PRELIMINARY STUDY OF ARSENIC CONTENT AND TOXICITY ASSESSMENT IN RICE FROM INDONESIA

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Abstract *Over 50 percent of the world's population consumes rice as a staple food. However, due to natural and anthropogenic activity, heavy metals, particularly arsenic (As), can be found in rice. This has become a worldwide concern because of the high level of consumption of rice and its processed products, as well as the long-term consequences. Information on dietary arsenic exposure in raw and cooked rice and its quality content in Indonesia is limited; however, its availability is essential for estimating toxicity intake. Therefore, an advanced, accurate, fast, relatively easy, and environmentally friendly arsenic analysis method, such as total X-ray fluorescence (TXRF) and a line equation approach, is needed. It is necessary to evaluate arsenic content and inorganic arsenic (i-As) in rice. The research aimed to assess preliminary dietary arsenic exposure, evaluate rice's inorganic arsenic concentration, and assess toxicity levels. Toxicity level estimation of arsenic exposure was done by determining the hazard quotient (HQ). The results showed that t-As and i-As in rice were 0.071-0.104 mg/kg and 0.050-0.073 mg/kg(wet base), respectively. The mean percentage of i-As reached 70% from the t-As,and the toxicity level of arsenic in cooked rice is low, as shown by the value of HQ < 1. The estimated result indicates no chronic, non-carcinogenic effect caused by these samples.*

INTRODUCTION

Rice (Oryza sativa) is a staple food that the majority of the world's population consumes, providing essential carbohydrates, proteins, vitamins, and minerals (1,2). Typically, rice is cultivated in flooded paddy fields so that the plants can absorb nutrients and other minerals from the water. Minerals mobilized from the soil can accumulate in rice grains, including essential trace elements required for the body's metabolic processes and toxic minerals such as heavy metals that can compromise the health of humans and animals (3,4). Heavy metals are generally harmful and significant environmental pollutants, and their toxicity is an issue of growing ecological, evolutionary, nutritional, and environmental significance. Heavy metals refer to any metallic element with a relatively high density (> 4 g/cm^3) that is toxic or harmful, even in low concentrations. However, compared to them, the chemical properties of heavy metals are the most influential factors. Heavy metals, such as arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), copper (Cu), and chromium (Cr), are prevalent, particularly in areas with high human pressure (5).

Arsenic, a highly toxic metal, is classified as a potent class-one non-threshold carcinogen in humans. It enters the environment through geogenic and anthropogenic activities, which are its primary sources. Elevated concentrations of arsenic have been observed in various countries, including Bangladesh, Brazil, China, France, India, Vietnam, and the United States. The ingestion of water contaminated with arsenic or the consumption of food items with heightened levels of arsenic accumulation is the primary pathway through which arsenic gains entry into the human body. Elevated concentrations of arsenic have been linked to both immediate and long-term adverse health outcomes in humans. These include skin lesions, cardiovascular diseases, neurological disorders, and cancer. Humans consume arsenic primarily through rice, wheat, barley, and maize grains. Over 3.5 billion individuals worldwide relyon rice as their primary source of nutrition. It is a significant source of inorganic arsenic in the human diet because it accumulates arsenic more efficiently than other cereal crops, particularly in its shoots and grains (6,7).

In contrast, seafood is the primary source of non-toxic organic arsenic (o-As), such as arsenobetaine and arsenosugars. Previous research reported that certain protein-rich rice varieties increase the bioavailability of arsenic because the thiol groups in these proteins bond tightly (As III). Depending on the redox state of the soil, inorganic arsenic exists in the trivalent arsenite (As III) and pentavalent arsenate (As V) oxidation states. Arsenate predominates in aerobic soils, whereas arsenite predominates in anaerobic conditions such as flooded paddy fields (6–8).

