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Arsenic mitigation strategy for rice, using water regime management

S.M. Imamul Huq, U.K. Shila and J.C. Joardar

Abstract

The response of two varieties of rice plant (BR-28 and BR-29) to arsenic accumulation added from two sources (As^{III} and As^{V}) under two different water regimes (100 and 75% of field capacity) were examined. Treatments added to soil were 0, 10, 20 and 40 mg As/kg soil. Plant samples were collected after 120 and 140 days from seed sowing, for BR-28 and BR-29 respectively. Delayed seedling emergence; reduced plant growth; yellowing and wilting of leaves; brown necrotic spots on old leaves; and, finally, reduced grain yield of the two varieties, confirmed the symptoms of As toxicity. Arsenic accumulation by plants growing with either As^{III} or As^{V} increased with increasing As treatment, irrespective of water regimes. However, the accumulation was greater in the arsenite-treated soil than that in the arsenate-treated one, indicating the higher phytoavailability of As^{III}. Most of the As taken up by plants was sequestered in the root, followed by straw and grain. In roots of BR-28, the maximum As accumulation from arsenite-treated soil was 17.6 mg/kg dry weight (d.w.) at 100% field capacity (f.c.), whereas at 75% f.c. it was 15.4 mg/kg d.w. and for the roots of BR-29 the values were 31.04 mg/kg d.w and 22.65 mg/kg d.w. at 100 and 75% of f.c. respectively. Arsenic in straw and grain was lower for plants of both varieties at 75% of f.c. However, there have been some varietal differences in the response to As^{III} or As^{V}. The paper discusses the possible management of moisture regimes to reduce the phytoavailability of arsenic, thereby mitigating its toxicity in the rice crop.

Key words: arsenate, arsenic, arsenite, mitigation, rice, water regime

INTRODUCTION

Contamination of groundwater by arsenic in the deltaic region, particularly in the Gangetic alluvium of Bangladesh, has become one of the world's most important natural calamities (Imamul Huq and Naidu 2005). This As-contaminated groundwater is used for irrigation, leaving a risk of the accumulation of this toxic element in soil and the eventual exposure of the food chain through plant uptake and animal consumption (Imamul Huq and Naidu 2005). Most of the irrigation needs of the 30 to 40% of the net cultivable areas are met from groundwater. Presently, 75% of the total cropped area and 83% of the total irrigated area are used for rice (*Oryza sativa* L.) cultivation (Dey *et al*. 1996). The background level of As in the soils of Bangladesh has been reported to be between 4 and 8 mg/kg. In areas irrigated with As-contaminated water, the soil level can reach up to 58 mg As/kg (Imamul Huq and Naidu 2003). About 90% of the inorganic arsenic present in groundwater has been found to be in the arsenite form (Imamul Huq and Naidu 2003). Under aerobic soil conditions, arsenate dominates, whereas in submerged soil conditions the predominant species is arsenite (Masscheleyn *et al*. 1991; Marin *et al*. 1992).

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Due to systematic use of As-contaminated groundwater for irrigation in Bangladesh a substantial amount of arsenic is being accumulated in topsoil and rice plants may take it up (Imamul Huq *et al.* 2001). In Bangladesh, Boro (the dry season rice) is the major recipient of irrigation water. Rice is especially susceptible to arsenic toxicity compared to upland crops, because of an increase in both the bioavailability and toxicity of As under the reducing conditions of submerged soil in paddy fields (Horswell and Speir 2006). Several surveys of arsenic in rice grain have been reported (Imamul Huq and Naidu 2003; Meharg and Rahman 2003; USAID 2003; Lauren and Duxbury 2005). Imamul Huq and Naidu (2003) estimated the dietary load on the basis of arsenic content in Bangladesh rice grain, and according to them, about 19% of the total population are at risk of exceeding the maximum allowable daily limit (MADL) value, which is 0.2 mg/day. The calculated daily human intake of arsenic from rice has been modelled for Bangladesh by Correll *et al.* (2006). According to them, with a drinking water intake of 3L per day with 0.05 mg As/L, and a high-As-rice (0.437 mg/kg) diet, the As load would be 0.304 mg per day. This exceeds the 0.22 mg/day threshold, indicating that rice contributed to approximately 65% of the 0.22 mg/day limit. Arsenic from rice is thus an important pathway of exposure in the Bangladesh food system. Management strategies to reduce As uptake by rice are, therefore, very pertinent and urgent. This research attempted to devise remedial measures to minimize As toxicity by oxidizing arsenite as well as by reducing the entry of As into the growing rice. As such, this research was undertaken to observe the impact of manipulation of water regimes to make a more oxidized rice rhizosphere and, at the same time, using two oxidation states of As, the response of two varieties of rice, *viz.*, BRRI dhan-28 (BR-28) and BRRI dhan-29 (BR-29), and to compare the uptake of As under the prevailing conditions by the two varieties.

