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ARSENIC UPTAKE AND SPECIATION IN *ORIZA SATIVA* L. GROWN IN WAHALKADA, SRI LANKA

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ABSTRACT

Arsenic uptake and speciation studies on brown rice, AT 307 were carried out for the samples collected from Wahalkada, Sri Lanka in where large number of patients with Chronic Kidney Disease (CKD) was reported. Total arsenic (total extractable arsenic) concentration values, and different arsenic species present in soil and different parts of paddy plants were determined using ICP-MS coupled with HPLC in order to understand the translocation of arsenic in different parts of paddy plants including rice grain. Here we report the results of arsenic speciation studies on rice (Oriza sativa L.) for the first time in Sri Lanka. Total arsenic content in soil was found to be 3.40 ± 0.03µg/g but As(V) and As(III) were found to be 1.05 ± 0.01 µg/g and in the not detectable range, respectively. Though soil contained a considerable amount of arsenic, rice grains contain only 0.33 ± 0.02 µg/g of total arsenic, approximately one tenth, was transferred from soil to rice grains in which negligible accumulation (about 0.02µg/g) of arsenic was observed after milky stage. The amount of As(III) and As(V) in matured rice grains were 0.10 ± 0.02 µg/g and 0.02 ± 0.01 µg/g, respectively but these concentration values are much less than the value (0.3 or 0.2 µg/g) to be proposed by WHO as the standard for inorganic arsenic in brown rice.

Key words: Arsenic, Arsenate, Arsenite, Chronic Kidney Disease, Wahalkada, Sri Lanka

1. Introduction

Arsenic uptake and speciation in rice (*Oriza sativa* L.) grown in Sri Lanka are of great concern in this study as rice is the major staple food consume by Sri Lankans. According to Department of Agriculture, rice provides 45% total calorie and 40% total protein requirement of an average Sri Lankan and the per capita consumption of rice fluctuates around 100 kg per year (De Silva and Yamao 2009). However, as reported, it depends on the price of rice, bread and wheat flour (De Silva and Yamao 2009). In order to fulfil consumers' requirements, rice are cultivated in all around Sri Lanka and also imported from other countries such as Bangladesh, Pakistan and India (http://www.indexmundi.com/agriculture).

Arsenic is a metalloid and can be released to the environment by different processes such as mining, coal burning, usage of pesticides, wood preserving arsenicals (Garelick et al., 2008). In Sri Lanka, arsenic contamination in aquatic and terrestrial environment is mainly due to the use of pesticides (Wijesekara et al., 2011). Arsenic is a well-known contaminant in irrigation water and the soil which causes a set of health problems commonly named as arsenicosis (McCarty et al., 2011). It is also known to cause bladder, lung and skin cancers (Bhattacharya et al., 2013). Previous studies have shown that the liver, kidney and prostate glands (in human or any other animal) are potential targets of arsenic-induced cancers (Indu et al., 2007).

The potential of harmful effects of arsenic depends on the chemical form and the extent of exposure (Meharg et al., 2002). The total arsenic concentration in any food is not the only determinant of its toxicity. Arsenic toxicity mostly depends on its speciation or species such as inorganic and organic arsenic. Inorganic arsenic species are more toxic than methylated organic arsenic due to its high bioavailability (Smith et al., 2008). The dominant forms of inorganic arsenic are arsenite, As(III) and arsenate, As(V). Arsenite is more toxic than arsenate and previous study by smith et al (2008) has shown that arsenite could efficiently absorb by rice root and reached rice grain due to its high water solubility and soil mobility (Smith et al., 2008). On the other hand, Dimethylarsinous acid (DMAA), monomethylarsinous acid (MMAA), Arsenobetaine, arsenocholine, tetramethylarsonium salts and arsenosugars are common organic arsenic species and some of them are metabolites of inorganic arsenic and some of which cause toxicity (Ng 2005). In this context, identification of arsenic species are rather important than total arsenic analysis in toxicity assessment.