Arsenic, a naturally occurring metalloid, is widespread as an environmental contaminant and predominantly enters the food chain through contaminated soil, water, and several commonly consumed foods. In paddy ecosystems, anthropogenic processes such as mining activities and the use of arsenic-contaminated groundwater for irrigation promote the accumulation of natural arsenic. In addition, insecticides, herbicides, feed additives, and wood preservatives containing arsenic are potential sources of arsenic in rice ecosystems (9). It could be the result of former mining or the use of fertilizers. Rice fields near factories or polluted rivers are also feared to contribute to the presence of arsenic in rice (10). Many researchers have investigated the impact of being exposed to arsenic in rice. These studiesshow that rice is the most common food exposedto chemicals, likely heavy metals. Rice is more likely to absorb inorganic arsenic (i-As) due to its cultivation in flooded soil. In populations not exposed to i-As through potable water, rice significantly contributes to i-As intake (8). Arsenic toxicity has posed a significant health risk and contributed to a severe threat to food security, notably in South and Southeast Asia, where rice is the primary source of calories.

Moreover, it is one of the most significant environmental contaminants due to its lengthy biological half-lives, non-biodegradability, and toxicity, even at low concentrations (11). The contamination of the human body with arsenic can originate from water, sediment, air, and food, both as a result of natural components and processing. The arsenic content of rice from the gold mining area (Gunung Pongkor Jawa Barat) is 0.352-3.216 mg/kg (12), and the arsenic range of rice in Aceh is 1.60 mg/kg (10). The arsenic in rice attracted researchers' attention to further testing in another area of Indonesia. Because white rice, as one of the primary food sources in Indonesia, has the opportunity to be contaminated with hazardous chemicals, improving the quality and increasing rice productivity require fertilizing and eradicating pests suspected of causing pollution. Coupled with activities that can affect pollution, such as mining coal, gold, iron, oil, and gas (10).

Considering the magnitude of the problem and the large number of individuals affected by arsenic toxicity, periodic monitoring of the arsenic content of rice is necessary. Information regarding the content of As in rice and its preparations is still minimal; it thus cannot describe the As content in rice found in Indonesia or the intake of As exposure, which can explain the toxicity hazard quotient (HQ). The availability of the latest information regarding the content of As in rice and its processed products is very much needed, considering that rice is one of the staple foods with the highest consumption level in Indonesia, reaching 0.214 kg/day per capita. Therefore, an advanced, accurate, fast, relatively easy, and environmentally friendly arsenic analysis method, such as X-ray Fluorescence (XRF), is needed. The Total X-ray Fluorescence (TXRF) method was chosen because it has a specific configuration with a critical angle of ~ 0.10, which minimizes the background and has a lowdetection limit. TXRF is an alternative method for analyzing arsenic in food with minimal preparation, fast, and accurate. Due to the limitations of inorganic arsenic analysis in Indonesia, it is necessary to estimate inorganic arsenic content using a line equation approach. Technology will probably be developed to reduce arsenic contamination in rice and the environment, and exposure control measures are required (6,8).

This study determined the total arsenic (t-As) concentrations in rice, the estimated i-As concentrations, the estimated t-As concentrations in cooked rice, and the estimated toxicity level. The primary objective of this study was to determine the arsenic concentrations in rice and the arsenic exposure risk posed by this source.

EXPERIMENTAL SECTION

Materials

This study's chemicals were analytical reagent grade. Standard reference material (SRM) National Institute Standard and Technology (NIST) 1568b Rice Flour and deionized water with a resistivity of 18.2 MΩ.cm^{-1} from water purification Millipore Direct-Q, Merck Certipur Gallium ICP standard traceable to SRM from NIST $Ga(NO_3)_3$ in HNO_3 2-3% 1000 mg/l, quartz glass (reflector), Merck Triton X-100 for gas chromatography, and Silicone solutionSERVA for siliconizing glass and metal in isopropanol, Merk Extran MA 01 liquid, alkaline, concentrate**.**

Instrumentation

TXRF S4-TStar (Bruker AXS Microanalysis GmbH, Germany) was used in this study. The instrumentation has Mo tubes operating at 1000 A and 50 KV, a multilayer monochromator, a silicon drift detector (SDD), and an energy resolution of <149 eV. Measurements are carried out for 1000 seconds, with quartz sample carriers as sample holders and reflectors.

