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In vitro assessment on the impact of soil arsenic in the eight rice varieties of West Bengal, India

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ABSTRACT

Rice is an efficient accumulator of arsenic and thus irrigation with arsenic-contaminated groundwater and soil may induce human health hazard via water–soil–plant–human pathway. A greenhouse pot experiment was conducted on three high yielding, one hybrid and four local rice varieties to investigate the uptake, distribution and phytotoxicity of arsenic in rice plant. 5, 10, 20, 30 and 40 mg kg^{-1} dry weights arsenic dosing was applied in pot soil and the results were compared with the control samples. 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*. In these five rice varieties accumulation of arsenic in grain exceeded the WHO permissible limit (1.0 mg kg^{-1}) at 20 mg kg^{-1} arsenic dosing. Irrespective of variety, arsenic accumulation in different parts of rice plant was found to increase with increasing arsenic doses, but not at the same rate. A consistent negative correlation was established between soil arsenic and chlorophyll contents while carbohydrate accumulation depicted consistent positive correlation with increasing arsenic toxicity in rice plant.

1. Introduction

Arsenic, a human toxin, is affecting more than 150 million people worldwide [1] and can be found in all living organisms, as well as in the air, soil and water. Human uptake of arsenic mainly occurs via the food chain (drinking water and dietary sources) and occupational exposure [2,3]. The epidemiological studies show that the chronic arsenic poisoning can cause serious health effects including cancers, melanosis (hyper pigmentation or dark spots and hypo pigmentation or white spots), hyperkeratosis (skin hardening), restrictive lung disease, peripheral vascular disease (black foot disease), gangrene, diabetes mellitus, hypertension and ischemic heart disease [4–7]. It is now well recognized that consumption of arsenic, even at low levels, leads to carcinogenesis [8].

The uptake of arsenic by agricultural plants is a function of availability of arsenic (content, water requirement, soil properties) as well as physiological properties [9,10]. Several crop plant species (rice, elephant foot yam, green gram, arum, amaranth, radish, lady's finger, cauliflower and Brinjal) were reported to accumulate arsenic in substantial quantities [11–18]. Rice is a more efficient accumulator of arsenic than any other cereal crops [19] and consumption of rice has been termed as an important source of inorganic arsenic intake to human body [13]. Some works had been previously done to analyze the effect of arsenic on different varieties of rice plant [11,20–22], but there was no specific work on the genotype variation of rice cultivated in West Bengal. Delowar et al. [23] reported that the accumulation of arsenic in rice grain was in the range 0.0–0.14 mg kg^{-1} which was cultivated with 0.0–20.0 mg l^{-1} of arsenic-contaminated water. In greenhouse pot experiments with higher concentrations of arsenic in soil, different rice varieties have showed significant differences in the accumulations of arsenic in straw, husk and grain parts [11,20]. Analyzing the two widely cultivated rice varieties in Bangladesh, Rahman et al. [11] 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. [21] 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). Presence of stable genetic differences in arsenic accumulation by rice plant was suggested by Norton et al. [22]. They also identified a number of local cultivars with low arsenic accumulation in grain. Azad et al. [24] observed an increase in the grain arsenic uptake of transplanted *Aman* rice with the increase of arsenic treatment in soil

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and found that 30–50 mg kg^{-1} arsenic containing soil produced rice grains with arsenic levels exceeding the WHO recommended permissible limit of 1.0 mg kg^{-1}. In our previous study, relatively higher translocation (0.194–0.393) of arsenic was observed in the Red *Minikit* (a high yielding rice variety) as compared to the translocation (0.099–0.161) by a local rice variety, *Megi* [14].

