

Arsenic pollution and subsequent bioaccumulation in *Oryza sativa* (rice) and *Corchorus capsularis* (jute): Environmental health perspectives

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ABSTRACT:

Arsenic is a metalloid that poses severe environmental threats due to its extravagant toxicity and colossal abundance. Bengal basin is regarded to be one of the most acutely arsenic affected zones of the world. The use of arsenic contaminated groundwater for irrigation purpose in crop fields can elevate arsenic concentrations in surface soil and in the plants. *Oryza sativa* Linn. (rice) and *Corchorus capsularis* Linn. (jute) are the two major crops of the Bengal basin. Both rice and jute are generally grown in submerged flooded conditions, where arsenic bioavailability is high in soil. The consumers of the edible parts from both plants therefore face an inevitable source of exposure to arsenic, with consequent accumulation and toxicity. The objective of the study was to observe the *in-vivo* temporal variation of arsenic bioaccumulation in the different parts of *O. sativa* and *C. capsularis*. The study was performed in and around Kalinarayanpur (23°22' N, 88°56' E), Nadia, West Bengal, India, a significant arsenic contaminated zone of Bengal Basin. Rice plant specimens (*Aman* rice, *Ratna* variety) of different age groups (1, 2 and 3 months old) were analyzed in HG-AAS for absorbed arsenic content in different parts. The accumulation of arsenic remained significantly high in the initial phase (1 month age); particularly in root zones it was 149.4 mg./kg., in stem basal part 22.5 mg./kg., in stem middle part 6.34 mg./kg., in stem apical part 2.78 mg./kg., in leaves 1.53 mg./kg and in the early phase of grains (apical parts in tillering stage) 0.29 mg./kg. in average. Interestingly, bioaccumulation of arsenic decreased drastically with time. In the 3 months old rice roots, arsenic bioaccumulated 31.4 mg./kg., in stem basal part 7.72 mg./kg., in stem middle part 0.52 mg./kg., in stem apical part 0.43 mg./kg., in leaves 0.12 mg./kg and in the grains 0.07 mg./kg. in average. Amount of arsenic bioaccumulation followed the decreasing order: root>basal stem>median stem>apical stem>leaves>grains in all the three age groups of the plant samples. The current investigation also found that *C. capsularis* followed a trend of arsenic bioaccumulation similar to *O. sativa*. In the initial phase (1 month), in jute roots, arsenic bioaccumulated 8.701 mg./kg., in stem basal part 6.08 mg./kg., in stem middle part 5.71 mg./kg., in stem apical part 3.92 mg./kg., in leaves 2.07 mg./kg. in average. However, in the 3 month old plants, the accumulation level in all plant parts dropped within the permissible limit and showed no threat. *O. sativa* had more accumulation potential than *C. capsularis*, but *C. capsularis* showed much higher efficiency of arsenic translocation in the above ground parts. As the permissible limit of arsenic in rice grains is only 1 mg/kg. according to WHO, it is quite alarming that such high concentrations of arsenic have been found in rice. The contamination level can reach the grain part in significant amount and can cause health hazards in more severely arsenic affected areas. Intensive investigation on a complete food chain is urgently needed in the arsenic contaminated zones for further risk assessments.

1. INTRODUCTION:

Arsenic (As) is a metalloid that poses serious environmental threats due to its behemoth toxicity and wide abundance. Arsenic contamination in groundwater has been reported in Bangladesh, India, China, Taiwan, Vietnam, USA, Argentina, Chile, and Mexico (Bhattacharya *et al.*, 2012). In many of these places the concentration has exceeded the permissible limit of 10 ppb (Bhattacharya *et al.*, 2012). The Bengal basin is regarded to be the most acutely arsenic affected geological province in the world (Mukherjee *et al.*, 2008). Bengal delta is one of the largest deltas of the world and accommodates an enormous volume of sediments deposited during the Tertiary and Quaternary periods (Bhattacharya *et al.*, 2007). During the late Holocene period, marshy or swampy lowlands developed in several parts of the Bengal Delta Plain and formed the peat and other sediments rich in organic matter (Umitsu, 1993).