Methods

Sample collection

Samples were collected from three areas in Sumatra and Java Island. Figure 1 illustrates the sampling location map. White rice samples were collected from granaries and traditional markets in Kecamatan Ambarawa Kabupaten Pringsewu (Lampung), Kecamatan Pucuk Kabupaten Lamongan (Jawa Timur), and Sukamelang (Subang, Jawa Barat).

Preparation sample

The rice samples were collected from granaries and traditional markets in three areas. The rice was weighted, then reduced to particle size, homogenized using a titanium eye blender (SB TI172G, Sharp), and filtered with 100 mesh (Retsch Test Sieve, 150 microns). The water content was determined by gravimetry using the Air oven method.

Water content determination

The determination of water content is carried out by the Air Oven Method (SNI 6125:2015). In the oven drying, the sample is heatedunder specific conditions. The weight loss is usedto determine the sample's water content. A 1-gram rice sample is weighed in a cup whose constant weight is known, then dried in an oven at 105°C for 3 hours or until the weight remains stable. The sample is stored in a desiccator, and after it has cooled, it is weighed. The water content of rice is calculated as a mass fraction. The water content was determined using the following equation 1 (13):

$$Water\ content = \frac{\mathrm{b-c}}{\mathrm{b-a}}\ x\ 100\% \qquad (1)$$

a = weight of the blank cup; b = weight of the sample and cup before drying; c = weight of the sample and cup after drying

Preparation T-XRF

Samples and SRM NIST 1568b Rice flour were weighed 100-200 mg into a 15-ml tube. Then, 5 mL of Triton X-100 1% and 10 µL of standard Ga 1000 mg/L are added. The sample was homogenized, and the suspensions were sonicated in an ultrasonic bath for 5 minutes to minimize agglomerations and then vortexed. The sample was pipetted to 10 µL on a siliconized quartz reflector and dried on a hotplate at a low temperature. TXRF determined the sample and SRM.

Toxicity level

To assess the carcinogenicity of the potential health risks from arsenic exposure, an estimated daily intake (EDI) for As exposure using Equation 2. This EDI is the basis for determining the hazard quotient (HQ) parameters, according to Equations 3.

$$EDI = \frac{(\mathrm{C_{As}\times IR\times ED\times EF})}{(\mathrm{BW\times AT})} \qquad (2)$$

$$HQ = \frac{\mathrm{EDI}}{\mathrm{R}_{fd}} \qquad (3)$$

Where C is the average concentration in As, IR is the rate of rice consumption (0.012 kg/day for children 13-24 months, 0.214 kg/day for adult women and men) (14,15), ED is the duration of exposure (71.2 years), EF is the frequency of exposure (365 days/year), BW is theaverage body weight (9.9 kg for children aged 13-24 months; 58.6 kg for adult female; 62.9 kg for an adult male) (15,16). AT is the meantime (365 days × ED for non-carcinogenic health risk), and Rfd is the oral reference dose (3×10^{-4} mg/kg/day) (17,18).

Figure 1. Sampling Location in Three Regions in Sumatra and Java Island (Source: 2023 Tom by Excel)

