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Accumulation of Arsenic in Tissues of Rice Plant (*Oryza sativa* L.) and its Distribution in Fractions of Rice Grain

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Abstract

A study was conducted to investigate the accumulation and distribution of arsenic in different fractions of rice grain (*Oryza sativa* L.) collected from arsenic affected area of Bangladesh. The agricultural soil of study area has become highly contaminated with arsenic due to the excessive use of arsenic-rich underground water (0.070±0.006 mg l^{-1}, *n*=6) for irrigation. Arsenic content in tissues of rice plant and in fractions of rice grain of two widely cultivated rice varieties, namely BRRI dhan28 and BRRI hybrid dhan1, were determined. Regardless of rice varieties, arsenic content was about 28 and 75 folds higher in root than that of shoot and raw rice grain, respectively. In fractions of parboiled and non-parboiled rice grain of both varieties, the order of arsenic concentrations was; rice hull > bran-polish > brown rice > raw rice > polish rice. Arsenic content was higher in non-parboiled rice grain than that of parboiled rice. Arsenic concentrations in parboiled and non-parboiled brown rice of BRRI dhan28 were 0.8±0.1 and 0.5±0.0 mg kg^{-1} dry weight, respectively while those of BRRI hybrid dhan1 were 0.8±0.2 and 0.6±0.2 mg kg^{-1} dry weight, respectively. However, parboiled and non-parboiled polish rice grain of BRRI dhan28 contained 0.4±0.0 and 0.3±0.1 mg kg^{-1} dry weight of arsenic, respectively while those of BRRI hybrid dhan1 contained 0.43±0.01 and 0.5±0.0 mg kg^{-1} dry weight, respectively. Both polish and brown rice are readily cooked for human consumption. The concentration of arsenic found in the present study is much lower than the permissible limit in rice (1.0 mg kg^{-1}) according to WHO recommendation. Thus, rice grown in soils of Bangladesh contaminated with arsenic of 14.5±0.1 mg kg^{-1} could be considered safe for human consumption.

Keywords: Arsenic, Accumulation, Rice (*Oryza sativa* L.), Brown rice grain, Polish rice grain.

Introduction

The rice cultivation is solely depended on underground water in Bangladesh, West Bengal, India, particularly in dry season, since the sources of surface water like river, dam, pond etc. of these regions becomes dry throughout the season. Natural release of arsenic from aquifer rocks has been reported to contaminate this underground water in Bangladesh and West Bengal, India (Fazal et al., 2001; Smith et al., 2000; Nickson et al., 1998; Nickson et al., 2000; Chakraborty et al., 2002; Hopenhayn, 2006; Harvey et al., 2002; Chowdhury et al., 1999; Chakraborti and Das, 1997). Long term use of arsenic contaminated underground water in irrigation may results in the increase of its concentration in agricultural soil and eventually in crop plants (Ullah, 1998; Imamul Huq et al., 2003; Rahman et al., 2007a; Rahman et al., 2007b). Survey on paddy soil throughout Bangladesh showed that arsenic concentrations were higher in agricultural soils of those areas where shallow tube wells (STWs) have been in operation for longer period of time and arsenic contaminated underground water from those STWs have been irrigated to the crop fields (Meharg et al., 2003). Onken and Hossner (1995) reported that plants grown in soil treated with arsenic had higher rate of arsenic uptake compared to those grown in untreated soil. Some other researchers (Abedin et al., 2002; Rahman et al., 2004; Rahman et al., 2007a) also reported elevated content of arsenic in tissues of rice when the plant was grown in soils contaminated with higher concentrations of arsenic.