MATERIALS AND METHODS

Sampling site

An area where groundwater As contamination has not been reported was sought. As such, a farmer's field in Dhamrai Thana near Dhaka, Bangladesh, was selected for soil sampling (Figure 1). The georeference of the sampling spot is 23° 54.776' N and 90° 10.938' E. The soil thus selected belongs to the soil series – Dhamrai; general soil type – non-calcareous grey floodplain soil (GST No. 6); USDA soil taxonomy – Typic Endoaquepts and FAO (UNESCO Legend) – Chromi-Eutric Gley Sol.

Collection and preparation of soil samples

The bulk soil samples representing 0–15 cm depth from the surface were collected by the composite soil sampling method as suggested by the United States Department of Agriculture soil survey staff (USDA 1951). The collected soil samples were air dried, and visible roots and debris were removed and discarded. Then a portion of the larger and massive aggregates were broken by gentle crushing with a wooden hammer, and screened through a 0.5 mm stainless steel sieve. The sieved samples were then mixed thoroughly to make the composite sample. These soil samples were used for various laboratory analyses. The bulk soil samples were screened through a 2 mm sieve and used for pot experiments.

Experimental set-up

A pot experiment was carried out in the net house (a semi-controlled mesocosm where the roof and walls are of wire gauge net, the light and moisture supply can be controlled) of the Department of Soil, Water and Environment, University of Dhaka, Bangladesh. In the experiment, two water regimes, *viz.* 100% and 75% of field capacity, were maintained. Two sources of arsenic – sodium meta arsenite ($NaAsO_2$) for As (III), and hydrated sodium meta arsenate ($Na_2HAsO_4.7H_2O$) for As (V) were used. Clay pots of 5 L sizes were used. The pots were filled with 5 kg of soil, and arsenic (both III and V), at concentrations of 0, 10, 20 and 40 mg/kg soil, was mixed with the soils before one week from sowing of the seeds. The field capacity of the soil was found to be 37.81%. To maintain the water regimes, the pots were measured on alternate days and the required amount of water was added. All experiments were done in triplicate. The pots were arranged randomly in the net house. The two varieties of rice seeds (BR-28 and BR-29) were collected from the Bangladesh Rice Research Institute (BRRI). The background level of As in the soil was 1.6 mg/kg. This was taken as the control. The nutritional (N, P and K) requirement was calcu-

