

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

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26 **Abstract**

27 A study was conducted to investigate the accumulation and distribution of arsenic in
28 different fractions of rice grain (*Oryza sativa* L.) collected from arsenic affected area of
29 Bangladesh. The agricultural soil of study area has become highly contaminated with
30 arsenic due to the excessive use of arsenic-rich underground water (0.070 ± 0.006 mg l⁻¹,
31 $n=6$) for irrigation. Arsenic content in tissues of rice plant and in fractions of rice grain of
32 two widely cultivated rice varieties, namely BRRI dhan28 and BRRI hybrid dhan1, were
33 determined. Regardless of rice varieties, arsenic content was about 28 and 75 folds higher
34 in root than that of shoot and raw rice grain, respectively. In fractions of parboiled and
35 non-parboiled rice grain of both varieties, the order of arsenic concentrations was; rice hull
36 > bran-polish > brown rice > raw rice > polish rice. Arsenic content was higher in non-
37 parboiled rice grain than that of parboiled rice. Arsenic concentrations in parboiled and
38 non-parboiled brown rice of BRRI dhan28 were 0.8 ± 0.1 and 0.5 ± 0.0 mg kg⁻¹ dry weight,
39 respectively while those of BRRI hybrid dhan1 were 0.8 ± 0.2 and 0.6 ± 0.2 mg kg⁻¹ dry
40 weight, respectively. However, parboiled and non-parboiled polish rice grain of BRRI
41 dhan28 contained 0.4 ± 0.0 and 0.3 ± 0.1 mg kg⁻¹ dry weight of arsenic, respectively while
42 those of BRRI hybrid dhan1 contained 0.43 ± 0.01 and 0.5 ± 0.0 mg kg⁻¹ dry weight,
43 respectively. Both polish and brown rice are readily cooked for human consumption. The
44 concentration of arsenic found in the present study is much lower than the permissible
45 limit in rice (1.0 mg kg⁻¹) according to WHO recommendation. Thus, rice grown in soils
46 of Bangladesh contaminated with arsenic of 14.5 ± 0.1 mg kg⁻¹ could be considered safe for
47 human consumption.

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49

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

52 **Introduction**

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

71 Because of groundwater contamination with high level of arsenic, scientists and
72 researchers become interested to investigate the effects of arsenic contaminated soil and
73 irrigation water on its accumulation and metabolism in rice (*Oryza sativa* L.). Recently,
74 some reports focused on the effects of arsenic contaminated soils and irrigation water on
75 its uptake in root, shoot, husk and grain of rice and its metabolism in rice at greenhouse
76 condition (Rahman et al., 2004; Rahman et al., 2007a; Abedin et al., 2002a; Abedin et al.,
77 2002b). However, field level investigation on this aspect is inadequate. Limited literatures

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

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

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

95

96 **Materials and methods**

97 **Sample Collection**

98 Samples of two rice varieties named BRR1 dhan28 and BRR1 hybrid dhan1 were collected
99 from three sampling points (2 m² of area) of selected plot in each of the two locations. Soil
100 samples were also collected from three points of 2 m² areas and 10-15 cm depth of the
101 selected plots using soil auger. Locations of the sampling area are shown in [Fig. 1](#).
102 Samples were collected during harvest and sun dried immediately after collection, tagged
103 properly, kept air tied in poly bag and brought to the laboratory for further analysis.

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

110

111 **Treatment of raw rice**

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

116

117 **i) Soaking and parboiling of raw rice**

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

129

130 **ii) Milling**

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

135 The brown rice, bran-polish and polish rice of both parboiled and non-parboiled rice were
136 weighted carefully and the data were calculated for per cent distribution of rice fractions
137 which are presented in [Table 1](#).

138

139 **Sample digestion procedure**

140 Soil and rice samples were digested with acid digestion following the heating block
141 digestion procedure. About 0.5 g of the sample was taken into clean, dry digestion tubes
142 and 5 ml of concentrate nitric acid was added to it. The mixture was allowed to stand over
143 night under fume hood. In the following day, the digestion tubes were placed on heating
144 block and heated at 60 °C for 2 h. Then, the tubes were allowed to cool at room
145 temperature. About 2 ml of concentrated perchloric acid was added to the plant samples.
146 For the soil samples (initial soil), 3 ml sulfuric acid was added in addition to the 2 ml
147 perchloric acid. Again, the tubes were heated at 160 °C for about 4 to 5 h. Heating was
148 stopped when the dense white fume of perchloric acid occurred. The digests were then
149 cooled and diluted to 25 ml with distilled deionized water and filtered through filter paper
150 (Whatman No. 42 for soil samples and Whatman No. 41 for plant samples) and stored in
151 30-ml polythene bottles.