So far, there is no federal limit for arsenic in most of consumable food in the world. Different countries have established maximum limits for total arsenic intake for the human body. For an example, China has established the maximum limit for inorganic arsenic intake in rice and rice based products as 0.2 µg/g (www.consumerreports.org › ... › 2012 ›) and in Australia it is 1 µg/g (Smith et al., 2008). In Sri Lanka, during late 1980s, the Sri Lanka Standards Institution (SLSI) had proposed the maximum limits of arsenic allowed for various types of food as 1 µg/g (Jayawardene 1987). Internationally, the World Health Organization met in 2014 to propose arsenic standards for rice. For white rice, whether the limit is 0.2 µg/g (inorganic arsenic) and for brown rice, the limit is 0.3 µg/g (total or inorganic arsenic), however, this is still under discussion (www.consumerreports.org › ... › 2012 ›).

Rice is one of the major crops grown in water flooded areas. Rice absorbs arsenic much more effectively because roots can absorb arsenic easily and store in grains. Many studies have been carried out throughout the world to investigate arsenic concentrations in rice to predict the extent of possible dietary intake of arsenic from this food source (Meharg et al., 2004; Mondal et al., 2010; Mondal et al 2008; Rahman et al., 2006; Rahman et al., 2007; Rahman et al., 2008; Williams et al., 2006; Williams et al., 2005; Williams et al 2007). Williams et al. (2007) have reported the arsenic concentration values of rice grain from different countries. According to their report, total mean arsenic concentration value in Indian white basmati rice collected from Indian super markets was 0.05 µg/g of dry weight within the range of 0.03–0.08 µg/g. They have also reported the mean inorganic arsenic concentration value as 0.04 µg/g (range of 0.02–0.05) showing that mean value of 56 % of inorganic arsenic (range of 36-67) was present in Indian white basmati rice. On the other hand, in Bangladesh, it was reported that the corresponding concentration values for total arsenic and the inorganic arsenic were 0.24 µg/g (range of 0.21–0.27) and 0.20 µg/g (range of 0.17–0.22), respectively accounting 82% of inorganic arsenic (range of 81-83) in that particular rice (Rahman et al., 2011).

As Sri Lankan farmers use huge amount of pesticides for different agricultural purposes, the fear of arsenic contamination in rice and water ways became a serious issue during past few years. As a result, 28 pesticides used in Sri Lanka were tested by Industrial Technology Institute and the Ministry of Technology and Research of Sri Lanka. Among 28 pesticides, three pesticides namely Glyposate, Carbofuran and Thiocyclam were found to contain arsenic levels of 0.334 µg/g, 0.166 µg/g, and 0.370 µg/g, respectively. These three pesticides were banned by the Sri Lankan government now (Wijesekara et al., 2011).

As mentioned earlier, the level of impact of arsenic on human health has been documented to a certain extend for many countries in the world except Sri Lanka, and only limited investigations have been carried out to identify the arsenic levels of rice in Sri Lanka. Jayasekera et al (2005) has investigated various trace elements, including arsenic in rice from Sri Lanka. According to their report, raw polished grains and parboiled grains consisted of total arsenic concentration values of 0.034 µg/g and 0.065 µg/g respectively. Meanwhile the Rice Research and Development Institute (RRDI) of the Department of Agriculture analyzed different rice varieties throughout the country. These results revealed that arsenic was not present in rice at detectable levels (Wijesekara et al., 2011). On the other hand, Jayasumana et al (2015) have reported that few new improved rice varieties found in Sri Lanka contained 0.021 – 0.540 µg/g where as few traditional rice varieties contained 0.012-0.064 µg/g of total arsenic.

Recently, there is an increase in patients of chronic kidney disease reported in certain areas of Sri Lanka including Wahalkada, Sri Lanka (www.arsenic.lk/content/Situation). Major causes for this kidney disease are still a puzzle even though the number of reported patients in these areas increases rapidly day by day. Many researchers have suspected that one cause can be the arsenic present in rice grown in those particular areas due to the usage of pesticides contaminated with arsenic (www.arsenic.lk/content/Situation). Therefore, misinformation about arsenic levels in rice caused a serious panic among Sri Lankans. As mentioned before, there is a need to investigate inorganic arsenic levels in rice other than total arsenic levels because inorganic arsenic species are more toxic than organic species.

Therefore, our study was planned to determine the arsenic uptake of paddy plants, *Oriza sativa* L, AT 307 brown rice variety grown in Wahalkada, Sri Lanka which explores the transfer of arsenic from soil to matured rice grains through roots, stems and rice in the milky stage. We also studied the different arsenic species present in each paddy parts and soil. In one way, the results will be very useful to know the concentration values of different arsenic species present in this variety of rice since there is no any prior investigation of speciation has been carried out in Sri Lanka. Secondly, it will be very helpful to find out the causes of the chronic kidney disease which is an urgent need to save lives of poor farmers in Sri Lanka.