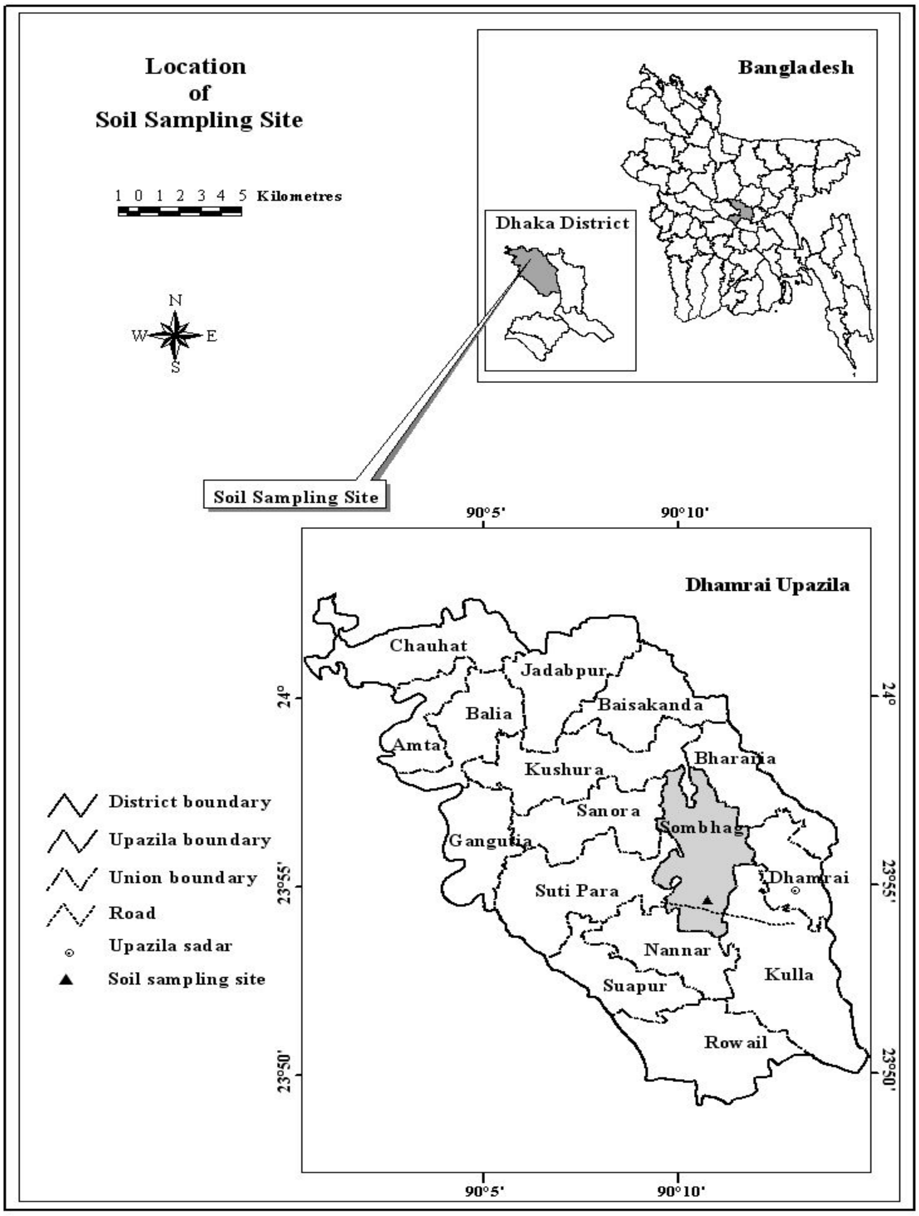


Figure 1. Map showing the location of the sampling site

lated on the basis of 'Soil Test Value Interpretation' as recommended by the Bangladesh Agriculture Research Council (BARC 1997). According to this recommendation the required amounts of N, P and K for the given soil were met from the fertilizer source urea, TSP and MP respectively. All of the TSP and MP and 1/3 of the urea fertilizer were applied at the time of soil preparation.

Method of rice cultivation

Seeds of BR-28 and BR-29 were dipped in water and kept overnight, and were then kept for two to three days in dark conditions for germination. The germinated seeds were sown directly in pots and were allowed to grow. The pots were thinned to three plants after the seedlings were established. Of the remaining 2/3 of urea fertilizer, 1/3 was applied after 35 days from seed sowing, and the final 1/3 was applied during the panicle initiation stage of the rice plants. Various cultural operations were made whenever necessary. Weeds were removed manually. Different plant agronomic characteristics were observed during the growth period.

Collection of plant samples

Plants of BR-28 and BR-29 were harvested after 120 days and 140 days from germination, respectively. The plants were harvested by manual uprooting. The grains of the two varieties were collected two days before the harvest. The harvested roots were washed with tap water to dislodge the adhering soil, and then several times with deionized distilled water to remove solutes from the ion free space. The aerial portions of the plants were also washed. The plant samples were separated into root, straw and grain. The collected plant samples were first air dried and then oven dried at 70° ±5°C for 48 hours. The dried plant samples were then ground, and were sifted through a 0.2 mm sieve. After harvest, soil samples from each pot were also collected from the rhizosphere, and these soil samples were prepared following the procedures as described earlier.

Laboratory analysis

Various physical, chemical and physiochemical properties of the soils were determined following procedures described in Imamul Huq and Alam (2005). Both plants and soil were analysed for total arsenic by hydride generation atomic absorption spectrometry (HG-AAS). The arsenic from the plant samples was extracted with HNO_3, and from the soil with aqua regia solution (Portman and Riley 1964). Certified reference materials were used throughout the digestion and analysed 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 ten samples was accompanied by reference standard samples to ensure strict QA/QC procedures.