152

153 **Total arsenic analysis**

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

156 Germany) using matrix-matched standards (Welsch et al., 1990). In each analytical batch,
157 at least two reagent blanks, one spike and three duplicate samples were included in the
158 acid digestion to assess the accuracy of the chemical analysis. The recovery of spike was
159 87.4% ($n = 6$). The precision of the analysis was also checked by certified standard
160 reference material (SRM) (1573a tomato leaf, NIST, USA). The arsenic concentration in
161 certified reference material was $0.112 \pm 0.004 \mu\text{g g}^{-1}$ while the measured arsenic
162 concentration was $0.120 \pm 0.009 \mu\text{g g}^{-1}$. The concentrations detected in all samples were
163 above the instrumental limits of detection ($\geq 0.0008 \text{ mg l}^{-1}$ in water). All glassware and
164 plastic bottles were previously washed by distilled DI water and dried.

165

166 **Chemicals**

167 Nitric acid (HNO_3) (70%), Sulfuric acid (H_2SO_4), Perchloric acid (HClO_4) and Sodium
168 arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) were purchased from Merck. Other chemicals were from
169 AnalaR. All the reagents were of analytical grade.

170

171 **Statistical analysis**

172 The experimental data were statistically analyzed. The test of significance (ANOVA) of
173 different parameters was calculated according to Duncan multiple range test (DMRT) at
174 5% level and correlation coefficient was computed by SPSS 10 for windows.

175

176 **Results and Discussions**

177 **Arsenic content in tissues of rice plant**

178 Arsenic concentrations in soil and water of study area were $14.5 \pm 0.1 \text{ mg kg}^{-1}$ and
179 $0.070 \pm 0.006 \text{ mg l}^{-1}$, respectively ($n=3$). Though the soil arsenic concentration was below
180 the maximum acceptable limit for agricultural soil recommended by the European
181 Community (EC) (20.0 mg kg^{-1} soil), its concentration in water was much higher than the

182 acceptable limit recommended by world health organization (WHO) (0.01 mg l^{-1}) (O'Neil,
183 1995; Smith, 1998). The arsenic concentration in drinking and irrigation water also
184 exceeded the Bangladesh standard of 0.05 mg l^{-1} .

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

197 In BRRI dhan28, mean arsenic concentrations (mg kg^{-1} dry weight) were 46.3 ± 1.4 in root,
198 1.7 ± 0.1 in straw and 0.6 ± 0.0 in raw rice. The BRRI hybrid dhan1 contained 51.9 ± 1.3 ,
199 1.9 ± 0.1 , and $0.7 \pm 0.2 \text{ mg kg}^{-1}$ dry weight in root, straw and raw rice, respectively ($n=3$)
200 (Table 3). Results indicate that regardless the rice variety, most of the arsenic accumulated
201 into plant tissues, remains in root which is about 28 and 75 times higher than that of straw
202 and raw rice, respectively. Abedin et al. (2002a) also observed that a very large amount of
203 arsenic retained in rice root compared to its content in straw and rice grain. Some other
204 literatures (Rahman et al., 2004; Rahman et al., 2007b; Duxbury et al., 2002; Meharg et
205 al., 2001; Rahman et al., 2006) also reported the same results. Why such a large amount of
206 arsenic remain in the roots of rice plant is interesting. Though the mechanism of arsenic
207 accumulation in rice plant is not well understood, Liu et al. (2004) reported that iron

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

212

213 **Arsenic distribution in fractions of rice grain**

214 Arsenic contents in fractions of rice grain are shown in Table 4. Arsenic contents in husk
215 of non-parboiled and parboiled BRR I dhan28 were 1.1 ± 0.2 and 0.7 ± 0.1 mg kg⁻¹ dry
216 weight, respectively. Its content in BRR I hybrid dhan1 were 1.6 ± 0.1 and 0.8 ± 0.2 mg kg⁻¹
217 dry weight, respectively ($n=3$).

