water. The background level of As in the soil was 1.6 mg/kg. This was taken as the control. Sodium metaarsenite was used as the source of arsenic. All experiments were done in triplicate. A total of 24 pots were used for the experiment. The rice plants were harvested after 120 days of growth. At the same time, algal samples and soil samples were collected from each pot. The soils were dried, crushed and stored in plastic bags for analysis.

The plant samples (roots, straw and grain) were collected at the time of harvesting of the crop (120 days after sowing). This was done manually by uprooting the plants carefully from the pot. The plants were washed first with tap water; then there were three 1 L distilled water washings, each for one minute, of the roots to evacuate the ions/solutes from the free space. Grains were plucked before uprooting. The plants were separated into roots and straw, and the fresh weight was taken. The samples were oven-dried at 70º ± 5ºC, and the dry weight was taken. The oven-dried samples were ground and passed through a 0.2 mm sieve.

The algae in each pot were also collected after harvesting of the crop. They were then digested for further analysis.

Soils from each pot were collected after harvesting of the crop. Plant, algae and soil samples were analysed for total arsenic, using a hydride generation atomic absorption spectrometer (HG–AAS). Certified reference materials were carried through the digestion and analysis as part of the quality assurance/quality control protocol. Reagent blanks and initial standards were used where appropriate to ensure accuracy and precision in the analysis of arsenic. Each batch of 20 samples was accompanied by reference standard samples to ensure strict QA/QC procedures.

RESULTS AND DISCUSSION

Visual observations were made and some agronomic parameters were recorded to see the effect, if any, of arsenic on the growth and yield of the rice plants. No symptoms of As toxicity were visible in the treated plants.

Effect of arsenic on plant growth and yield

Of the various growth and yield parameters, plant heights (cm), fresh weight of plants (g) and grain yield (t/ha) were considered in the present investigation. Regression analysis between the yield parameters and the treatments applied was carried out to determine the R^2 value, and analysis of variance (ANOVA) of the data was carried out to determine the F-value. The test of significance of treatments was calculated by LSD. No marked differences were observed in the mean height of rice plants for the As treatments, and between the two culture sets. However, Barrachina *et al*. (1995) observed that progressive accumulation of arsenic in plants, with time of exposure to As treatments, caused plant height reduction. A reduction in plant heights with increasing As concentration has also been reported in rice plants (Yamare 1989). In our case, the application rate was much lower than those used by the authors mentioned – i.e. we were using a value similar to that found in field conditions. However, there was a slight decrease in the fresh weights of the rice. At 2.0 mg/L As treatment, rice showed a decrease of more than 16% from the control for algae+ treatment; whereas, at the same application rate, but without the presence of algae, the decrease was around 8% over the control. An ANOVA test was carried out for fresh weight, and the p-values were found to be 0.277 and 0.003 for the treatment effect and for the algal effect, respectively. The low p-value confirms the significant effect of algae on rice plant fresh-matter production, and also a significant difference between rice-algae+ plants and rice-algae– plants. The grain yield (t/ha) did not noticeably decrease as a result of higher As doses. At lower doses of As in the irrigation water (0.5 and 1 mg/L), the rice yield increased slightly, compared to the control, in both rice-algae+ and rice-algae– treatments, although the increase was not significant. The maximum yields of rice plants grown with and without algae were observed, for the 0.5 mg/L treatment, to be 7.07 t/ha and 6.89 t/ha, respectively. At a high As dose (2.0 mg/L), the rice yield decreased compared to lower doses of As, either for rice-algae+ or rice-algae– plants. This reduction was probably due to the toxic effect of arsenic on the physiological function of the plants. Hossain *et al*. (2005) found yield reductions of more than 40 and 60% for BRRI Dhan-28 and Iratom-24, with 20 mg/kg As, compared to the control.

Arsenic content in individual rice plants

From the results, it is evident that in both algae-treated and non-treated plants, arsenic accumulation increased with increasing arsenic concentration in the irrigation water. Arsenic content was higher in roots than in shoots. Plants grown without algae contained higher amounts of arsenic compared to those grown with algae. When grown without algae, the highest concentration of arsenic in the roots was found to be 100.2 mg/kg (2.0 mg As/L dose) and the lowest was obtained for the control (3.18 mg As/kg). No detectable arsenic (the detectable level of As in the machine used – Varian Spectra 220 – is 0.0002 mg/kg) was found in grains plus husks either in rice-algae+ or rice-algae– plants. The mean values (for three replicates) of arsenic concentration in roots and shoots of rice-algae+ and rice-algae– are presented in Figures 1 and 2. It is important to note that there was some As accumulation in the control plants, and perhaps this was due to the presence of water-extractable As in the soil.