Because of groundwater contamination with high level of arsenic, scientists and researchers become interested to investigate the effects of arsenic contaminated soil and irrigation water on its accumulation and metabolism in rice (*Oryza sativa* L.). Recently, some reports focused on the effects of arsenic contaminated soils and irrigation water on its uptake in root, shoot, husk and grain of rice and its metabolism in rice at greenhouse condition (Rahman et al., 2004; Rahman et al., 2007a; Abedin et al., 2002a; Abedin et al., 2002b). However, field level investigation on this aspect is inadequate. Limited literatures

are found on arsenic accumulation in different fractions of rice gain as well as its retention in cooked rice following the traditional cooking methods used by the populations of arsenic epidemic areas.

Being rice one of the major food crops in many countries, the populations of different countries cook rice differently. Majority of the people of Bangladesh and West Bengal, India, parboil raw rice before cooking though, the people of some other countries like Thailand, Japan and China cook rice without parboiling. Moreover, rice is milled to remove the husk (hull) before cooking. Some times, the bran polish (the outer thin layer of milled rice) becomes detached from the rice grain during milling. Thus, the total arsenic in raw rice grain does not correspond to the definite amount of arsenic retained in cooked rice.

The objective of the present study was to determine arsenic distribution in different fractions of both parboiled and non-parboiled rice. The studies would help to determine the amount of arsenic retained in cooked rice and to assess the possible amount of arsenic taken by the populations of arsenic epidemic areas from rice. As far we know this is the first report on the distribution of arsenic in different fractions of parboiled and non-parboiled cooked rice grain.

Materials and methods

Sample Collection

Samples of two rice varieties named BRRI dhan28 and BRRI hybrid dhan1 were collected from three sampling points (2 m^2 of area) of selected plot in each of the two locations. Soil samples were also collected from three points of 2 m^2 areas and 10-15 cm depth of the selected plots using soil auger. Locations of the sampling area are shown in Fig. 1. Samples were collected during harvest and sun dried immediately after collection, tagged properly, kept air tied in poly bag and brought to the laboratory for further analysis.

Water samples were collected from STWs nearby the rice field. Water has been irrigated from those STWs for rice cultivation. The populations of near by villages are also drink water from those STWs. Water was collected in polyethylene bottles from a uniform rate of discharging water, usually 10-20 min after pumping, which were filtered through 0.45 Millipore filter paper. About 90 ml of water was collected from each STW and preserved in the refrigerator adding 10 ml 2M hydrochloric acid in them.

Treatment of raw rice

Rice has been processed differently for cooking in different countries. In this study, two common cooking methods, usually practiced by the populations of arsenic epidemic areas of Bangladesh and West Bengal, India were followed. The rice cooking methods are shown schematically in Fig. 2.

i) Soaking and parboiling of raw rice

About 800 g of sun dried raw rice was soaked in 1400 ml water for 36 h at room temperature (25±2 ºC). Soaked raw rice was sieved through wire net and water was discarded. The quantity of water absorbed by rice was determined by measuring the amount of discarding water. After that, the soaked raw rice was taken in a silver pot and about 250 ml of water was added to the rice so that about 25% grains remained under water. The pot was heated on an electric heater at 100 ºC for about 1.5 h. The water was started to boil and steam was generated. Raw rice was parboiled by boiling water as well as steam generated from the water. The completion of parboiling of raw rice was determined by slightly opening the lemma and palea of rice grain. Parboiled rice was then sieved by wire net and water was discarded. The sieved parboiled rice was then sun dried to about 14% moisture content.

ii) Milling

Sun dried parboiled and non-parboiled rice was dehulled in rice mill. Hull/husk and brown rice were collected after milling. Brown rice was further milled in a rice testing mill (RTM) to remove bran-polish. The bran-polish and polish rice were collected separately and stored in paper packet for chemical analysis.

The brown rice, bran-polish and polish rice of both parboiled and non-parboiled rice were weighted carefully and the data were calculated for per cent distribution of rice fractions which are presented in Table 1.