Since groundwater arsenic contamination was first reported in year 1983 from 33 affected villages in four districts in West Bengal, the number of villages has increased to 3417 in 111 blocks in nine districts till 2008 in West Bengal alone (Bhattacharya *et al.*, 2007). Chakraborti and his group analyzed over 1,25,000 water samples for studying arsenic contamination in the Bengal basin and reported that arsenic levels in the affected areas of the basin vary between <1 and 1300 mg/l (Chakraborti *et al.*, 2002, 2004). The use of As contaminated groundwater for irrigation of crops in this region elevates arsenic concentration both in the surface soil and the plants (Meharg and Rahman, 2003; Roychowdhury *et al.*, 2005).

Oryza sativa Linn. (rice) and *Corchorus capsularis* Linn. (jute) are the two major crops of the Bengal basin. *O. sativa* is the paramount food crop in this part of the world. *C. capsularis*, on the other hand, is economically important for extraction of fibers and is also consumed as food by rural people (Shukla and Pai, 2005). Previous studies have reported bioaccumulation of arsenic by *O. sativa* and *C. capsularis* in arsenic contaminated zones (Meharg and Rahman, 2003; Das, 2007). Both rice and jute are generally grown in submerged flooded condition, where arsenic bioavailability is high in soil (Duxbury and Panaullah, 2007). Rice is much more efficient at assimilating arsenic into its grain than other staple cereal crops (Williams *et al.*, 2007). Contamination of different varieties of rice by arsenic has been widely reported in North America, Europe, Taiwan, China, Bangladesh and India (Robberecht *et al.*, 2002). In fact, food surveys on the daily arsenic intake in the US and Europe showed, second to fish products, rice is a major dietary source of arsenic (Robberecht *et al.*, 2002; Schoof *et al.*, 1999). The consumers of the edible parts from both plants therefore face an inevitable source of exposure to arsenic, with consequent accumulation and toxicity.

The present study was aimed towards estimation of temporal dependence of arsenic bioaccumulation in the different parts of *O. sativa* and *C. capsularis* with reference to arsenic levels in the diverse strata of soil and in the groundwater used for irrigation.

2. MATERIALS AND METHODS:

2.1. Site of the study:

The study was performed in and around Kalinarayanpur (23°22' N, 88°56' E), Nadia, West Bengal, India. The region, while being among the maximally affected zones of the country due to arsenic pollution (Chakraborti *et al.*, 2002), is greatly involved in agriculture, particularly in the cultivation of *O. sativa* and *C. capsularis*. The agricultural system in this region is mostly dependent on irrigation with arsenic contaminated groundwater obtained from a depth ranging from 70 ft. to 600 ft. through tube-wells. Huge amount of groundwater is used for agricultural irrigation. Most of this groundwater is contaminated with arsenic, which is deposited in the soil in contact with the irrigation water throughout the year.

2.2. Analysis of arsenic content in *O. sativa* and *C. capsularis*:

Levels of arsenic in the two plants were determined by HG-AAS. The plants (grown in agricultural fields irrigated with arsenic contaminated water) were collected carefully without damaging the roots. Two age groups (1 month and 3 months old) were selected for studying the extent of arsenic

bioaccumulation in rice and jute plants in the initial and final phase of growth. Following a thorough washing of all samples with water, roots of each one were measured to note their respective lengths.

Each plant specimen was analyzed for absorbed arsenic content in different parts: roots, stem (basal), stem (median), stem (apical), leaves and grains (only in *O. sativa*). These plant parts were separated carefully and dried in a hot air oven at 70 °C for 72 h. The dried samples were grounded in a grinder and 100 ml of deionized water was added to each grounded sample. All samples were homogenized with a mechanical homogenizer, and the resultant homogenates were filtered. The filtrates were evaporated at 80-90 °C in a hot-air oven followed by the addition of 4 ml of 37% (w/w) HNO₃ (APHA, 1985). All steps following the addition of HNO₃ in the protocol for arsenic estimation in groundwater (as described above) were then employed to analyze arsenic in each of the selected plant parts by HG-AAS (Hydride generation Atomic Absorption Spectroscopy).