2. Methodology

2.1 Study area and sample collection

Study area is located at Wahalkada in Anuradhapura district, Sri Lanka as marked in Figure 1.

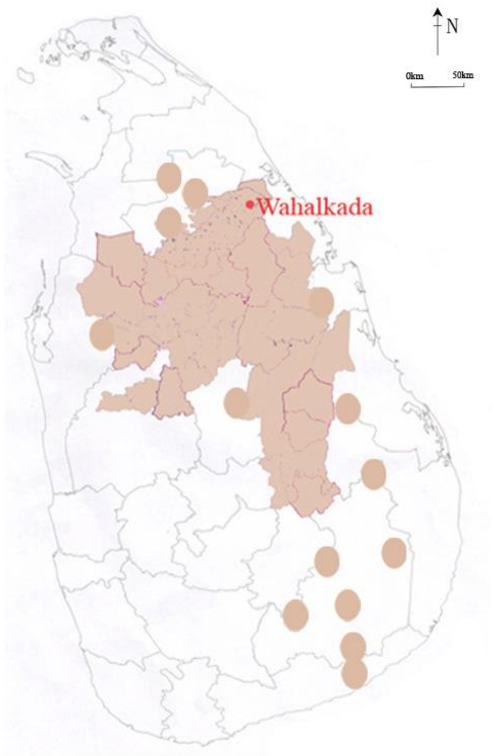

Fig 1.The identified areas with chronic kidney disease reported in Sri Lanka including Wahalkada.

AT 307 *Oriza sativa L*, a brown rice variety, was cultivated using urea, potassium chloride (commonly referred to as Muriate of Potash or MOP**),** and TSP (tri sodium phosphate) as fertilizers and Matari, M 50 (M.C.P.A 40%) as weedicides. Twenty rice samples at the milky stage and the matured stage (after one month) were collected randomly from twenty places in the same slot where paddy plants were grown. Roots and stems of the same paddy plants by which rice was harvested and the soil samples from spots where relevant paddy plants were grown were collected.

2.1 Sample preparation

All collected root and stem samples were cleaned and washed with tap water followed by three times with double distilled water. These samples were cut into small pieces and freeze dried (LABCONCO) for three days. Collected rice samples were peeled manually. Soil samples were collected where paddy plants pulled off. Soil samples were oven dried at 70°C and kept in sealed zip lock bags. Freeze-dried samples and rice samples were finely ground to a homogenous powder using an IKAAII micro mill along with liquid nitrogen.

2.2 Extraction of species

Approximately 0.2g of sample was weighed into each 50 mL poly tetrafluoroacetate (PTTE) digestion vessel (CEM, USA), and 10 mL of 2% v/v HNO_3 (Aristar, BDH) was added (for soil samples, Aqua-regia was added). Samples were heated in a microwave oven (MARS, CEM, USA) with a time temperature program consisting of 95°C for 10 minutes. Samples were cooled down to room temperature and supernatants were removed after centrifuging at 5000 rpm for 10 minutes. Collected supernatants were filtered through Minisart RC 150.2 µm filter papers and diluted to 1:1 using phosphate mobile phase before feeding HPLC-ICP-MS.

2.2.1 HPLC-ICP-MS analysis

The HPLC system consisted of a Perkin-Elmer series 200 mobile phase delivery system.
Column conditions used for the separation of anionic arsenic species were as follows.
Column: Hamilton PRP-X 100 anion exchange column (250 mm × 4.6 mm, 10 µm)
Mobile phase: 20 mM ammonium phosphate (Merck, Germany), buffer at 4.5, flow rate at 1.5 ml min^{-1}.
Column temperature: 40°C
Injection volume: 20 µl
The eluent from HPLC column was directed by PEEK (Polyethylene ether ketone) capillary tubing in to nebulizer of a Perkin Elmer Elan DRC-eICP-MS, used to monitor the intensity of the signal of arsenic at m/z 75.