The experimental data were statistically analysed by using the common statistical software MINITAB 13.0. The amount of As uptake (mg/100 plants) by different plant parts and the plant as a whole were calculated. The uptake was calculated using the As concentration in the dry matter and the dry weight of plant parts, and the result was expressed as mg/100 plants.

Uptake (As) = Concentration (As) in dry matter × dry weight of plant part

RESULTS AND DISCUSSION

Some characteristics of the soil

Some important basic properties of the selected soil are presented in Table 1.

Table 1. Some basic properties of the selected soil

Soil properties	Value
Particle size analysis	
Sand	4.58%
Silt	67.93%
Clay	27.49%
Textural class	Silty clay
pH	6.40
EC	0.07 µs
Organic matter	1.50%
Field capacity	37.81%
Moisture content	26.39%
Total N	0.15%
Available N	50.10 mg/kg
Total P	0.05%
Available P	0.10 mg/kg
Total K	7.95 meq/100 g soil
Available K	0.01 meq/100 g soil
Arsenic	1.60 mg/kg
Total S	0.19%
Available S	24.00 mg/kg

Agronomic parameters

Symptoms of any abnormality in the rice plants were noted during the experiment in order to assess the phytotoxicity of As. Both varieties showed severe symptoms of toxic effects at higher As concentrations and the symptoms became more pronounced with time of exposure of the plants to arsenic stress. The symptoms were: delayed seedling emergence; reduced plant growth; yellowing and wilting of leaves; and, finally, reduced grain yield. Brown necrotic spots were also observed on old leaves of the plants growing at 20 and 40 mg/kg As treatment in both the varieties. Red-brown necrotic spots on old leaves, tips and margins of rice, due to arsenic toxicity, have also been reported (Aller *et al.* 1990; Marin *et al.* 1992). Plant heights (BR-28 and BR-29) were measured from time to time and finally at maturity. At the initial stage of growth, plant height did not differ from the control plants. But at maturity, plant heights decreased with increasing arsenic treatment. Plants of both the rice varieties grown on 40 mg As/kg-treated soil under the two moisture regimes (100% and 75% of field capacity) showed the minimum plant height. This decreasing trend of plant height with increasing As treatment was not statistically significant for any of the variables. It was clear that arsenic did not readily cause plant height reduction, but the progressive accumulation of arsenic in plants with time of exposure caused the plant height reduction. A reduction in plant height with increasing As concentration has also been reported in rice plants (Yamare 1989; Barrachina *et al.* 1995; Islam 1999). Fresh as well as dry matter production of the two varieties decreased with increasing As treatment under both moisture regimes. Maximum weights were noted for the control plants, whereas the minimum values were for plants growing at 40 mg/kg As treatment. No differences in either fresh weight or dry weight were observed which were due to varietal difference or differences in the moisture regimes. The grain yield (unhusked) decreased remarkably as a result of higher As doses. A yield reduction of more than 40 and 60%, for BR-28 and Iratom-24 respectively, in As-treated soil has been reported (Hossain *et al.* 2005). BR-28 growing on arsenite-treated soil showed a significant grain yield difference for both the moisture regimes, again indicating the greater phytotoxicity of As^{III}.

Arsenic accumulation

Arsenic concentrations in different parts (root, straw and grain) of the rice plants of the two varieties are presented in Figures 2 and 3. It is important to note that in control plants of BR-29 there were some As accumulation, and perhaps that could be due to the presence of the background As (1.6 mg/kg) in soil.

100% field capacity

Root

Arsenic concentration in roots of both the varieties increased with increasing As treatment. Arsenite salt contributed more towards As accumulation in the two varieties. At 40 mg As/kg soil treatment, the concentration of As was 17.6 mg/kg d.w. for As^{III} treatment, while the value was 13.4 mg/kg d.w. for As^{V} treatment in the roots of BR-28 (Figure 2). However, the maximum values were found to be 31.04 mg/kg d.w. and 20.59 mg/kg d.w. in roots of BR-29 after the same treatment from As^{III} and As^{V} sources respectively (Figure 3). However, arsenic concentration in the roots of BR-28, either from As^{III} or As^{V}, was not significant. On the other hand, arsenic concentration in roots of BR-29 from both sources was significant ($R^2 = 98.1$, $p = 0.009$ and $R^2 = 99.0$, $p = 0.005$ for As^{III} and As^{V} sources respectively). In general, the roots accumulated higher As than the other parts. However, the As concentration in the roots of BR-29 was higher than in those of BR-28 for both As sources.