Sample digestion procedure

Soil and rice samples were digested with acid digestion following the heating block digestion procedure. About 0.5 g of the sample was taken into clean, dry digestion tubes and 5 ml of concentrate nitric acid was added to it. The mixture was allowed to stand over night under fume hood. In the following day, the digestion tubes were placed on heating block and heated at 60 ºC for 2 h. Then, the tubes were allowed to cool at room temperature. About 2 ml of concentrated perchloric acid was added to the plant samples. For the soil samples (initial soil), 3 ml sulfuric acid was added in addition to the 2 ml perchloric acid. Again, the tubes were heated at 160 ºC for about 4 to 5 h. Heating was stopped when the dense white fume of perchloric acid occurred. The digests were then cooled and diluted to 25 ml with distilled deionized water and filtered through filter paper (Whatman No. 42 for soil samples and Whatman No. 41 for plant samples) and stored in 30-ml polythene bottles.

Total arsenic analysis

Total arsenic was determined by hydride generation atomic absorption spectrophotometer (HG-AAS) (Perkin-Elmer AAnalyst 100 fitted with flow injection system, FIAS 100,

Germany) using matrix-malched standards (Welsch et al., 1990). In each analytical batch, at least two reagent blanks, one spike and three duplicate samples were included in the acid digestion to asses the accuracy of the chemical analysis. The recovery of spike was 87.4% (*n* = 6). The precision of the analysis was also checked by certified standard reference material (SRM) (1573a tomato leaf, NIST, USA). The arsenic concentration in certified reference material was 0.112±0.004 μg g^{-1} while the measured arsenic concentration was 0.120±0.009 μg g^{-1}. The concentrations detected in all samples were above the instrumental limits of detection (≥ 0.0008 mg l^{-1} in water). All glassware and plastic bottles were previously washed by distilled DI water and dried.

Chemicals

Nitric acid (HNO_3) (70%), Sulfuric acid (H_2SO_4), Perchloric acid ($HClO_4$) and Sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) were purchased from Mark. Other chemicals were from AnalaR. All the reagents were of analytical grade.

Statistical analysis

The experimental data were statistically analyzed. The test of significance (ANOVA) of different parameters was calculated according to Duncan multiple range test (DMRT) at 5% level and correlation coefficient was computed by SPSS 10 for windows.

Results and Discussions

Arsenic content in tissues of rice plant

Arsenic concentrations in soil and water of study area were 14.5±0.1 mg kg^{-1} and 0.070±0.006 mg l^{-1}, respectively (*n=3*). Though the soil arsenic concentration was below the maximum acceptable limit for agricultural soil recommended by the European Community (EC) (20.0 mg kg^{-1} soil), its concentration in water was much higher than the

acceptable limit recommended by world health organization (WHO) (0.01 mg l^{-1}) (O'Neil, 1995; Smith, 1998). The arsenic concentration in drinking and irrigation water also exceeded the Bangladesh standard of 0.05 mg l^{-1}.

In the present study, arsenic distribution in tissues of rice plant was found to be 96% in root, 3% in straw and 1% in raw rice of BRRI dhan 28. However, the straw of BRRI hybrid dhan1 contained a little higher amount of arsenic than that of BRRI dhan 28 (Fig. 2). From the results it seems to be that, translocation of arsenic from root to shoot (straw) of hybrid rice variety is a little higher than that of non-hybrid variety. Arsenic translocation from straw to rice grain did not differ significantly for the variations of rice strain. This might be because the fresh shoot biomass production of hybrid variety was higher than that of non-hybrid variety and the bioaccumulation of metals and other nutrients are related to the total biomass production. The bioaccumulation of metals is also related to the rate of transpiration. Larger shoot biomass enhances the transpiration of larger amount of water which might results in the translocation of larger amount of arsenic along with other nutrient elements to the above ground parts of rice plant.