2.3. Statistical analyses:

All analyses were carried out in triplicates. Data were presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed by one-way ANOVA to determine significant differences between groups at $P < 0.05$. Estimated correlations were tested for significance by Student's t-test at the same confidence limit. MATLAB ver. 7.0 (Natick, MA, USA), SPSS ver. 9.05 (Chicago, IL, USA) and Microsoft Excel 2007 (Roselle, IL, USA) were used for the statistical and graphical evaluations.

3. RESULTS AND DISCUSSIONS:

The current study revealed that there was a time-dependent decline of arsenic content in the diverse parts of *O. sativa* (Fig. 1). In all the plants samples of two different age groups (i.e. 1 month and 3 months old plants), maximum arsenic accumulation was observed in the roots followed by stem parts, leaves and grains. The accumulation of arsenic remained significantly high in the initial phase (i.e. 1 month age); particularly in root zones it was 149.4 mg./kg., in stem basal part 22.5 mg./kg., in stem middle part 6.34 mg./kg., in stem apical part 2.78 mg./kg., in leaves 1.53 mg./kg and in the early phase of grains (apical parts in tillering stage) 0.29 mg./kg. in average (Fig. 1). The possible reason behind it is the existence of oxidized zone around the rice root zone which helps to form oxidized iron plaque. Iron plaque can efficiently bind arsenic and can reduce its translocation to the above ground tissues (straw, husk and grain) of the plant (Norra *et al.*, 2005). The microbial communities of the rhizosphere can also solubilize ferric iron in the rhizosphere by exuding siderophores to the root-plaque interface, which is another possible reason for increased bioavailability of arsenic in rice plants (Kraemer, 2004). The concentration of arsenic in rice roots can reach as high as 160 mg/kg. due to the formation of iron plaques, as reported in a previous study and with synchrony with the present work (Bhattacharya *et al.*, 2007). It has also been reported that the accumulation of arsenic in rice plants is highest in the root zones and decrease significantly in the upper parts of the plant (Liu *et al.*, 2004). This was verified by the results of the present study, which showed a root>basal stem>median stem>apical stem>leaves>grains trend of arsenic accumulation in *O. sativa*. As the permissible limit of arsenic in rice plant grains is 1 mg/kg. according to WHO, it is quite alarming that such high concentration of arsenic has been found in rice. Interestingly, bioaccumulation of arsenic decreased drastically with time and in the mature 3 months old plants, the accumulation levels were much lower than that of the initial 1 month old phase. In the 3 months old rice roots, arsenic bioaccumulated 31.4 mg./kg., in stem basal part 7.72 mg./kg., in stem middle part 0.52 mg./kg., in stem apical part 0.43 mg./kg., in leaves 0.12 mg./kg and in the grains 0.07 mg./kg. in average respectively. In both growth phases, the grain parts accumulated arsenic well below the permissible limit. In a previous study, approximately 0.081% and 1.57% of arsenic are accumulated in rice grain from arsenic-deposited land and contaminated soil and about 0.029 and 0.133 mg. of arsenic are accumulated in rice grain per kg. of plant and kg. of paddy, respectively in Nadia district (Roychowdhury, 2008). The arsenic concentrations in the edible parts of a plant depend on the availability of the soil arsenic and the accumulation and translocation ability of a plant (Huang *et al.*, 2006). The study can indicate that the contamination level can reach the grain part in significant amount and can cause health hazards in more severely arsenic affected areas. Interestingly, Roychowdhury (2008) found that in Murshidabad, there was a substantial increase of arsenic accumulation in rice after cooking (in raw rice the range of arsenic was found to be 0.043-0.662 mg./kg. whereas in the cooked rice the range was 0.198-1.03) which can indicate that arsenic can create further health hazards because of cooking rice in arsenic contaminated water.