Roots

Treatment of soil with arsenic solution increased As concentration in roots over the control in both rice-algae+ and rice-algae– plants. When the concentration of As in solution increased, the As concentration in roots also increased, and it was higher than that in the shoots. The highest concentration of As in roots was 66.23 mg/kg at 2.0 mg/L treatment for rice-algae+ and 100.2 mg/kg for rice-algae– plants. The pot experiment showed that algae could reduce accumulation of arsenic in rice plants by as much as 74%. An ANOVA test was carried out for the root arsenic, and it was found to be highly significant, with an F-value of 5036.96 for treatment and 7299.89 for algal effect, with p values of 0.00 in both cases. ANOVA indicated that arsenic accumulates preferentially in roots, and that this accumulation increases with increasing concentration of the element in the growth medium. The statistical analyses also revealed that the presence of algae has been instrumental in reducing the entry of As into the rice roots. To further substantiate the observation, a paired t-test was performed. The value (t = 2.63) indicated that there was a significant difference in the As accumulation between algae+ and algae– treatments.

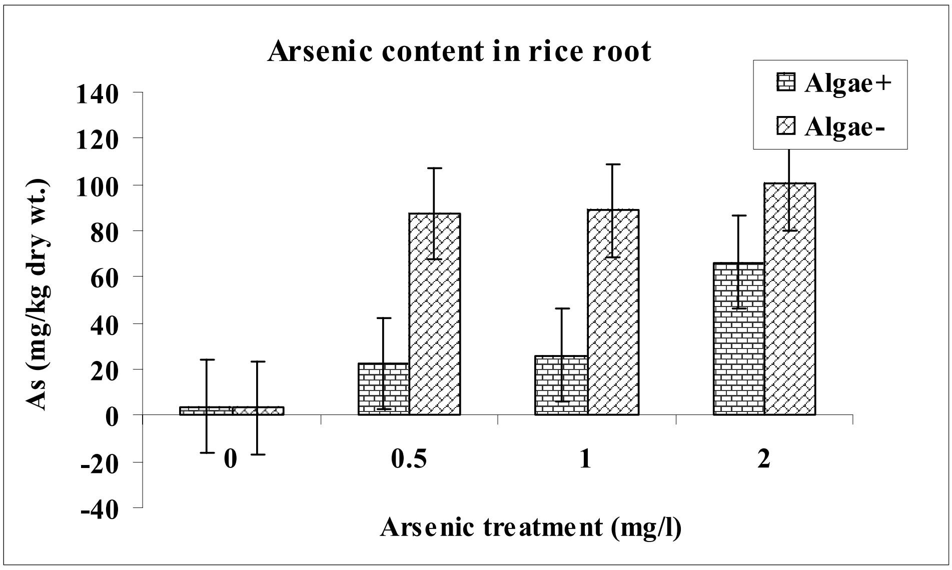

Figure 1. Arsenic content in the roots of rice-algae+ and rice-algae– plants

This again showed that the presence of algae in the rice-growing medium can substantially reduce the accumulation of As, thereby making the crop less toxic.

Straw

Treatment of soil with arsenic solution also increased As concentration in straw over the control, in both rice-algae+ and rice-algae– plants. When the concentration of As in solution increased, the As concentration in straw also increased, and it was lower than that of the roots. The highest concentration of As in straw was 6.62 mg/kg with the 2.0 mg/L treatment for rice-algae+ and the 8.1 mg/kg treatment for rice-algae– plants. The pot experiment showed that algae could reduce accumulation of arsenic in rice straw by as much as 33%. An ANOVA test was done for the straw arsenic, and highly significant F-values – 102.68 for treatment and 13.66 for algal effect, with p values of 0.00 in both cases – were obtained. ANOVA indicated there was a higher accumulation of arsenic in straw with increasing rate of arsenic application to rice, and that the application of algae could substantially reduce accumulation of As in shoots. To further substantiate the findings, a paired t-test was performed. The value ($t = 2.34$) indicated that there was a significant difference in the As accumulation between algae+ and algae– treatments, proving again that the presence of algae in the rice-growing medium can substantially inhibit the accumulation of As, thereby keeping the crop less toxic.

Uptake of arsenic

The uptake of As by the entire crop was determined as the concentration of As (in mg/kg) in the total dry matter produced by the rice crop. The amount of As uptake by different plant parts (roots and straw) and the whole plant are presented in Table 3. The total uptake of As by plant roots at 2.0 mg of As/L in irrigation water was the highest, with the values being 162.93 mg/kg dry wt. (rice-algae+) and 269.54 mg/kg dry wt. (rice-algae–) respectively for the two conditions. The total uptake of As by plant straw at 2.0 mg of As/L was 24.43 mg/kg dry wt. (rice-algae+) and 32.72 mg/kg dry wt. (rice-algae–). The results showed that the presence of algae in the growth medium was responsible for reducing the uptake of arsenic in rice roots by as much as 74%, and in straw by as much as 40.11%. When total plant uptake was considered, it was observed that the total uptake of As by the plants treated with a dose of 2.0 mg of As/L in irrigation water was the highest, the values being 187.4 mg/kg dry wt. (rice-algae+) and 302.3 mg/kg dry wt. (rice-algae–). It thus appears that As uptake was much higher for plants grown in the absence of algae than for those grown in the presence of algae with the same treatment.