2.2.2 Statistical Analysis

Statistical analyses were performed using SPSS 21. Before a comparison of means, normality and homogeneity of variance of the data were verified using Shapiro–Wilk test and Levene's test, respectively. Significant difference between soil and different parts of the paddy plant for As species were compared by analysis of variance (ANOVA) followed by a Tukey–Kramer's post-hoc comparisons for parametric data and by Kruskall–Wallis test with Mann–Whitney U-tests for post-hoc analysis for non-parametric data. Before using non-parametric test, logarithmic transformation was applied to test the normality and homogeneity of variance. If data did not meet assumptions, non-parametric tests were applied.

3. Results and Discussion

Studied soil sample consists of considerable amount of detectable total arsenic, that is, 3.40µg/g. But this amount is comparatively very less than the soil concentration values reported in West Bengal, India (11.35 µg/g), Bangladesh (7.31-27.28 µg/g), China (6.04 µg/g) and Nepal (6.1-16.7 µg/g) (Roychowdhury et al., 2002; Das et al., 2004; Huang et al., 2006; Dahal et al., 2008).

Table 1 summarizes concentrations of total extractable arsenic and different arsenic species present in different parts of the paddy plant. According to table 1, rice grains consist of only 0.33 µg/g of extractable arsenic. This concentration value is higher than the reported values from Italy (0.19 µg/g), India (0.07 µg/g), but lower than USA (0.33 µg/g), China (0.36µg/g) and Bangladesh (0.61µg/g) [20]. Very recent studies carried in Sri Lanka by Jayasumana et al (2015) reported 0.54 µg/g of total arsenic, which is somewhat higher than the value reported in this study.

Table 1. Concentrations of total arsenic and different arsenic species present in different paddy parts. ND is the abbreviation for the concentration less than the detectable limit of 0.01 µg/g.

Sample	Total As (µg/g)	As(III) (µg/g)	As(V) (µg/g)	MMAA (µg/g)	DMAA (µg/g)
Roots	1.28 ± 0.04	0.60 ± 0.03	1.05 ± 0.01	ND	ND
Stems	0.24 ± 0.02	ND	0.14 ± 0.02	ND	ND
Rice in milky stage	0.31± 0.02	0.13 ± 0.11	0.07 ± 0.02	ND	ND
Rice grain (matured)	0.33 ± 0.02	0.10 ± 0.02	0.02 ± 0.01	ND	ND

In order to understand arsenic distribution in different parts of paddy plants, the transfer factor between two phases (parts) was calculated using the following expression as written for the rice and soil,

$$\text{Transfer factor of rice:soil} = \frac{\text{Arsenic concntration of rice}}{\text{Arsenic concentration of soil}}$$

Similarly transfer factor for roots : soil, stem : soil, milky stage: soil, and rice grain : soil were calculated and tabulated in Table 2. Noticeable feature seen in values of transfer factors between various parts is the low value (0.09) for the rice grain: soil, which indicates the considerably low translocation of arsenic from soil to rice grain though soil which consists of significant amount of arsenic. This is a significant factor for Sri Lankans who consume this brown rice variety grown in this area despite the value of extractable arsenic in rice grain, 0.32 µg/g, is questionable as there has not yet established maximum limit for arsenic intake in rice in Sri Lanka.

Table 2. Transfer factors between soil and different paddy tissues.

Ratio	Transfer factors (Total Arsenic)
Roots : Soil	0.376
Stem : Soil	0.073
Rice in milky stage : Soil	0.090
Rice grain : Soil	0.093

According to the Table 1, roots contain the highest amount of arsenic compare to the other parts of the paddy plant, which is 1.28 µg/g. As shown in Table 2, transfer factor of roots : soil is 0.38. It accounts that about one third of arsenic is transferred from soil to roots. Even though the exact mechanism of higher accumulation of arsenic in roots was not well established, Liu et al (2004) have suggested that iron oxide layers around roots binds arsenic and hence reduces the accumulation of arsenic in above ground tissues. Small transfer factor of stem: soil, 0.07 which revealed lesser translocations of arsenic up to stem from soil. Increase in transfer factors from 0.07 → 0.09 → 0.093 (stem → rice in the milky stage → rice gran) evidence that the accumulation of arsenic is more favourable in rice than the stem.