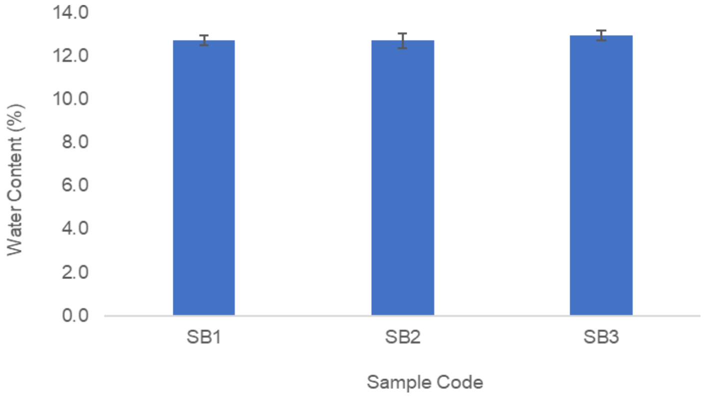

Figure 2. The Water Content of Rice, Using The Air Oven Method at 105 ^{0}C

RESULTS AND DISCUSSION

Water content

Determining the water content of food samples is a fundamental and vital step in analytical procedures. It determines the value of the nutrient intake and heavy metal toxicity in the body (19). However, water content is also complicated because it requires accurate and precise data (20). The water content in this study was determined on rice samples using the air oven method (AOAC, 2006, in SNI 6128:2015). The results of the water content determination are shown in Figure 2.

The average water content of rice is 12.8±0.13, as shown in Figure 2. The water content of rice in this study was comparable to that of Wibowo and Indrasari (2009) and Soerjandoko (2010) (21,22) for rice from the West Java region (12.1–12.4%) and Ciherang rice varieties (11.3–12.70%). Following the Indonesian National Standard (SNI) for SNI 6128:2015 rice, the water content must be less than 14% for premium quality, medium one and two, and a maximum of 15% for medium three when heated in an oven and weighed gravimetrically.

Quality Control

Table 1 presents the results of the quality control evaluation of SRM NIST Rice Flour 1568a by T-XRF. Table 1**.** It shows that the results obtained were in good agreement with certificate values. Analytical accuracy, described as % recovery, was 75–120%, while analytical precision, described as % CV, was < 0.67 CVHorwitz. Both analytical accuracy and precision were acceptable according to AOAC guidelines (23,24).

Table 1. Quality Control Assessment Using SRM NIST Rice Flour 1568a

Element	Result mg/Kg	Certified Value mg/Kg	Accuracy %	Precision %CV	Acceptable Accuracy	Acceptable Precision
As	0.275 ± 0.035	0.285 ± 0.014	96.5	12.7	75-120	13.02

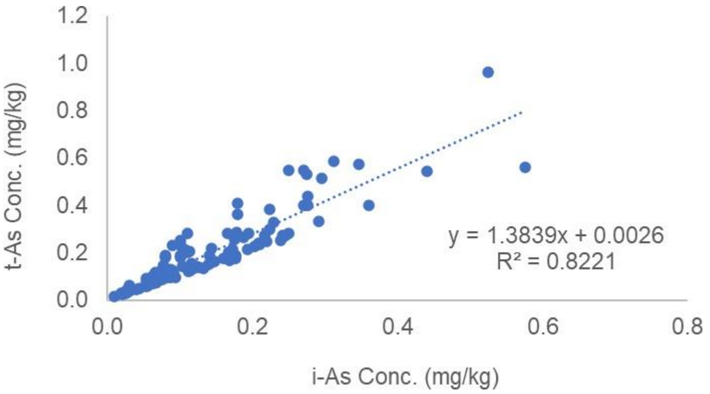

Figure 3. Correlation of (i-As) and (t-As) Levels in Rice From Various Countries (7,25–30)

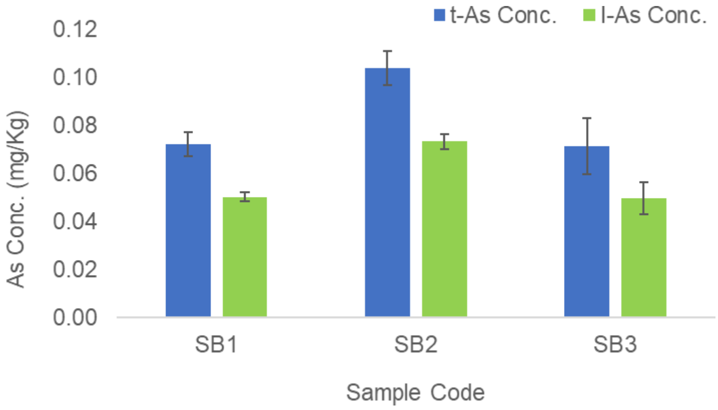

Figure 4. t-As and i-As Concentration in Rice (Wet Base)