218 Bran polish has been removed from brown rice during milling to make polish rice. The
219 bran-polish rice of non-parboiled and parboiled BRR I dhan28 contained 0.9 ± 0.1 and
220 0.6 ± 0.2 mg of As kg⁻¹ dry weight, respectively. On the other hand, brown rice of non-
221 parboiled and parboiled of the rice variety contained 0.8 ± 0.1 and 0.5 ± 0.0 mg of As kg⁻¹
222 dry weight, respectively ($n=3$) (Table 4). The results show significantly higher amount of
223 arsenic in bran-polish compared to that in brown rice and fractions of BRR I hybrid dhan1
224 contained higher amount of arsenic than those of BRR I dhan28.

225 Polish rice is readily cooked for human consumption in which arsenic concentrations were
226 found to be 0.4 ± 0.0 and 0.3 ± 0.1 mg kg⁻¹ dry weight in non-parboiled and parboiled rice of
227 BRR I dhan28 variety, respectively. Arsenic concentrations in non-parboiled and parboiled
228 polish rice of BRR I hybrid dhan1 were 0.4 ± 0.1 and 0.5 ± 0.1 mg kg⁻¹ dry weight,
229 respectively (Table 4). Though there is no standard level of arsenic concentration in south
230 Asian food grains, the above concentrations of arsenic in rice fractions are below the
231 standard level recommended by the UK and Australia (1.0 mg kg⁻¹ dry weight) (Warren et
232 al., 2003). However, fractions of non-parboiled rice contained higher amount of arsenic
233 compared to those of parboiled rice suggest that parboiling of raw rice may result in the

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

240 There have been some reports on arsenic content in tissues of rice ([Rahman et al., 2004](#);
241 [Abedin et al., 2002a](#); [Marin et al., 2003](#); [Meharg et al., 2001](#)) and in cooked rice ([Bae et](#)
242 [al., 2002](#); [Roychowdhury et al., 2002](#)) though its distribution in fractions of parboiled and
243 non-parboiled rice grain is not discussed in literatures. [Roy Chowdhury et al. \(2002\)](#)
244 reported 0.21 and 0.37 mg kg⁻¹ dry weight of arsenic in raw and cooked rice, respectively.
245 [Rahman et al. \(2004\)](#) also reported 0.4 mg of As kg⁻¹ in raw rice grown on soils containing
246 20 mg As kg⁻¹. [Abedin et al. \(2002a\)](#) reported 0.42 mg of As kg⁻¹ in rice grain when 8.0
247 mg l⁻¹ of arsenic contaminated water was irrigated. Arsenic content in raw rice, collected
248 from arsenic epidemic area of the present study (mean soil arsenic concentration of the
249 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](#)
250 [3](#)), which is much higher than those of previous reports. Moreover, among the fractions of
251 non-parboiled rice grain, arsenic concentration was highest in husk (35-40%) followed by
252 bran-polish (28-29%) and brown rice (20-25%). Polish rice gain contained the lowest
253 amount of arsenic (11-12%). In fractions of parboiled rice grain, arsenic contents were 29-
254 32% in husk, 24% in brown rice, 28-29% in bran-polish and 15-19% in polish rice ([Fig.](#)
255 [4](#)). Regardless of rice strain, arsenic distribution in rice fractions followed the trend; husk
256 > bran-polish > brown rice > polish rice. Milling of raw rice significantly reduces the
257 arsenic concentrations in the grain ([Duxbury et al., 2002](#); [Rahman et al., 2006](#)) which
258 decrease the possibility of arsenic intake in human body. The present study also supports
259 the previous reports. This might be because milling removes the outer bran-polish layer of

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

265 The present study revealed that parboiling (cooking of raw rice before removing the husk)
266 decreased arsenic concentrations in fractions of rice grain (Table 4). Roy Chowdhury et al.
267 (2002) and Bae et al. (2002) reported higher arsenic concentrations in cooked rice than
268 that of raw rice. Bae et al. (2002) suggested that cooked rice could be an important source
269 of arsenic, if it is boiled with extensive arsenic contaminated water. They proposed two
270 possible causes of increased arsenic concentrations in cooked rice are; i) arsenic in the
271 water by which the raw rice was cooked is chelated by rice grain, ii) arsenic becomes
272 concentrated during the cooking process because of evaporation. The result of the present
273 study is not in agreement with the previous studies of Bae et al., (2002). In parboiling
274 process, excessive water has been used which is discarded after parboiling (Fig. 2).
275 Arsenic from raw rice may dissolve in water during boiling and discarded with boiling
276 water.