Straw

Arsenic concentration in straw followed the same pattern as for roots. The magnitude of the increasing trend varied considerably between the varieties. The maximum As concentration (7.41 mg/kg d.w.) was found in straw of BR-28 treated with 40 mg As/kg soil from As^{III}, while from As^{V} it was 5.08 mg/kg d.w. (Figure 2). This concentration was found to be significant ($R^2 =$ 95.3, $p = 0.024$) for As^{III}, but insignificant for As^{V}. The maximum arsenic concentration (4.86 mg/kg d.w.) in straw of BR-29 was also for As^{III}-treated soil (Figure 3). The corresponding value for As was 4.25 mg/kg d.w. in As^{V}-treated soil. The accumulation did not differ significantly for the two sources in this variety. As accumulation in straw was higher for As^{III}-treated soil in both the varieties.

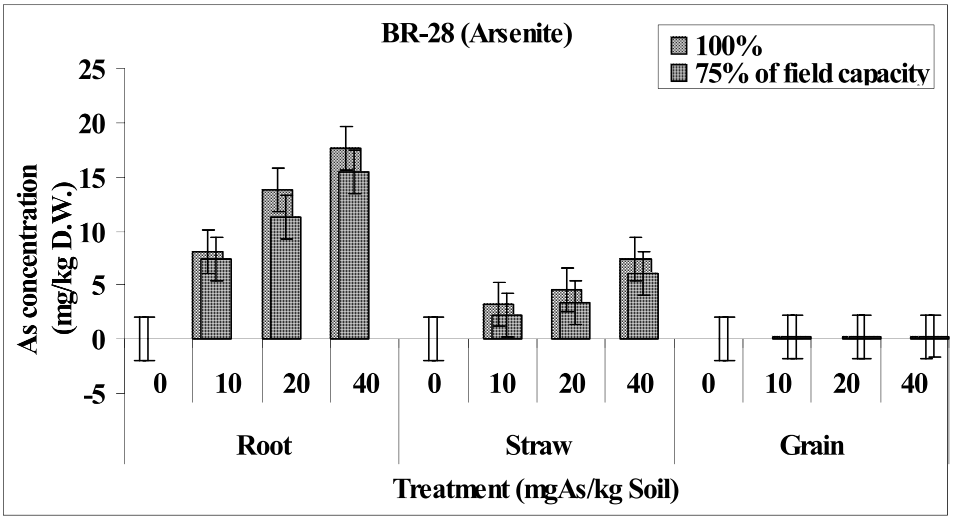
Grain

Arsenic accumulations in rice grain were similar for the two sources of arsenic, though there was a variation between the varieties. The maximum value was found for BR-29, and the concentration increased with increasing As treatment. However, in none of the varieties did the grain As concentration exceed the maximum permissible limit of 1.0 mg of As/kg (National Food Authority 1993). This Australian standard is taken as a reference, as no standard has yet been adopted in Bangladesh. Arsenic accumulation in grain was not significant for any of the variables.