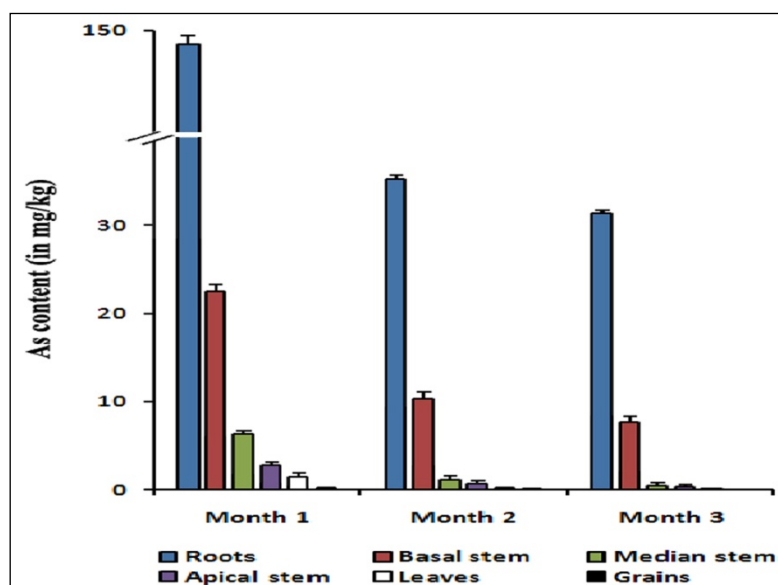


Figure 1. Diverse levels of arsenic bioaccumulation in different parts of *O. sativa*. A distinct time-dependent decrease in the levels of accumulated arsenic has been observed. Data are given in mean $\pm$ SD for $n = 3$ samples ($P < 0.05$).

Arsenic toxicity can reduce the rate of photosynthesis in rice plants, which, in turn can reduce the chlorophyll content, and can affect the growth and yield of rice (Rahman *et al.*, 2007). Rice yield has been reported to decrease by 10% when the concentration of arsenic in soil is as high as 25 mg/kg (Xiong *et al.*, 1987). It has been shown that in T-Aman rice (*Oryza sativa* L.), the grain and straw yields were significantly reduced by the artificial introduction of arsenic in soil (Azad *et al.*, 2009). Trivalent arsenic has adverse effects on the photosynthetic efficiency and growth of the rice seedlings because of the involvement of MAP kinase cascade along with ROS and NO production in arsenic induced stress signal transduction in plants (Rao *et al.*, 2011). In our study, the extent of accumulation indicated the fact that it may affect the productivity and quality of rice plants in the arsenic-contaminated zones, which in turn can affect the economic growth of the Bengal delta, as rice is the main cash crop of this region.

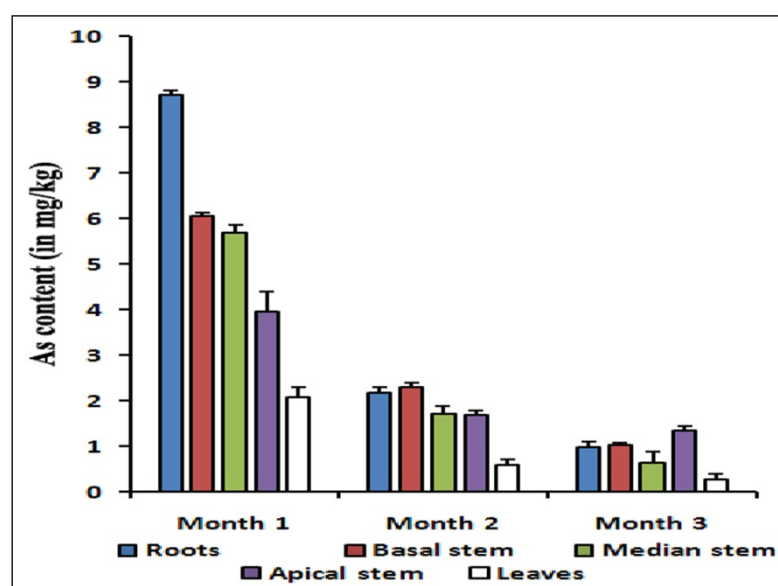


Figure 2. Diverse levels of arsenic bioaccumulation in different parts of *C. capsularis*. A distinct time-dependent decrease in the levels of accumulated arsenic has been observed. Data are given in mean $\pm$ SD for $n = 3$ samples ($P < 0.05$).