In BRRI dhan28, mean arsenic concentrations (mg kg^{-1} dry weight) were 46.3±1.4 in root, 1.7±0.1 in straw and 0.6±0.0 in raw rice. The BRRI hybrid dhan1 contained 51.9±1.3, 1.9±0.1, and 0.7±0.2 mg kg^{-1} dry weight in root, straw and raw rice, respectively (n=3) (Table 3). Results indicate that regardless the rice variety, most of the arsenic accumulated into plant tissues, remains in root which is about 28 and 75 times higher than that of straw and raw rice, respectively. Abedin et al. (2002a) also observed that a very large amount of arsenic retained in rice root compared to its content in straw and rice grain. Some other literatures (Rahman et al., 2004; Rahman et al., 2007b; Duxbury et al., 2002; Meharg et al., 2001; Rahman et al., 2006) also reported the same results. Why such a large amount of arsenic remain in the roots of rice plant is interesting. Though the mechanism of arsenic accumulation in rice plant is not well understood, Liu et al. (2004) reported that iron

oxides (iron plaques), formed around the rice root, bind the arsenic and check its translocation to the above ground tissues of the plant. Arsenic concentrations in tissues of rice plant generally follow the trend; root > straw > husk > grain (Abedin et al., 2002a; Rahman et al., 2004; Xie et al., 1998; Odanaka et al., 1987; Marin et al., 1992).

Arsenic distribution in fractions of rice grain

Arsenic contents in fractions of rice grain are shown in Table 4. Arsenic contents in husk of non-parboiled and parboiled BRRI dhan28 were 1.1±0.2 and 0.7±0.1 mg kg^{-1} dry weight, respectively. Its content in BRRI hybrid dhan1 were 1.6±0.1 and 0.8±0.2 mg kg^{-1} dry weight, respectively (*n*=3).

Bran polish has been removed from brown rice during milling to make polish rice. The bran-polish rice of non-parboiled and parboiled BRRI dhan28 contained 0.9±0.1 and 0.6±0.2 mg of As kg^{-1} dry weight, respectively. On the other hand, brown rice of non-parboiled and parboiled of the rice variety contained 0.8±0.1 and 0.5±0.0 mg of As kg^{-1} dry weight, respectively (*n*=3) (Table 4). The results show significantly higher amount of arsenic in bran-polish compared to that in brown rice and fractions of BRRI hybrid dhan1 contained higher amount of arsenic than those of BRRI dhan28.

Polish rice is readily cooked for human consumption in which arsenic concentrations were found to be 0.4±0.0 and 0.3±0.1 mg kg^{-1} dry weight in non-parboiled and parboiled rice of BRRI dhan28 variety, respectively. Arsenic concentrations in non-parboiled and parboiled polish rice of BRRI hybrid dhan1 were 0.4±0.1 and 0.5±0.1 mg kg^{-1} dry weight, respectively (Table 4). Though there is no standard level of arsenic concentration in south Asian food grains, the above concentrations of arsenic in rice fractions are bellow the standard level recommended by the UK and Australia (1.0 mg kg^{-1} dry weight) (Warren et al., 2003). However, fractions of non-parboiled rice contained higher amount of arsenic compared to those of parboiled rice suggest that parboiling of raw rice may results in the

decrease of arsenic concentrations in rice fractions. During parboiling, arsenic might have released from straw and rice grain to the boiling water and the discarding of boiling water may result in the decrease of its concentrations rice. Though rice has not been parboiled before milling in many countries, the populations of arsenic epidemic areas of Bangladesh and West Bengal, India have been consuming parboiled rice. Thus, parboiling of rice grain before cooking may reduce the magnitude of arsenic intake in human body.