Arsenic-contaminated groundwater is a severe problem in West Bengal, India [25]. Nine out of total nineteen districts of West Bengal has groundwater arsenic contamination [14,15,26]. In the rural areas of West Bengal arsenic-contaminated groundwater is not only used for drinking purpose, but also employed for irrigation of various crops and especially rice [17]. Bhattacharya et al. [15] and Samal et al. [17,27] in their studies on arsenic accumulation in crops of West Bengal concluded that if the common trend of using arsenic-contaminated groundwater for irrigation continues, there is a high possibility of increase of arsenic levels in crops, including rice in near future. Thus a greenhouse pot experiment was conducted to investigate the uptake and distribution of arsenic in the different fractions of rice plant with increasing soil arsenic treatments (5, 10, 20, 30 and 40 mg kg^{-1} dry weights) on the eight selected rice varieties (three high yielding varieties- *Ratna*, IR 50 and IET 4094; one hybrid variety- *Gangakaveri* and four local varieties-*Kanakchur*, *Tulsa*, *Kerala Sundari* and *Danaguri*). Further, two important biochemical parameter – chlorophyll (both '*a*' and '*b*') and carbohydrate (total as well as reducing sugar) contents were analyzed on arsenic-treated rice varieties and the results were compared with the control samples to determine the responses of arsenic accumulation in rice plant metabolites. This study would help to recognize the rice varieties which are resistant to arsenic phytotoxicity that would have significant impact on agriculture, environment and public health.

2. Materials and methods

2.1. Description of experimental condition

The pot culture experiment on rice (*Oryza sativa* L.) varieties was carried out in a greenhouse at the Department of Environmental Science, University of Kalyani campus. 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 unwanted natural calamities (such as heavy rainfall, northwester wind, etc.) and disturbances by animals. The climate of this region of West Bengal is characterized by high temperatures during summer (March–June), high rainfall during monsoon (July–October) and relatively low temperatures during winter (November–February).

2.2. Soil collection and pot preparation

Soil was collected from the University of Kalyani campus at a depth of 0–15 cm. Initial arsenic content of the collected soil prior to arsenic treatment was found to be 1.87 ± 0.09 mg kg^{-1} dry weights (Table 1). After collection, the soil was air dried for 7 days and massive aggregates were broken by gentle crushing. The unwanted materials such as dry roots, grasses, stones, 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 [28]. About 10 kg of soil was taken in total 144 pots comprising of five different arsenic treatments (5, 10, 20, 30 and 40 mg kg^{-1} dry weights) along with one control treatment (no arsenic dosing), each with three replications for the eight 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 [29].

Table 1
Physico-chemical properties of initial pot soil.

Soil parameters	Range
Clay (%)	73–78
Sand (%)	5.7–6.8
Silt (%)	19–24
Texture	Clay loam
pH	7.7 ± 0.27
Organic carbon (%)	0.92 ± 0.09
Total nitrogen (%)	0.17 ± 0.02
Available phosphorous (mg kg^{-1})	15 ± 1.2
Total arsenic (mg kg^{-1})	1.9 ± 0.09

The tap water, used for irrigation, contained arsenic below the detection limit (<0.0003 mg 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 [24].

2.3. Selection of rice varieties and seedling transplantation

In order to meet the increasing demand for feeding large population, the Indian Government encouraged the farmers to cultivate high yielding rice varieties instead of local rice varieties [30]. But, due to continuous economical strain in the cultivation of high yielding rice varieties, farmers have again started to cultivate some local rice varieties. Among those local rice varieties four (*Kanakchur*, *Tulsa*, *Kerala Sundari* and *Danaguri*) were selected through germination test. Three high yielding rice varieties (*Ratna*, IET 4094 and IR 50) and one hybrid rice variety (*Gangakaveri*), highly cultivated in West Bengal, were also selected for this study.

Rice seedlings of 21 days old were carefully uprooted from nursery-bed and transplanted into pots under flooded condition. Eight seedlings, 6 in. apart from each other, were transplanted into each pot. The seedlings, which died within 7 days of transplantation, were discarded and replaced by new seedlings.

2.4. Sample collection, preservation and digestion

The full-grown rice plants were carefully uprooted at their maturity stage (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 [11], 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.

2.5. Analysis of total arsenic

The total arsenic was analyzed by flow injection hydride generation atomic absorption spectrometer (FI-HG-AAS, Perkin Elmer