Chapter 16
A Greenhouse Pot Experiment to Study Arsenic Accumulation in Rice Varieties Selected from Gangetic Bengal, India

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16.1 Introduction

It is predicted that around 100 million people living in the Ganga-Meghna-Brahmaputra plain are at the risk of serious arsenic toxicity through exposure of contaminated groundwater (Chakraborti et al. 2008). Groundwater arsenic contamination in the Gangetic Bengal has been termed as the largest mass poisoning in the history of human kind (Smith et al. 2000). Arsenic pollution has spread in fourteen out of total nineteen districts of Gangetic Bengal (Chakraborti et al. 2009). Application of arsenic-contaminated groundwater for irrigation in Gangetic Bengal has shown to influence accumulation of arsenic in rice, the major staple food in West Bengal (Meharg 2004; Signes-Pastor et al. 2008; Meharg et al. 2009; Bhattacharya et al. 2010a; Samal et al. 2011; Banerjee et al. 2013; Santra et al. 2013). Rice is an efficient accumulator of arsenic than any other cereal crops (Su et al. 2010) and consumption of rice has been termed as an important source of inorganic arsenic intake to human body (Meharg et al. 2009).

Greenhouse pot experiments conducted with Bangladeshi rice varieties have showed significant differences in the accumulation of arsenic (Azad et al. 2009, 2013; Norton et al. 2009). Delowar et al. (2005) reported that the accumulation of arsenic in rice grain was in the range 0–0.14 mg kg^{-1} which was cultivated with 0–20 mg l^{-1} of arsenic containing water. Analyzing two widely cultivated rice varieties

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in Bangladesh, Rahman et al. (2007) reported that the BRRI *dhan* 28 and BRRI hybrid *dhan* 1 had difference in the amount of arsenic accumulation (0.5 ± 0.0 and 0.6 ± 0.2 mg kg^{-1} dry weight of arsenic, respectively). Rahman et al. (2008) by studying five different hybrid as well as non-hybrid rice samples concluded that the arsenic translocation from root to shoot (straw) and husk was higher in the hybrid variety (BRRI hybrid *dhan* 1) as compared to those of non-hybrid varieties (BRRI *dhan* 28, BRRI *dhan* 29, BRRI *dhan* 35 and BRRI *dhan* 36). Azad et al. (2009) observed an increase in the grain arsenic uptake of transplanted aman rice with the increase of arsenic treatment in soil. Abedin et al. (2002b) found that 30–50 mg kg^{-1} arsenic containing soil produced rice grains with arsenic levels exceeding the WHO recommended permissible limit of 1 mg kg^{-1}. In our previously conducted study on some other rice varieties, all the studied high yielding and hybrid varieties (*Ratna*, IET 4094, IR 50 and *Gangakaveri*) were found to be higher accumulator of arsenic as compared to all but one local rice variety, *Kerala Sundari* (Bhattacharya et al. 2001). Azad et al. (2013) have recently reported the accumulation of arsenic in the range 0.06–0.47 mg kg^{-1} through a greenhouse pot experiment conducted in Bangladesh.

Thus, a greenhouse pot experiment was conducted to investigate the accumulation and distribution of arsenic in the different fractions of rice plant with increasing soil arsenic treatments (5, 10, 20 and 30 mg kg^{-1} dry weights) on six selected rice varieties (four high yielding varieties MTU 7029, IET 5656, MTU 1010 and CNHR 3, and two local varieties *Nayanmani* and *Danaguri*). The major objective of the present study was to identify the rice varieties that are resistant to arsenic phytotoxicity. The findings would have significant impacts on agriculture and public health of arsenic-contaminated 14 districts of Gangetic Bengal.

16.2 Materials and Methods

16.2.1 Experimental Condition

The pot culture experiment on different rice (*Oryza sativa* L.) varieties was carried out in a greenhouse at the Department of Environmental Science, University of Kalyani. The experimental site was selected on the basis of having good sunshine throughout the day. Although the experiment was conducted in a greenhouse, the environmental conditions inside the greenhouse were not controlled. The greenhouse was only used to protect the experiment from natural calamities (such as heavy rainfall, northwester wind, etc.) and disturbances by animals.