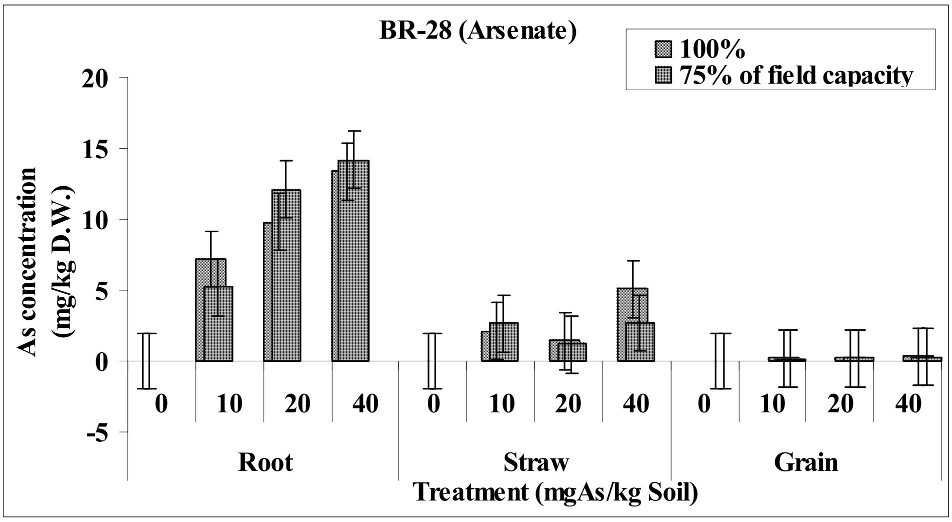
75% field capacity

Root

Under this moisture regime too, arsenic concentration in the roots of both the varieties increased with increasing As treatment. However, the values were relatively lower than what was observed under a 100% moisture regime. The maximum As concentration in roots of BR-28 was 15.4 mg/kg d.w. for As^{III}, and 14.2 mg/kg d.w. for As^{V} at 40 mg As/kg soil (Figure 2). Arsenic concentration in roots was significant ($R^2 = 90.6$, $p =$



(a)



(b)

Figure 2. Arsenic concentration in different parts of BR-28 under the two moisture regimes as affected by (a) arsenite (As^{III}); (b) arsenate (As^{V})

0.048) for As^{III} only. Similarly, the roots of BR-29 accumulated the most As from the As^{III} soil; the value being 22.65 mg/kg d.w. (Figure 3a). In As^{V}-treated soil the value was 22.36 mg/kg d.w. (Figure 3b). For the latter variety, arsenic accumulation in roots was significant for both As^{III} ($R^2 = 98.9, p = 0.006$) and As^{V} ($R^2 = 98.3, p = 0.008$). Of the two rice varieties, BR-29 accumulated more As in its roots from both As^{III} and As^{V} sources. In general, as with the 100% moisture regime, the roots of both BR-28 and BR-29 accumulated the maximum amounts of As.

Straw

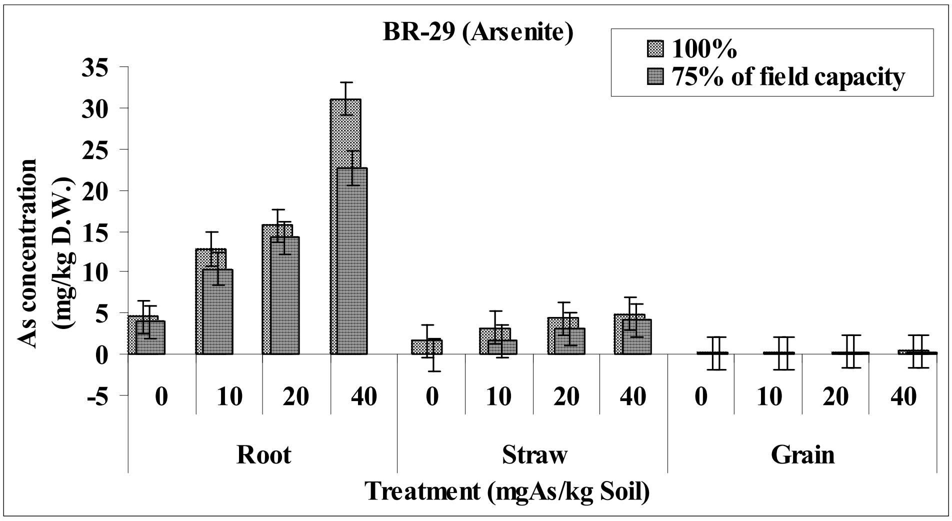
The As concentration in straw of both the varieties increased with increasing As in the growth medium. The magnitude of the increasing trend varied considerably between the varieties. The maximum As concentration (6.01 mg/kg d.w.) was found in BR-28 treated with As^{III}, while the value was 2.69 mg/kg d.w. for As^{V} (Figure 2). A similar trend was also observed for BR-29 (Figure 3). The As^{III}-treated plants accumulated 4.15 mg As/kg d.w., while for As^{V} plants it was 3.58 mg/kg d.w. Arsenic accumulation in the straw of BR-29 was only significant ($R^2 = 92.1, p = 0.04$) for As^{III}. Of the two rice varieties, BR-28 accumulated more As from As^{III}, while BR-29 showed a preference towards As^{V}.