277

278 **Conclusion**

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

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

289

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395 **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

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397 ^a About 600 g raw rice was taken for the measurement of the fractional distribution
398 of non-parboiled and parboiled rice.

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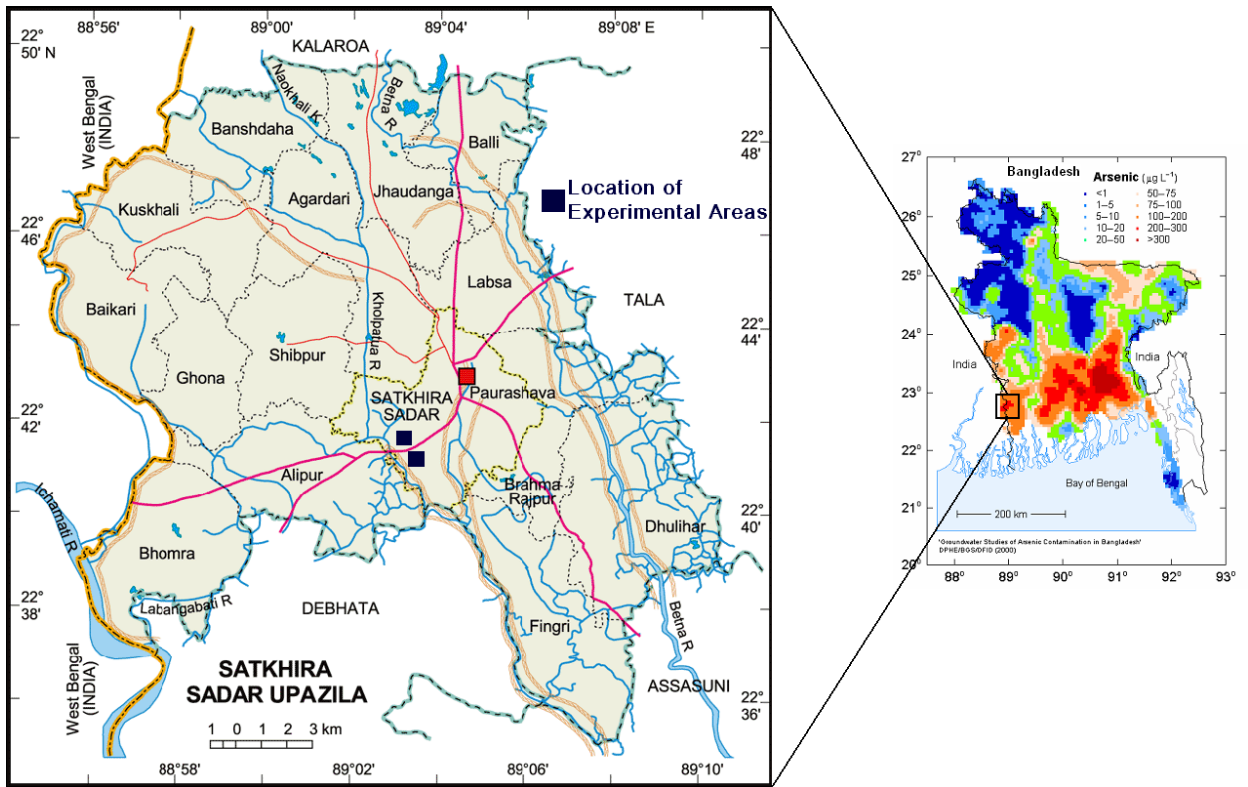
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410 Fig. 1: Site map of sampling locations; Itagasa and Guddirdangi village of Satkhira sador

411 thana in Satkhira district is on of the severely arsenic affected areas in

412 Bangladesh. The sampling area was located at 22°40' - 22 ° 42' altitudes and

413 89°02' - 89°04' longitude.

