Bangladesh J Microbiol, Volume 22, Number 2, December 2005, pp 148-151

Original Article

Accumulation of Arsenic in Green Algae and Its Subsequent Transfer to Soil-Plant System

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[Received 16 November 2005; Accepted 10 December 2005]

An experiment was conducted to study the response of algae to accumulation of arsenic (As) from water and subsequent transfer of this arsenic to soil-plant system. The experiment consisted of two phases; the first phase included the *in vitro* algae cultivation under imposed treatments; the second phase included the release of the accumulated arsenic through mineralization of decomposed algae in soil and transfer of the released arsenic to *Ipomea aquatica*. The algae accumulated arsenic from the growth media and the accumulation increased with increasing rates of arsenic application. With control, the cellular arsenic content was nil whereas at 5-mg As/l application rate (a total of 115 mg As), the value was 1,400.40 mg/l upon mineralization, these algae added significant amount of arsenic to soil. Algae growing on a 5 mg As/l added as high as 10.60 mg As/kg soil. Plants growing on the algae-treated soil also accumulated quite a substantial amount of arsenic in it, the value surpassing the acceptable range of 1.5 mg/kg dry weight. The above-ground portion of the plants accumulated more arsenic than the root. There has been a time effect on the mineralization rate and subsequent accumulation of arsenic in plant.

Keywords: Arsenic, Green algae, Mineralization, Plant uptake

Introduction

In Bangladesh, shallow tube-wells are widely used for irrigation during dry season. Widespread use of arsenic contaminated ground water for irrigation suggests that ingestion of irrigated crops could be another major exposure route for As. Phytotoxicity due to increased As in soil and water has long-term impact on agricultural yield[1].

High-yielding variety (HYV) of rice is introduced to cope up with the growing cereal demand. About 86% of total groundwater withdrawn is utilized in agricultural sector[2]. Due to abrupt use of arsenic contaminated groundwater for irrigation in Bangladesh substantial amount of arsenic is being accumulated in topsoil and rice plants might take it up[3-4].

Since rice is the staple food in this country, any adverse effects on nutrient content of rice due to arsenic contaminated irrigation water would enhance the malnutrition problem[5]. More than 75% of the extracted ground water is used for irrigating about 40% of the net cultivable area of the country[2]. In Bangladesh, Boro, the dry season rice is the major recipient of irrigation water.

In our country, algal growth in irrigated rice fields is a common feature. Growth of algae in rice fields has been considered a natural fertilization process. After decomposition, these algae in rice fields add nitrogen and other nutrients to the soil[6].

While arsenic-contaminated groundwater is used for rice cultivation, algae thus growing in these rice fields are reported to take up among others, the arsenic present in the water[7]. Upon decomposition the arsenic thus accumulated is mineralised and enrich the soil with the toxic element. This condition is likely to create arsenic toxicity in topsoil that might be transferred subsequently to crop/vegetables. The arsenic present in groundwater will thus be recycled to the water-soil-crop system. Huq *et al.*[7] have observed that the presence of arsenic in the algal growth medium substantially increased the arsenic in algal tissue and enrich the soil with the element upon mineralization. It is possible that this mineralised-arsenic might subsequently be taken up by the following crop.

With these views in mind, a laboratory experiment with green algae and Kalmi (*Ipomea aquatica*) plant were set up to study the bioaccumulation of arsenic by algae from arsenic-contaminated water, the mineralization of the bioaccumulated-arsenic in soil and the transfer of the mineralised arsenic to plants.