There have been some reports on arsenic content in tissues of rice (Rahman et al., 2004; Abedin et al., 2002a; Marin et al., 2003; Meharg et al., 2001) and in cooked rice (Bae et al., 2002; Roychowdhury et al., 2002) though its distribution in fractions of parboiled and non-parboiled rice grain is not discussed in literatures. Roy Chowdhury et al. (2002) reported 0.21 and 0.37 mg kg^{-1} dry weight of arsenic in raw and cooked rice, respectively. Rahman et al. (2004) also reported 0.4 mg of As kg^{-1} in raw rice grown on soils containing 20 mg As kg^{-1}. Abedin et al. (2002a) reported 0.42 mg of As kg^{-1} in rice grain when 8.0 mg l^{-1} of arsenic contaminated water was irrigated. Arsenic content in raw rice, collected from arsenic epidemic area of the present study (mean soil arsenic concentration of the area was about 14.5±0.1 mg kg^{-1}), have been found to be 0.6±0.0-0.7±0.2 mg kg^{-1} (Table 3), which is much higher than those of previous reports. Moreover, among the fractions of non-parboiled rice grain, arsenic concentration was highest in husk (35-40%) followed by bran-polish (28-29%) and brown rice (20-25%). Polish rice gain contained the lowest amount of arsenic (11-12%). In fractions of parboiled rice grain, arsenic contents were 29-32% in husk, 24% in brown rice, 28-29% in bran-polish and 15-19% in polish rice (Fig. 4). Regardless of rice strain, arsenic distribution in rice fractions followed the trend; husk > bran-polish > brown rice > polish rice. Milling of raw rice significantly reduces the arsenic concentrations in the grain (Duxbury et al., 2002; Rahman et al., 2006) which decrease the possibility of arsenic intake in human body. The present study also supports the previous reports. This might be because milling removes the outer bran-polish layer of rice grain

which concentrates a significant amount of arsenic then that of the inner polish rice. But it is important to investigate why the arsenic concentrations decreased consequently in the inner fractions of rice grain. The outer fractions of rice (like husk) might act as translocation barrier to arsenic for which it could not move into the inner fractions (like grain or polish rice).

The present study revealed that parboiling (cooking of raw rice before removing the husk) decreased arsenic concentrations in fractions of rice grain (Table 4). Roy Chowdhury et al. (2002) and Bae et al. (2002) reported higher arsenic concentrations in cooked rice than that of raw rice. Bae et al. (2002) suggested that cooked rice could be an important source of arsenic, if it is boiled with extensive arsenic contaminated water. They proposed two possible causes of increased arsenic concentrations in cooked rice are; i) arsenic in the water by which the raw rice was cooked is chelated by rice grain, ii) arsenic becomes concentrated during the cooking process because of evaporation. The result of the present study is not in agreement with the previous studies of Bae et al., (2002). In parboiling process, excessive water has been used which is discarded after parboiling (Fig. 2). Arsenic from raw rice may dissolve in water during boiling and discarded with boiling water.

Conclusion

Results of this investigation reveal that the total amount of arsenic in raw rice is not taken in human body. During the processing of raw rice for human consumption, some fractions of rice such as husk and bran-polish are removed which contain a significant amount of arsenic. Arsenic concentration in polish rice is also reduced due to parboiling of the raw rice before milling. Thus the arsenic concentration in polish rice is much lower than that of in raw rice. Moreover, cooking of polish rice also reduces the arsenic concentration in cooked rice (Rahman et al., 2006). Regardless of rice variety, arsenic content in fractions

of parboiled and non-parboiled rice grain follow the order; rice hull > bran-polish > brown rice > raw rice > polish rice. Arsenic content was higher in non-parboiled rice grain than that of parboiled rice.

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Table 1: Fractional distribution (% dry weight) of non-parboiled and parboiled rice [a]

Rice fractions	% dry weight			
	BRRI dhan28		BRRI hybrid dhan1	
	Non-parboiled	Parboiled	Non-parboiled	Parboiled
Brown rice	77.1	75.9	77.9	77.8
Polish rice	69.8	68.6	67.0	67.2
Hull/Husk	22.7	23.8	21.6	21.9
Bran polish	7.3	9.3	10.8	10.5

[a] About 600 g raw rice was taken for the measurement of the fractional distribution of non-parboiled and parboiled rice.