While a very few studies were done on the arsenic bioaccumulation in *O. sativa*, not much research has been performed on arsenic accumulation in *C. capsularis* till date. The current investigation found that *C. capsularis* followed a trend of arsenic bioaccumulation similar to *O. sativa*. Fig. 2 demonstrates that there was a significant temporal dependence of the decline of arsenic levels in the different parts of the plant. In the initial phase of growth (i.e. in 1 month), in jute roots, arsenic bioaccumulated 8.701 mg./kg., in stem basal part 6.08 mg./kg., in stem middle part 5.71 mg./kg., in stem apical part 3.92 mg./kg., in leaves 2.07 mg./kg. in average respectively. However, in the 3 month old plants, the accumulation level in all plant parts is with the permissible limit and showed no threat. The roots were found to accumulate the maximum amount of arsenic, similar to *O. sativa*, however, the level of such accumulation was about 15-25 times higher in *O. sativa*. Interestingly, when compared to rice plants, jute plants showed much higher efficiency of arsenic translocation in the above ground parts, as can be evident from Fig. 1 and 2 (the ratios of arsenic bioaccumulation in different parts of jute plants is much higher compared to rice plants).

This is the first ever report of time-dependent decrease in arsenic bioaccumulation in *O. sativa* and *C. capsularis*. Since all parts of both plants showed gradual decline in arsenic levels over three months, it was pertinent to hypothesize that there might be some physiological mechanisms working in those plants that may lower the arsenic level with increasing age.

An explanation of this phenomenon might be the presence of a potential detoxification mechanism involving complexation of arsenite and arsenate through production of thiol rich peptides phytochelatins (Hartley-Whitaker *et al.*, 2001). There may also be some active processes which can help to reduce inorganic arsenic species to less toxic methylated arsenic compounds (Nissen and Benson, 1982). Again, there might be an age-dependent increased efflux of arsenic species from the root into the rhizosphere (Sharples *et al.*, 2000). It has been reported that in yeasts, arsenic tolerance is provided by three contiguous genes in the cluster ACR1, ACR2 and ACR3: ACR1 encodes a putative transcription factor; ACR2 encodes an arsenate reductase; and ACR3 encodes a plasma membrane AsIII-efflux transporter. This mechanism ensures As(V) reduction and its removal from the cytosol to the external medium (Tripathi *et al.*, 2007). There is a possibility of the presence of such arsenic removal system in rice and jute plants which may be another possible reason in the decline of arsenic bioaccumulation with time.

4. CONCLUSION:

The study underscored the fact that arsenic bioaccumulation is a function of availability of the metalloid as well as the physiological traits of the accumulating plants; the accumulation levels of arsenic in *O. sativa* being much higher in comparison to *C. capsularis*. Hence, intensive studies on the different crops grown in arsenic contaminated zones are the paramount priority. Additionally, the edible parts of the tuber crops grown in the arsenic contaminated zones are exposed continuously to soil and irrigation water contaminated with arsenic, which, in turn, can increase arsenic level in the tuber part. The extent of arsenic bioaccumulation in these edible plants should be monitored carefully.

Rice straw is often used as a cattle feed which represents another entry route of arsenic into the food chain, as rice straw typically contains much higher amount of arsenic than grains, as evident from our study. Cattle population also used to drink water contaminated with arsenic in those areas, which, in turn, can further increase the toxicity level in food chain (Abedin *et al.*, 2002). Cattle manure is often used as fuel in household purposes, which can also increase the contamination risk (Pal *et al.*, 2007). Besides, the dry straw often been used by people as fuel, which can release arsenic in air as oxides, and can cause pollution and health hazards. The overall scenario indicates clearly that the bioavailability of arsenic in rice and other edible plants must be addressed to understand the importance of arsenic exposure from these food sources. Intensive investigation on a complete food chain is urgently needed in the arsenic contaminated zones, which should be our priority in future researches.

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