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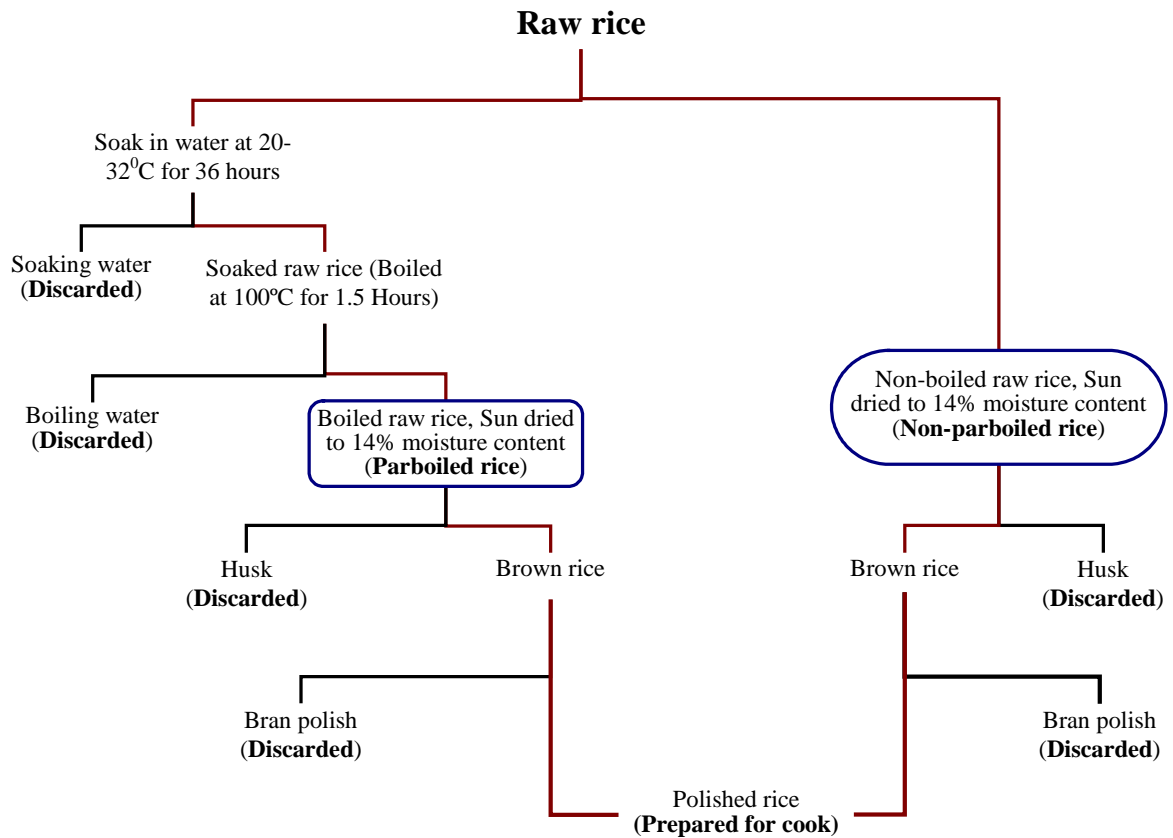
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Fig. 2: Flow diagram showed the sequential steps followed by the population of arsenic

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epidemic areas of Bangladesh for rice cooking. They usually follow two types

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of polished rice, parboiled and non-parboiled, for cooking which are

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processed in two different ways shown above.