Results of statistical analysis shows that the concentrations of total extractable arsenic in studied soil, roots, stems and matured rice grain, are significantly different while there is no significant different in the concentrations of rice in the milky stage and the matured rice.

The concentrations of different arsenic species present in different parts of the paddy plants are given in the Table 1 accounts that there is no detectable organic arsenic species, DMAA (Dimethylarsinous acid) and MMAA (Monomethylarsinous acid) present in any part of the studied paddy plants as well as in soil. This is accordance with the earlier report that organic arsenic compounds were mainly found in marine organisms (Taleshi et al., 2010) even though there reported values contain considerable amount of organic arsenic in some rice varieties grown in other countries (Williams et al., 2005; Zavala et al., 2008; Mehang et al., 2009; Batista et al., 2011). Williams et al (2005) have reported that white long grain rice variety grown in USA contains 0.05-0.26 µg/g of DMAA while brown long grain rice variety contains 0.4-0.15 µg/g. They have also reported that the chinigura, a local aromatic rice variety of Bangladesh consists of 49% organic arsenic among total arsenic.

According to the Table 1, considerable amount of inorganic arsenic species (As(III) and As(V)) are present in studied paddy parts but soil contains only As(V). The concentration of As(III) in soil is below the detection limit of 0.01µg/g. Comparison of total arsenic and arsenic species in the root with the rice grain shows that 25% of total arsenic in the root has transferred to the grain but only 17% and 3 % of As(III) and As(V) have transferred to the grain, respectively. This accounts the values 0.10 and 0.02 µg/g for As(III) and As(V), respectively. Total inorganic concentration value (0.12 µg/g) is much less than the values to be proposed as the standard for inorganic arsenic by WHO, that is 0.3 or 0.2 µg/g (www.consumerreports.org › ... › 2012 ›).

Statistical analysis carried out on concentrations for As(III) in each studied paddy parts and soil is shown in Figure 2. The values in roots and matured rice grains are significantly different whereas those in matured rice grain and rice in the milky stage are not significantly different.

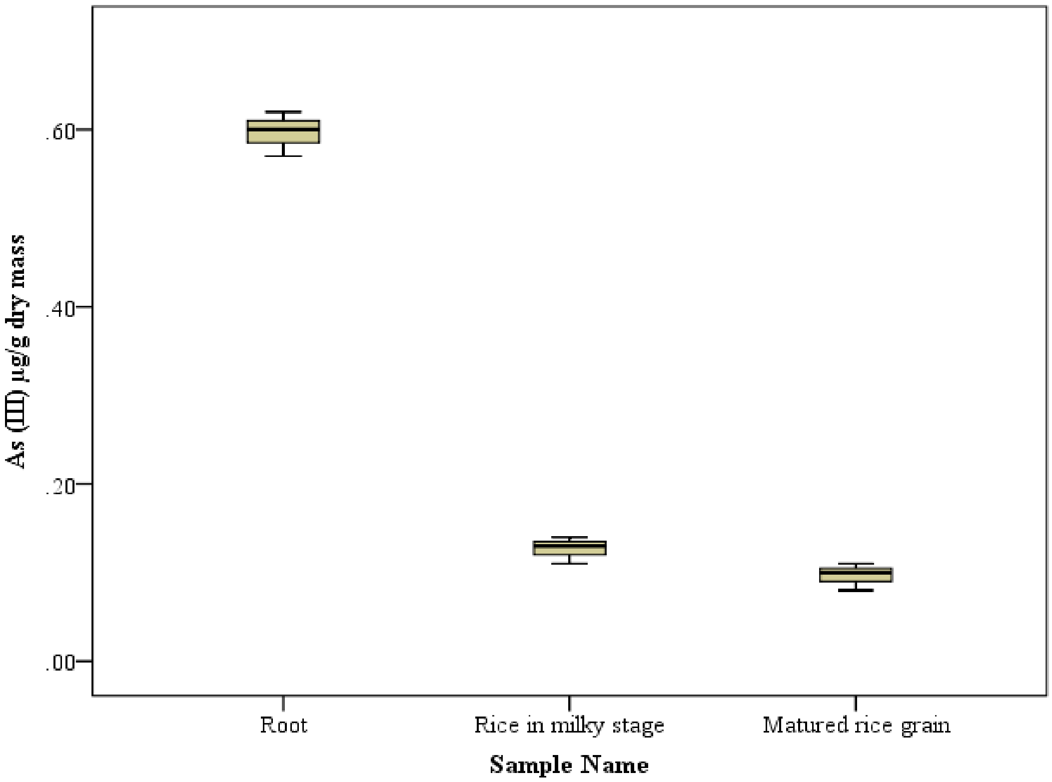

Fig 2: The concentrations of arsenic species (III) in roots, rice in milky stage, and matured rice. N = 3 per sample. (Tukey-Kramer; $p \leq 0.05$).