16.2.2 Soil Collection and Pot Preparation

Soil was collected from the campus of University of Kalyani at a depth of 0–15 cm. The physico-chemical properties of soils used for pot experiments are given in Table 16.1. Initial arsenic content of the soils prior to treatment was 2.3 ± 0.07 mg kg^{-1}

Table 16.1 Physico-chemical properties of experimental pot soil

Soil parameters	Range
Clay (%)	66–69
Sand (%)	9–15
Silt (%)	17–24
Texture	Clay loam
pH	7.8 ± 0.18
Organic carbon (%)	0.92 ± 0.13
Total nitrogen (%)	0.14 ± 0.02
Available phosphorous (mg kg^{-1})	14 ± 1
Total arsenic (mg kg^{-1})	2.3 ± 0.07

dry weights. After collection, the soil was air dried for 7 days and aggregates were broken by gentle crushing. The materials such as dry roots, grasses, stones and plastics were removed and the soil was thoroughly mixed to homogenize. Earthen pots (40 × 40 cm) were used for rice cultivation. The pots were designed to prevent the loss of water soluble arsenic from pots (Rahman et al. 2007). About 10 kg of soil was taken in total 90 pots comprising four different arsenic treatments (5, 10, 20 and 30 mg kg^{-1} dry weights) along with one control treatment (no arsenic dosing), each with three replications for the six different rice plant varieties. The arsenic was applied in the form of sodium arsenate (Na_2HAsO_4), which can easily convert to arsenite under reducing and submerged condition of paddy soil (Abedin et al. 2002a). Chemical fertilizers or nutritional solutions were not added to pot soil.

The tap water, used for irrigation, contained arsenic below the detection limit (<0.03 μg l^{-1}). Thus, there was no chance of arsenic input from the tap water to the pot soil. After the application of arsenic, soils were left in the pots for 2 days without irrigation. Then tap water was used to irrigate the pots to make the soil clay suitable for rice seedling transplantation. About 3–4 cm water level above the soil surface was maintained in the pots before and after seedling transplantation. The water level was maintained in each pot throughout the growth period. Irrigation was stopped before 10 days of harvest (Azad et al. 2009).

16.2.3 Selection of Rice Varieties and Seedling Transplantation

Four high yielding rice varieties MTU 7029 (*Swarna*), IET 5656, MTU 1010 and CNHR 3 and two local varieties *Nayanmani* and *Danaguri* were selected through germination test for this greenhouse pot experiment. Rice seedlings of 21 days old were carefully uprooted from nursery-bed and transplanted to pots under flooded condition. Eight seedlings, six inches apart from each other, were transplanted to each pot. The seedlings, which died within 7 days of transplantation, were discarded and replaced by new seedlings.

16.2.4 Sample Collection, Preservation and Digestion

The full-grown rice plants were carefully uprooted at their maturity (90–120 days after transplantation). Then the collected samples were separated into different parts and washed thoroughly with arsenic-free water to remove soil and other contaminants, followed by rinsing with de-ionized water with continuous shaking for several minutes. Finally, the samples were dried in the hot air oven at 60 °C for 72 h and stored in airtight polyethylene bags at room temperature with proper labeling. Proper care was taken at each step to minimize any contamination.

The samples were digested following the heating block digestion procedure (Rahman et al. 2007), diluted to 25 ml with de-ionized water and filtered through Whatman No. 41 filter papers and finally stored in polyethylene bottles. Prior to sample digestion all glass apparatus were washed with 2 % HNO_3 followed by rinsing with de-ionized water and drying.

16.2.5 Analysis of Total Arsenic

The total arsenic was analyzed by flow injection hydride generation atomic absorption spectrometer (FI-HG-AAS, Perkin Elmer AAnalyst 400) using external calibration (Welsch et al. 1990). The optimum HCl concentration was 10 % (v/v) and 0.4 % $NaBH_4$ (Merck, Germany; synthesis grade; 96 %) produced the maximum sensitivity. For each sample three replicates were taken and the mean values were obtained on the basis of calculation of those three replicates. Standard Reference Material (SRM) from National Institute of Standards and Technology (NIST), USA was analyzed in the same procedure at the start, during and at the end of the measurements to ensure continued accuracy. The observed arsenic concentrations of SRM Rice Flour (1568A) showed >97 % recovery.

16.3 Results and Discussion

The experimental soil belonged to clay loam type (Table 16.1). The soil was found to be slightly basic in nature (pH 7.8 ± 0.18) and with 2.3 ± 0.07 mg kg^{-1} initial arsenic content. The background value of arsenic in the non-irrigated soils of the study area was reported to be 2.3–3.1 mg kg^{-1} dry weights (Bhattacharya et al. 2010b).