Grain

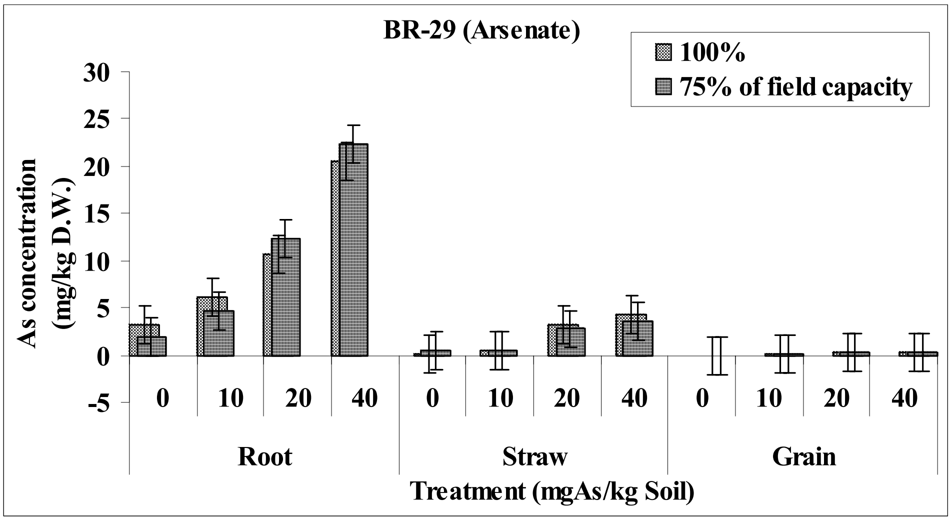
The arsenic concentrations in grain were similar, irrespective of the variety and source of arsenic. The maximum value was found for 40 mg As/kg-treated soil. The grain As concentration did not exceed the maximum permissible limit of 1.0 mg of As/kg – similar to what was observed for the moisture regime at 100% of field capacity. Nor did they vary significantly in their As concentrations.

Comparison among the plant parts

Maximum As accumulation was observed in roots, followed by straw and grain. Similar observations have been reported (Marin *et al.* 1992; Xie and Huang 1998). Abedin *et al.* (2002) showed the rice tissue As concentration in the order: root > straw > husk > grain. Roots of BR-29 accumulated more As than BR-28 under both moisture regimes, and more from As^{III} than As^{V}. This means that, in BR-29, As concentrated more



(a)



(b)

Figure 3. Arsenic concentration in different parts of BR-29 under the two moisture regimes, as affected by (a) arsenite (As^{III}); (b) arsenate (As^{V})

in the roots and translocated less to the upper parts of the plant, whereas in BR-28, As was translocated to the upper parts of the plants, giving rise to a greater risk of As ingestion. On the other hand, although As in straw was less translocated, grains of BR-29 accumulated more As than BR-28. Transfer factor values (greater than 0.1) indicated that the rice plant has a strong affinity with As accumulation (Farrago and Mehra 1992) for all treatments. Transfer factor values also showed greater affinity for As^{III} than for As^{V}, irrespective of the variety.

Comparison between two arsenic sources
Arsenic accumulation in all parts of both varieties was higher for As^{III} than for As^{V} under either moisture regime (100% and 75% of field capacity). However, there was no significant difference in arsenic accumulation between the two arsenic sources except for the rice variety BR-29 under the moisture regime of 100% field capacity (t = 3.12, p = 0.053 and t = 3.27, p = 0.047 for root and straw respectively).

Comparison between the two varieties
Arsenic accumulation varied between the different plant parts of the two varieties, but there was no significant difference in As accumulation between the two varieties except for roots at 100% of field capacity (t = 3.27, p = 0.047) for BR-29. Roots of the rice variety BR-29 concentrated more As than BR-28, resulting in less in the straw, whereas in grain the As concentration was higher for BR-29 than BR-28 under both moisture regimes, irrespective of the source of the arsenic.

Comparison between the two moisture regimes
In both varieties, As accumulation was found to be reduced at 75% of field capacity. Reducing moisture did not cause any significant yield difference, though As accumulation in any parts of the two varieties could be reduced.

Arsenic uptake

It was found that As uptake increased with increasing As treatment in both the As (As^{III} and As^{V}) treated plants under both moisture regimes irrespective of variety. However, As uptake was higher in the As^{III}-treated plants than in As^{V}. It was clear that in all parts of both varieties, As uptake was higher at 100% field capacity. Arsenic uptake by the whole plants was significant only in As^{III} plants at 100% field capacity (R^2 = 98.1, p = 0.01) for BR-28. Thus, it appeared that BR-28 is more susceptible to As accumulation than BR-29. Moreover, it became apparent that reducing the moisture level could substantially abate As accumulation in rice plants, thereby helping to reduce its entry into the food chain to some extent.

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