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Materials and Methods

First phase: Bioaccumulation of arsenic in algae

Seeds of green algae (*Pithopora*) of the phytophthera family were collected from the Botanical Garden of the University of Dhaka, Dhaka. A stock of the algae was cultured by growing them in a nutrient solution with composition as described earlier[7]. After two months of culture, 50 ml of the algal culture was transferred to 5 l medium containing 5 different arsenic treatments (0, 0.05, 0.5, 1.0 and 5.0 mg/l As in water). Arsenic was applied as solution of sodium meta-arsenite. There were three replications for each treatment. The algae were cultured in the arsenic containing media for a further 90 days under full sunlight. During this period, different volumes of arsenic solution at the specified concentration were added to keep the water level in the pots at 5-l level. By the end of the culture period, the treatments received a total of 0, 1.15, 11.5, 23.0 and 115.0 mg arsenic respectively for the 5 treatments. The algae were harvested by filtration and were allowed to dry in air. A portion of these algae were oven-dried and dusted by a blender for further analysis. The air-dry portion was used for the second phase of the study.

Second phase: Mineralization of arsenic in soil and its transfer to Kalmi

The soils used in the present experiment were collected from Dhamrai near Dhaka at 0-150 mm depth. The soil is a typical Haplaquent and formed on non-calcareous grey floodplain and belongs to the Dhamrai series. The soil samples were processed following standard procedure. About 2 kg-sized pots were used for the incubation study. Each pot was filled with 1 kg of air-dried soil, 2.5 g (5.5 ton/ha) of the harvested algae were mixed properly in each pot and the soils were watered with tap water. The soils remained wetted for 45 days. All experiments were replicated three times.

Growing of plant in soil

Seeds of Kalmi (*Ipomea aquatica* L) were collected from the Bangladesh Agricultural Research Institute (BARI), Ghazipur. Ten grams of Kalmi seeds were sown in each pot 5 days after the soils were wetted. Watering of the pots was made twice daily at morning and afternoon to keep the moisture level at optimum. Soil samples from each pot were collected at an interval of 15, 30 and 45 days. The soils were dried, crushed and stored for analysis. Half of the plants in the pots were sampled 30 days after emergence of seedlings and the rest were sampled after 45 days. This was done manually by uprooting the plants carefully from the pot. The plants were washed first with tap water. The roots were washed three times by immersing each time in 1 litre distilled water for 1 min to free the ions/solutes from the free space. The plants were separated into root and shoot and the fresh weight and dry weight (dried in oven at 80° ± 5°C for 48 h) were noted. The oven-dried samples were ground and passed through a 0.2 mm sieve and used for analysis.

Analysis of the samples (algae, soil and plant)

At the time of collection and before the set up of the experiment, soil and algal samples were analysed to see their initial nutrients status (Table 1). Algal and plant samples (root and leaf) were digested with concentrated HNO_3 in a block digester for 6 h at 100°C, whereas soil samples were digested with aqua-regia ($HCl:HNO_3 = 3:1$) for 4 h in a sand bath at 105° ± 5°C[8]. Arsenic was analysed by hydride generation atomic absorption spectrometric (HG-AAS) techniques[7]. Standard methods were followed for the analyses of other elements[8].

Table 1. *Some properties of initial algae and soil at the time of collection*

Property	Algae	Soil
pH	-	7.10
EC	-	0.07 μS
Sand	-	6.96%
Silt	-	49.18%
Clay	-	43.86%
Texture	-	Silty clay
Moisture Content	-	4.12%
Organic Carbon	-	0.91%
Total nitrogen	0.10%	0.12%
Total phosphorus	0.19%	0.02%
Total potassium	0.32%	0.39%
Total sulphur	0.15%	0.21%
Arsenic	ND	1.5 mg/kg
Cadmium	ND	ND
Lead	240.5 mg/kg	34.0 mg/kg
Zinc	5.22 mg/kg	2.12 mg/kg