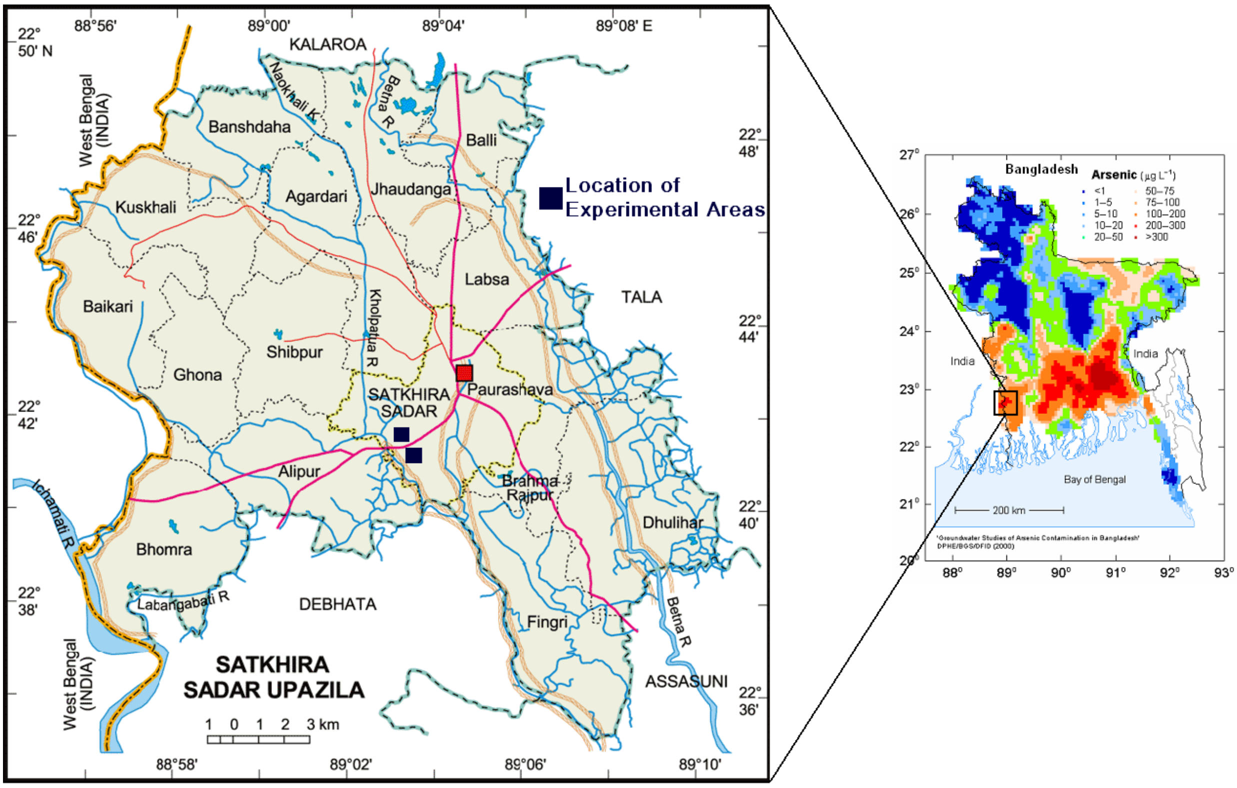

Fig. 1: Site map of sampling locations; Itagasa and Guddirdangi village of Satkhira sador thana in Satkhira district is on of the severely arsenic affected areas in Bangladesh. The sampling area was located at 22º40´- 22 º 42´ altitudes and 89º02´- 89º04´ longitude.