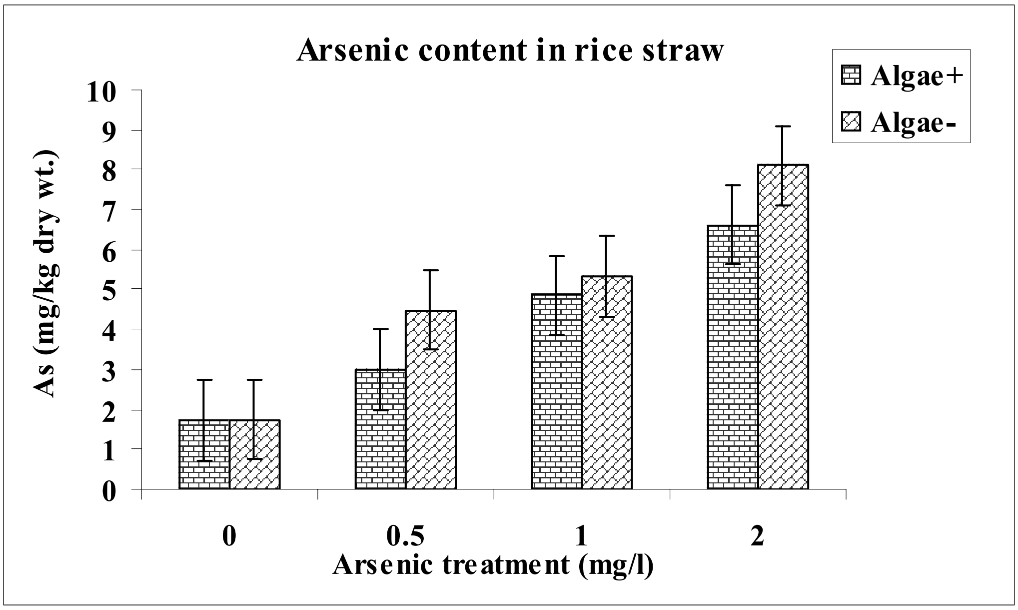

Figure 2. Arsenic content in the straw of rice-algae+ and rice-algae– plants

Table 3. Comparison of arsenic uptake by roots, straw and the whole plant for + or – algae (mg/kg)

Treatment (mg As/L)	Root			Straw			Plant as a whole		
	Algae+	Algae–	% decrease compared to algae–	Algae+	Algae–	% decrease compared to algae–	Algae+	Algae–	% decrease compared to algae–
	As (mg/kg)			As (mg/kg)			As (mg/kg)		
0	10.94	11.77	7.05	7.52	9.66	22.15	18.50	21.40	13.55
0.5	63.56	275.56	76.93	12.69	21.19	40.11	76.30	240.90	68.33
1.0	66.89	266.90	74.94	18.62	24.05	22.58	85.50	296.80	71.19
2.0	162.93	269.54	39.55	24.43	32.72	25.34	187.40	302.30	38.01

Arsenic in soil after harvest of the rice plants

After the harvesting of the rice plants, the soils were analysed for arsenic, and the results are shown in Table 4. The total soil As content after harvesting was highest for 2.0 mg of As/L in the irrigation water: i.e. 6.47 mg/kg (rice-algae+) and 6.8 mg/kg (rice-algae–). The data indicated that the algae could accumulate substantial amounts of the arsenic applied to the soil, and could thereby reduce the concentration of As in soil and also reduce the uptake of arsenic by the growing rice.

Table 4. Arsenic in soil and algae (in parentheses) as affected by various arsenic treatments

Treatment	Soil As (mg/kg)	
(mg As/L)	Algae+	Algae–
0	1.01 (2.16)	1.66
0.5	2.14 (3.35)	2.71
1	3.76 (8.05)	4.18
2	6.47 (16.42)	6.8

Arsenic accumulation in algae

The arsenic content (nitric acid extractable) in algae after harvesting is also presented in Table 4. When the concentration of As in the solution was increased, the As concentration in the algae also increased. The highest concentration of As in the algae was 16.42 mg/kg after the 2.0 mg/L treatment, and the lowest was 2.16 mg/kg after the control treatment. The pot experiment showed that the algae accumulated more than 85% of the As from the growth media as compared to the control treatment. An ANOVA test was done for the As content in the algae and it was found to be highly significant, with an F-value of 887.84 and a p value of 0.00. ANOVA indicated a higher accumulation of arsenic in the algae with an increasing rate of arsenic application, and, thus the application of algae could substantially reduce the accumulation of As in rice. The result indicates that algae could accumulate arsenic if it is present in solution. The higher the concentration of arsenic in solution, the greater the accumulation will be.

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