Similar trend was observed for concentrations for As(V) in each studied paddy parts and soil. Concentrations of As(V) present in soil, roots, stems and matured rice grains are significantly different while there is no such a significant different in concentrations of As(V) in matured rice grains and the rice in the milky stage.

For the comparison, we summarize the concentrations of total arsenic and inorganic arsenic of different rice varieties reported in few other countries where data are available in Table 3. Concentration values of rice grain in present study, 0.33 µg/g of total arsenic and 0.12 µg/g of inorganic arsenic are considerably higher than those, 0.03-0.08 µg/g of total arsenic and 0.02-0.05 µg/g of inorganic arsenic, in Indian rice variety reported by Williams et al (2005). It is much less when compared with the values reported for rice varieties from China and Bangladesh where account 0.46-1.18 µg/g of total arsenic and 0.25-0.76 µg/g of inorganic arsenic, and 0.41-0.98 µg/g of total arsenic and 0.23-0.39 µg/g of inorganic arsenic, respectively. Most noticeable comparison that could be extracted from Table 3 is that negligible difference in values of both total and inorganic arsenic concentrations observed in present study and those reported rice verities from USA, Brazil and Italy (Williams et al., 2005; Zavala et al., 2008; Mehang et al., 2009; Batista et al., 2011). (Williams et al., 2005; Zavala et al., 2008; Mehang et al., 2009; Batista et al., 2011).

Table 3. Comparison of total, inorganic and organic arsenic levels in rice verities reported form few other countries.

Country	Total As (µg/g)	**Inorganic As (µg/g)**		**Organic As (µg/g)**		Reference
		As(III)	As(V)	DMAA	MMAA	
USA	0.331 (0.201-0.710)	0.131 (0.097-168)	0.008 (<0.005-0.013)	0.173 (0.036-0.572)	0.001 (0.000-0.013)	Zavala et.al (2008)
USA	0.21-0.34	0.105 (0.060-0.140)	ND	0.090 (0.010-0.150)	ND	Williams et.al (2005)
China	0.360	0.210	ND	0.090	0.010	Mehang.et.al (2009)
Italy	0.190	0.100	ND	0.050	ND	Williams et.al (2005)
Brazil	0.348 (0.271-0.428)	0.146 (0.139-0.151)	0.042 (0.037-0.051)	0.127 (0.070-0.206)	0.011 (0.000-0.018)	Batista et.al (2011)
India	0.070	0.040	ND	ND		Williams et.al (2005)
Bangladesh	0.610	0.280	ND	0.170	0.010	Mehang.et.al (2009)
Sri Lanka	0.330	0.100	0.020	ND	ND	Present study

4. Conclusion

Total arsenic and inorganic arsenic concentrations in studied brown rice variety, AT 307 are 0.33 µg/g and 0.12 µg/g respectively. These concentrations are much below the maximum permissible limits proposed by other countries and the value to be proposed by WHO. There is no detectable organic arsenic species in both studied soil and all studied paddy parts.

Therefore, arsenic present in studied brown rice variety, AT 307, grown in the Wahalkada area in Sri Lanka may not be the major cause for the Chronic Kidney Disease reported in the area.

Analysis of the distribution of arsenic among different paddy parts after the up taking from the soil showed that there was a small tendency to accumulate arsenic in rice after the milky stage. The lower arsenic content in matured rice grains compared to the soil and smaller transfer factor of rice : soil grains are due to the higher retention of up taken arsenic in roots which contain the 38% of arsenic detected in soil.

Results of our statistical analysis clearly showed that total arsenic and As(V) in studied soil, roots, stems and matured rice grain are significantly different while these concentration values in rice in the milky stage and the matured rice grains are not significantly different. Similarly, our calculations clearly showed that As(III) present in soil, roots and rice grains are significantly different.

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