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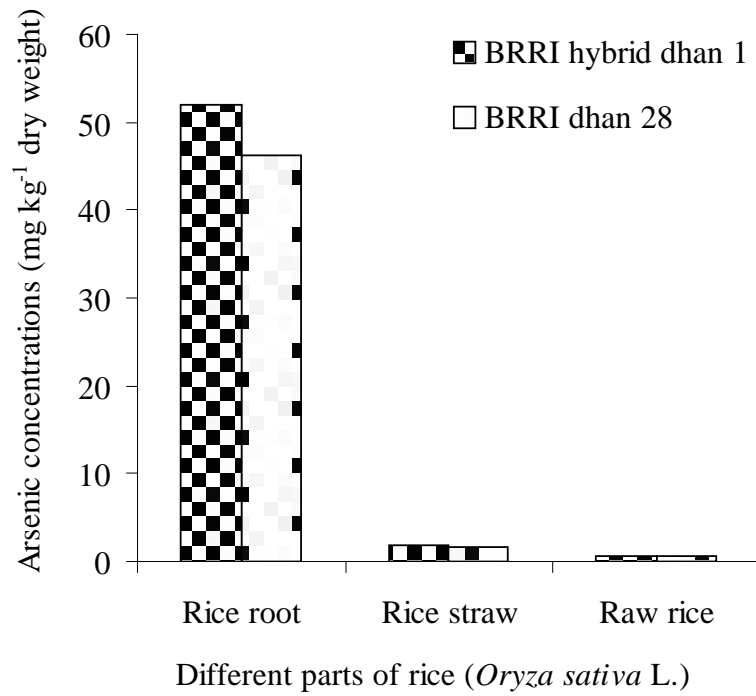
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439 Fig. 3: Arsenic distribution in different parts of rice plant (*Oryza sativa* L.)

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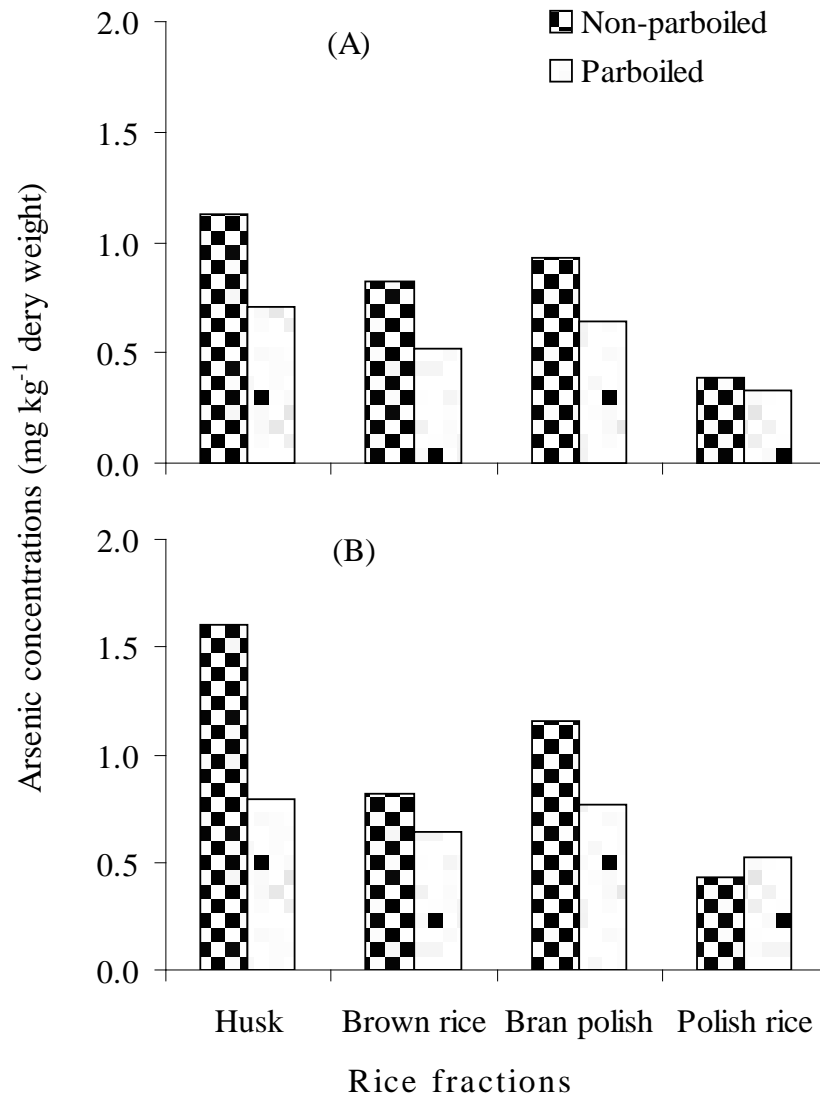
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455 Fig. 4: Arsenic distribution in fractions of parboiled and non-parboiled rice (*Oryza sativa*

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L.). BRR dhan28 (A); BRR hybrid dhan1 (B)