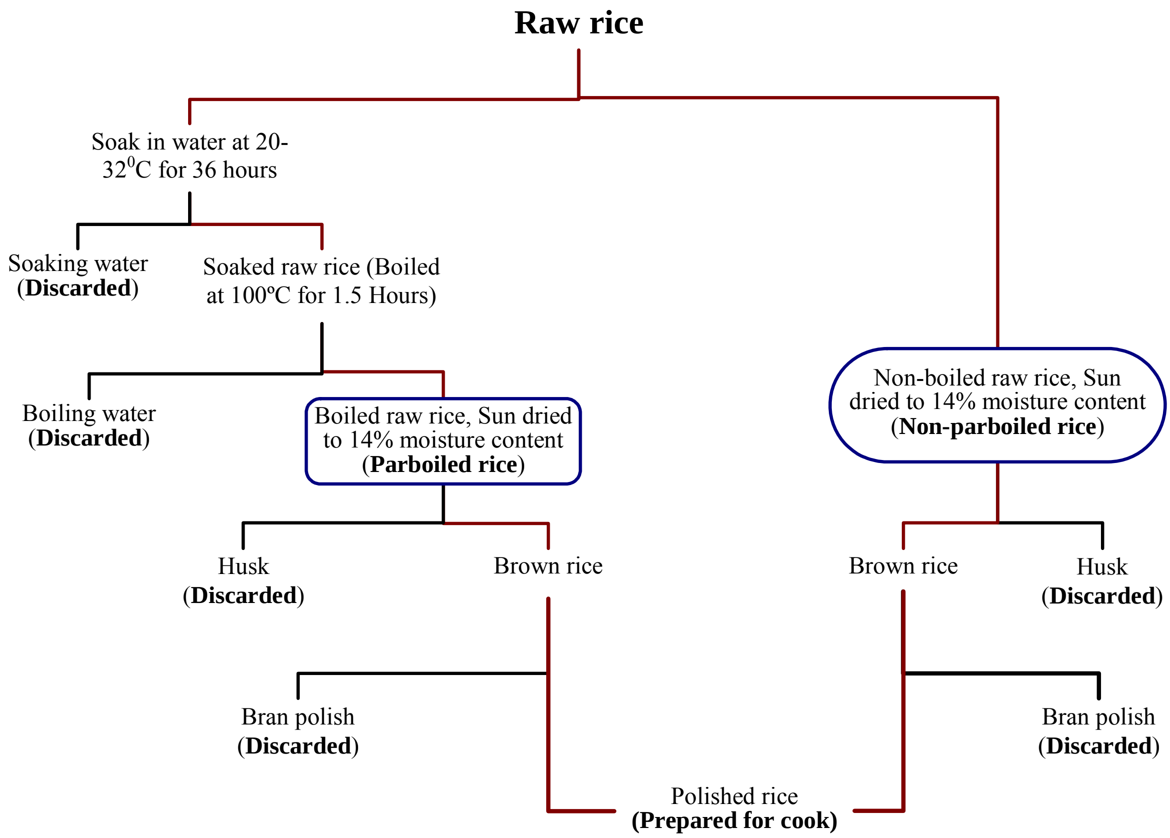

Fig. 2: Flow diagram showed the sequential steps followed by the population of arsenic epidemic areas of Bangladesh for rice cooking. They usually follow two types of polished rice, parboiled and non-parboiled, for cocking which are processed in two different ways shown above.