ND, not detected

Results and Discussion

Arsenic bioaccumulation by algae

The bioaccumulation of arsenic increased gradually with the increasing arsenic concentration in water (Table 2). Algae did not show any detectable quantity of arsenic when treated without it. However, even at 0.05 mg/l arsenic treatment (total amount applied 1.15 mg arsenic) the corresponding algal accumulation was 12.17 mg/kg whereas at 1.0 mg/l and 5.0 mg/l arsenic treated water (total application 23 mg and 115 mg respectively), the algae showed an accumulation of 340.13 mg and 1,400.40 mg arsenic per kg dry matter respectively. Algae possess the ability to take up toxic heavy metals from the environment resulting in higher concentrations than that in the surrounding water[9]. Similar accumulation of arsenic by a fern has been reported by Ma *et al.*[10]. It needs to be mentioned however, that plants take up As^{3+} passively with water, but As^{5+} is actively taken up by some algae species[11]. The metal content of the algae depends on the metal concentrations in the ambient water as well as metal bioavailability[12-13]. Population densities, limiting nutrients, pH, temperature, salinity, presence of chelators, etc. are some governing factors to metal uptake by algae[9].

Table 2. *Applied total arsenic in water and arsenic content in algae in different arsenic treatment*

Arsenic treatment (mg/l)	Applied total arsenic in water (mg)	Arsenic accumulation by algae (mg/kg)
T (0.0)	0	0
T (0.05)	1.15	12.17
T (0.5)	11.5	138.1
T (1.0)	23	340.13
T (5.0)	115	1,400.40

The regression analysis between applied arsenic and arsenic uptake by algae showed that the relationship was significantly positive ($Y = 0.0121x + 0.0124$, $r = 0.9978$**).

Mineralization of arsenic in soil

The release of arsenic through mineralization of arsenic treated algae at different days of incubation is shown in Figure 1. It is clear that the algae growing on arsenic contaminated water and bioaccumulating it had subsequently released the arsenic to soil upon decomposition during the process of mineralization. The release trend however was different with days of incubation.

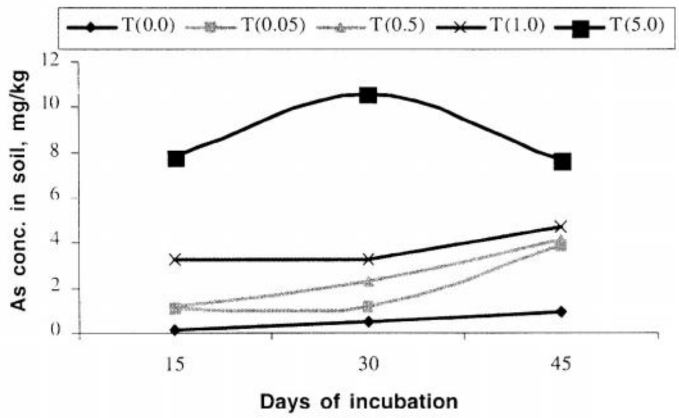

Figure 1. *Mineralization of arsenic in soil from algae treated with different arsenic concentration at different days of incubation.*

Mineralization of arsenic in soils was the maximum from algae grown in medium treated at the rate of 5.0 mg/l at any day of incubation. However, the release was at its peak at 30 days. Similar observation was made by Huq *et al.*[7] in a different occasion. The mineralization rate for any algal arsenic increased with days of incubation. It worthy to mention here that the background concentration of arsenic in the soil under study was 1.5 mg/kg. In soils receiving algal treatment the arsenic levels were much higher than this value which clearly is an indication that algae growing in arsenic contaminated environment will accumulate it and subsequently release it to the soil. A large portion of this recycled arsenic is not likely to be washed out only by flood or rain water in oxidized condition due to high affinity to iron (Fe), aluminium (Al) and other elements in soil[14]. Hence, arsenic accumulation in surface soil might attain a much more toxic level and some recent data show the top 0-15 cm soil to contain more arsenic (>58 mg/kg) than that of 15-30 cm soil, and it is high in areas where ground water contamination is reported and where irrigation is practiced[3].

The regression analysis between arsenic content in applied algae and in the soil samples of different days of incubation showed highly significant relationship (15 days, $r = 0.9849$**; 30 days, $r = 0.9769$**; 45 days, $r = 0.8677$**). The analysis of variance (ANOVA) indicated that both algal arsenic content as well as days of incubation had significant positive contribution to As release in soil (F for As in algae was 22.14 and for days of incubation it was 2.33, $LSD_{(0.95)}$ being 3.77).