The impact of soil arsenic treatments on accumulation of arsenic in different fractions of the six selected rice varieties are shown in Fig. 16.1. The uptake of arsenic in rice plants was observed to vary with the different local and high yielding rice varieties. This finding is concurrent with the earlier observations by Delowar et al. (2005), Williams et al. (2006) and Bhattacharya et al. (2013). With gradual increase in concentrations of arsenic treatments in pot soil, the accumulation of

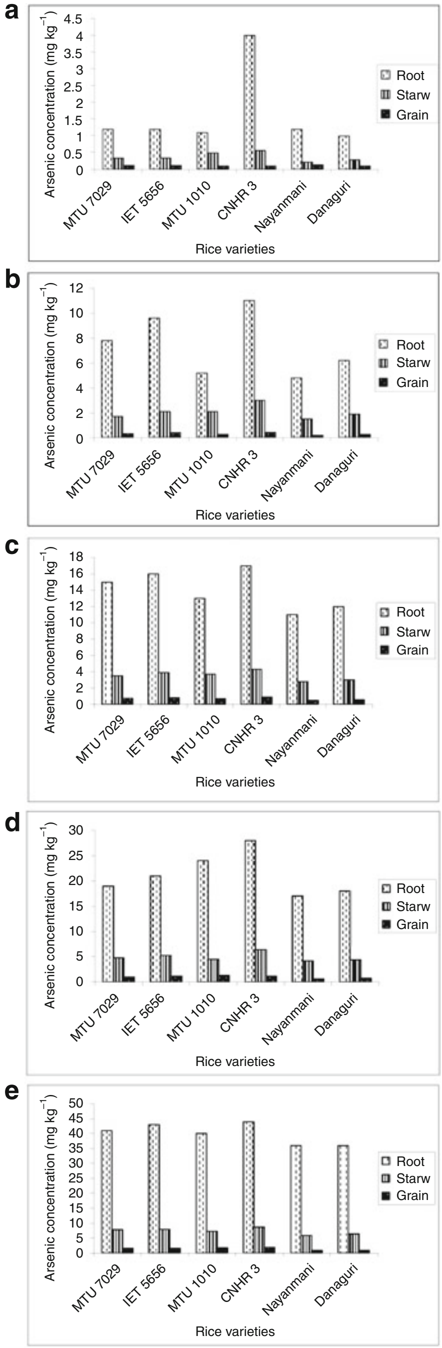

Fig. 16.1 Effect of soil arsenic treatments in pot soil [(**a**) control, (**b**) 5, (**c**) 10, (**d**) 20 and (**e**) 30 mg kg^{-1}] on arsenic accumulation in various parts of the six selected rice (*O. sativa* L.) varieties of Gangetic West Bengal, India

arsenic in different fractions of rice plant was found to increase at dissimilar rates in different rice plant varieties. It is also evident from the results that arsenic accumulated predominantly in root of the rice plant, irrespective of its variety. Iron plaques are commonly formed on the root surfaces of aquatic plants including rice by releasing oxygen to their rhizosphere through aerenchyma. This results in the oxidation of ferrous iron to ferric iron and the precipitation of iron oxides on the root surfaces (Armstrong 1964). Composition of iron oxides were later reported to be dominantly of ferrihydrite (63 %), followed by goethite (32 %) and siderite (5 %) (Hansel et al. 2001). All these precipitated iron oxides have strong adsorptive capacity for arsenate. According to Liu et al. (2004a), the formation of iron plaques around root surfaces of the rice plant has a significant influence on binding arsenic and reducing its translocation to the above ground tissues (straw, husk and grain) of the plant. The presence of iron plaque was found to sequester arsenic and form a buffer zone that alters the entry of arsenic into plants (Liu et al. 2004b). For example at 10 mg kg^{-1} arsenic dosing in pot soil the accumulation of arsenic in root was in the range 11 ± 1.2–017 ± 3.1 mg kg^{-1} dry weights. It was followed by the accumulation in the straw (2.8 ± 0.52–4.3 ± 0.85 mg kg^{-1} dry weight of arsenic) and grain (0.48 ± 0.15–0.90 ± 0.15 mg kg^{-1} dry weight of arsenic) parts of rice plant. The decreasing trend of accumulation of arsenic in rice plant parts (root > straw > grain) as detected in the present study is in good agreement with the previous findings by Rahman et al. (2007) and Bhattacharya et al. (2010a, 2013).

The results clearly show that rice straw is a moderate accumulator of arsenic. The rate of arsenic accumulation in rice straw was noticed to be concurrent with increasing soil arsenic treatments (Fig. 16.1). Previously, a significant correlation ($r = 0.961$) had been observed by us between average arsenic contents in straw part of different rice varieties and arsenic doses in pot soil (Bhattacharya et al. 2013). In rural West Bengal, rice straw is the most favoured and economical food given to cattle. Thus, accumulation of arsenic in rice straw induces additional risk of arsenic entry to human through cattle milk (Ulman et al. 1998; Datta et al. 2010) and meat (Rana et al. 2012; Bundschuh et al. 2012). Much higher arsenic accumulation ability in rice straw by hybrid rice varieties as compared to non-hybrid varieties had been also reported by Abedin et al. (2002a); Rahman et al. (2007).