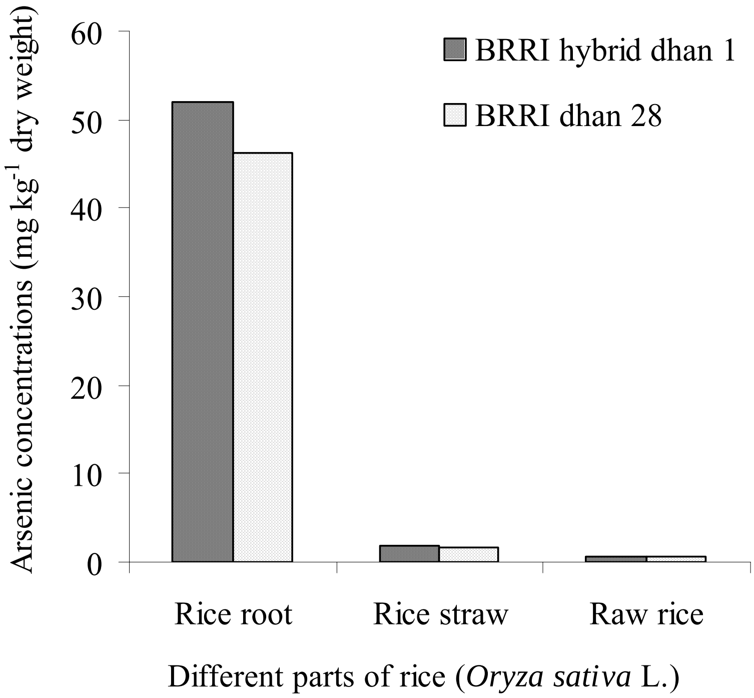

Fig. 3: Arsenic distribution in different parts of rice plant (*Oryza sativa* L.)

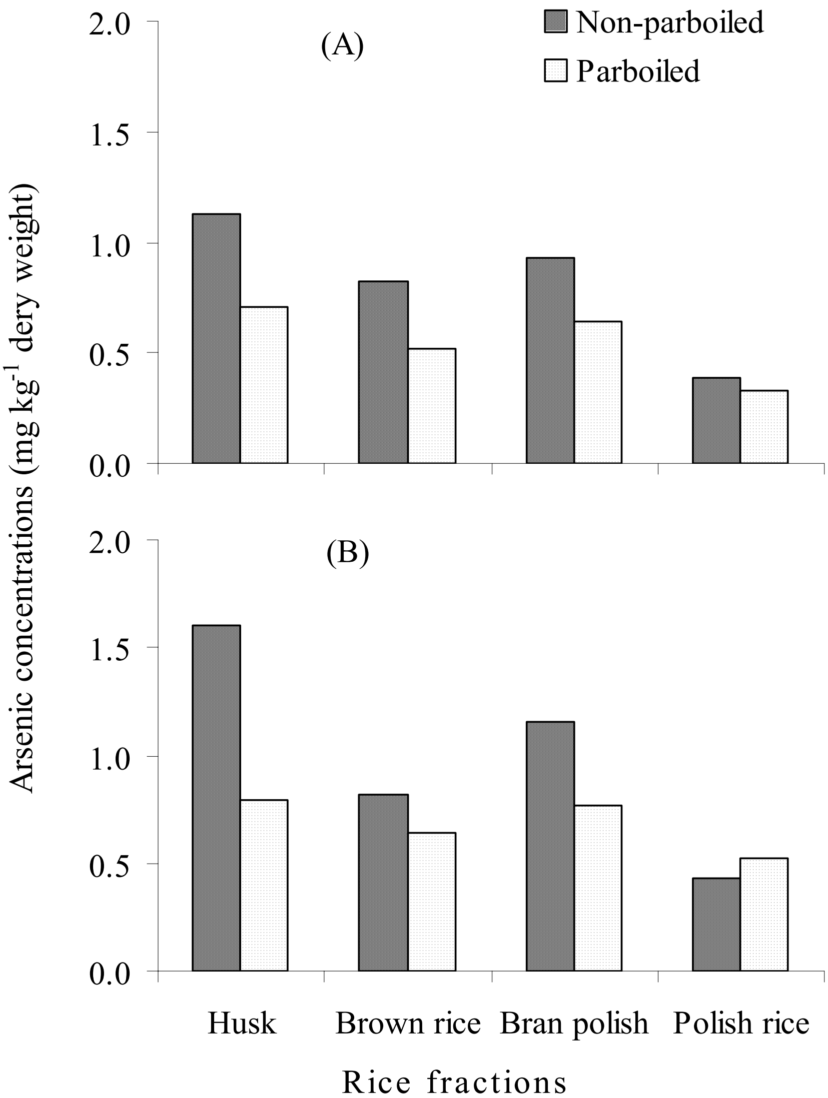

Fig. 4: Arsenic distribution in fractions of parboiled and non-parboiled rice (*Oryza sativa* L.). BRRI dhan28 (A); BRRI hybrid dhan1 (B)