Plant uptake of the mineralised arsenic

The uptake of mineralised arsenic by different plant parts (shoot and root) at 30 days and 45 days of incubation are presented in Figure 2 and 3. It is apparent that the plants growing on soil treated with algal arsenic have taken up arsenic and the uptake pattern differed with time. The total accumulation of arsenic (μg/100 plants) in the plant shoot (Figure 2) at 30 days of growth

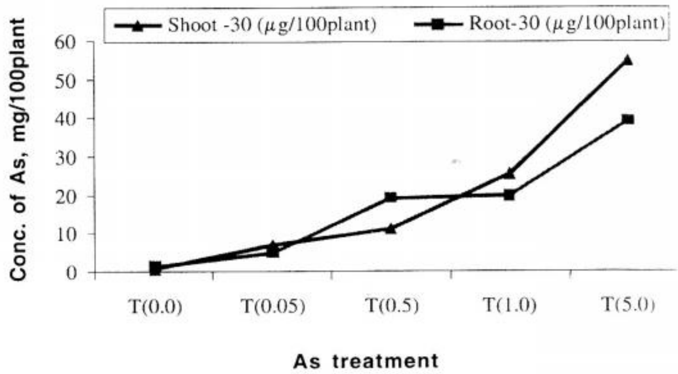

Figure 2. *Arsenic (As) accumulation in different plant parts after 30 days of growth.*

was maximum in soils at the 5.0-mg/l arsenic treatment rate. This could be related to the fact that mineralization rate was at the maximum during this period for the same treatment (Figure 1). At the 5.0 mg/l arsenic treatment the accumulation of arsenic in the roots at 30 days was also at the maximum. When the uptake by the whole plant was considered, it was observed that with increasing rates of arsenic application to the algae, the uptake also increased; this indicates that algae accumulated increased amount of arsenic from the growth media and released it to the soil on mineralization. The uptake showed an increasing trend with increasing number of growing days (Figure 3).

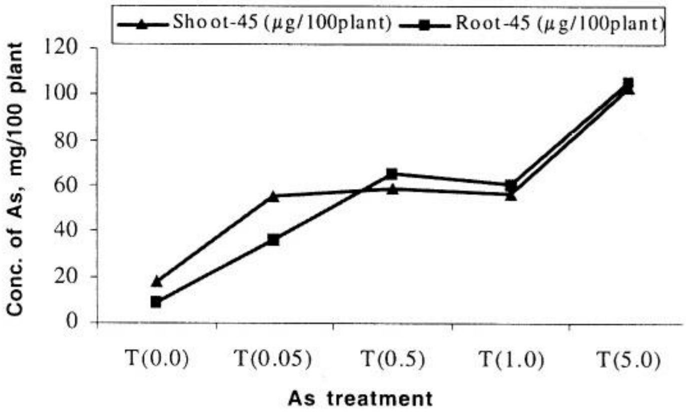

Figure 3. *Arsenic accumulation in different plant parts after 45 days of growth.*

The ANOVA indicated that both algal arsenic contents released in soil and days of incubation had significant positive contribution to arsenic uptake by plant leaf (F for shoot arsenic is 38.40 and for algal arsenic content is 13.03). The regression analysis between applied arsenic and arsenic in shoot at 30 days and 45 days showed that the relationship was highly significant (S_{30}, $r = 0.957^{**}$; S_{45}, $r = 0.7663^{**}$). Correlation coefficients were highly positive for S_{30} and S_{45} ($r = 0.91^{**}$) and R_{30} and R_{45} ($r = 0.98^{**}$).

Acknowledgement

The authors acknowledge the technical help rendered by Mr. JC Joardar and Ms. S Parvin of the Bangladesh-Australia Centre for Environmental Research (BACER-DU), University of Dhaka.

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