The average arsenic concentration in the paddy field soil of West Bengal was reported to be just below 10 mg kg^{-1}, the global average arsenic level in agricultural soil (Das et al. 2002; Bhattacharya et al. 2010a, b; Samal et al. 2011). The comparison of arsenic accumulation in grain of the six rice varieties in the present study at 10 mg kg^{-1} arsenic dosing showed that CNHR 3, a high yielding rice variety was the highest accumulator of arsenic (0.90 ± 0.15 mg kg^{-1} dry weight) while *Nayanmani*, a local rice variety was the lowest accumulator (0.48 ± 0.15 mg kg^{-1} dry weight). At 10 mg kg^{-1} of arsenic dosing in pot soil the accumulation of arsenic in rice grain in any of the studied sample did not exceed 1 mg kg^{-1} (WHO permissible limit). But, with the increasing concentration of arsenic added to the pot soil, the accumulation of arsenic in rice grain was found to increase, but at dissimilar rate (Fig. 16.1). At the maximum level of arsenic dosing in pot soil (30 mg kg^{-1}), comparison of arsenic accumulation in grain of the different rice varieties showed that CNHR 3 still remains as the highest accumulator of arsenic (1.9 ± 0.53 mg kg^{-1} dry weight) as compared to the *Nayanmani* rice variety with accumulation as low as 0.84 ± 0.18 mg kg^{-1} dry weight of arsenic. Figure 16.1 shows that the high yielding rice varieties (CNHR 3, MTU 1010, MTU 7029 and IET 5656) are on an average higher accumulator of arsenic as compared to the studied two local rice varieties, *Nayanmani* and *Danaguri*. Uptake of arsenic upto 2 mg kg^{-1} by an *Aman* rice variety had been reported by Huq et al. (2011). Table 16.2 describes the comparison among the previous works on the accumulation of arsenic in rice grain using a greenhouse pot experiment with the present findings.

Apart from *Nayanmani* and *Danaguri* the accumulation of arsenic in rice grain was found to exceed the WHO recommended permissible limit in rice (1 mg kg^{-1}) at 20 mg kg^{-1} arsenic dosing in pot soil, which is very much close to the reported highest content of arsenic (19.4 mg kg^{-1}) in soil of West Bengal (Roychowdhury et al. 2005). This surpassing of the 1 mg kg^{-1} limit by the four out of six studied rice varieties at the 20 mg kg^{-1} arsenic dosing is considerably alarming. The arsenic content of the paddy field soil of West Bengal and that of irrigation water was previously accounted to be significantly correlated (Bhattacharya et al. 2010b). Thus, an eminent possibility of increase of arsenic concentration in the paddy field soils of the entire arsenic-contaminated areas of West Bengal can be hypothesized from the present study. Moreover, if the situation is not immediately taken care of, it can be predicted that consumption of arsenic-contaminated rice may become the potent route for arsenic entry into human body along with the drinking water pathway.

Table 16.2 Accumulations of arsenic in rice grain in the present study compared with that reported from other green house pot experiments

Arsenic in soil (mg kg^{-1})	Arsenic in rice grain (mg kg^{-1})	Reference
Control-8	0.15–0.42	Abedin et al. (2002a)
0–20	0–0.14	Delowar et al. (2005)
Control-30	0.24–0.75	Rahman et al. (2007)
6.4–80	0.2–0.3	Rahman et al. (2008)
0–50	0.69–1.6	Azad et al. (2009)
0–20	0.22–0.81	Khan et al. (2010)
Control-0.5 mg l^{-1} (in irrigation water)	0.01–2	Huq et al. (2011)
1.9–40	0–2.6	Bhattacharya et al. (2013)
3.7–14.6	0.06–0.47	Azad et al. (2013)
2.3–30	0.1–1.9	Present study

16.4 Conclusions

The potentiality of arsenic contamination in groundwater of Gangetic Bengal is increasing day by day and enhancing the human health risk from arsenic toxicity via water-soil-plant-human pathway. Arsenic was found to accumulate in the range 0.10–1.9 mg kg^{-1} dry weight in rice grain with 2.3–30 mg kg^{-1} dry weight arsenic treatment in soil. Thus, prompt management strategy needs to be taken by the Government in encouraging cultivation of less arsenic accumulating rice varieties (e.g., *Nayanmani* and *Danaguri*) in arsenic-contaminated areas of West Bengal. Along with it, rice varieties that require huge irrigation water are found to accumulate higher amount of arsenic (e.g., CNHR 3, MTU 1010, MTU 7029 and IET 5656) which should be avoided. More emphasis is to be given for cultivation of crops accumulating very low amount of arsenic. This will support the economy of the farmers and also reduce the potential entry of arsenic into human food chain.

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