Arsenic in rice

Numerous investigations have been conducted to determine the quantity of arsenic in rice kernels across various regions and global locations. According to several sources, rice contains 0.014 to 0.96 mg/kg of total arsenic (t-As) and 0.010 to 0.57 mg/kg of inorganic arsenic (i-As). The mean values for t-As and i-As, based on a dataset of 118 data points, are 0.201 and 0.143 mg/kg, respectively. The equation of the line shown in Figure 3 is $y = 1.3839x + 0.0026$, and the correlation coefficient, R^2, is 0.8221. There has a strong correlation between the levels of i-As andt-As, according to the studies (7,25–30). The i-As content in the rice sample can be computed by utilizing the line equation presented in Figure 3. The i-As range of SB1-SB3 is shown in Figure 4.

Figure 4 shows that the estimated value of i-As in rice is 70% of t-As. The results obtained are within the range where the i-As fraction of t-As exhibited a range of variability from 50% to nearly 92% in rice, as reported in the references (31). This finding is consistent with a previous study conducted in Asian nations, which established a linear correlation between total arsenic and inorganic arsenic, accounting for an average of 78% of the total arsenic content (32). In contrast, the rice produced in the United States demonstrates a hyperbolic correlation between inorganic and total arsenic. The relationship between inorganic arsenic and total arsenic in US rice is hyperbolic, whereby the maximum values of inorganic arsenic are 0.15 mg/kg, and the maximum values of total arsenic exceed 0.4 mg/kg. According to the European Food Safety Authority (2009), i-As is carcinogenic and highly concentrated in rice.

Additionally, the FAO/WHO (2010) states that acute exposure to high levels of i-As can result in symptoms such as vomiting, abdominal pain, and diarrhea. Long-term exposure to i-As is associated with various health issues, including the development of skin lesions, diabetes, hypertension, and cardiovascular diseases. The concentration of inorganic and organic arsenic in rice grains is subject to variability, contingent upon factors such as rice variety, growth conditions, and geographical origin. The augmentation of dissolved arsenic levels in grain is plausibly attributed to irrigation water, as indicated by previous research (31–33). The level of arsenic in rice can be reduced by employing techniques such as rinsing the rice and cooking it in water with a lower arsenic content (8).

The present study reveals that the levels of arsenic detected suit those reported in various regions, including Jakarta, Spain, India, Jamaica, Qatar, Taiwan, the Himalayas, Bangladesh, and Belgium, specifically in basmati and wild rice (Table 2). However, the results of this investigation exhibit a lower magnitude compared to the corresponding figures reported in Italy, Kazakhstan, Portugal, Belgium (about white rice), Japan, and Korea. Such phenomena can be attributed to the fluctuation in the concentration of arsenic in cereals, particularly rice, which is contingent upon their geographical location and provenance. The observed variation in arsenic levels in raw rice across diverse Asian nations suggests that the results are consistent with those documented in other global regions. The elevated levels of arsenic detected in rice grains from fields in Bangladesh and India can be attributed to significant variations in the amount of arsenic in the soil, influenced by irrigation water, as documented in reference (39).

The level of arsenic present in rice is subject to various factors, including but not limited to geographic location, growing circumstances, soil composition, pollution levels in irrigation water, agricultural practices, genetic diversity, soil biogeochemistry, and culinary practices. In previous research, the variability of arsenic content in rice collected from the Murshidabad district of West Bengal, India, ranged from 0.04 to 0.61 mg/kg. A significantly elevated level of arsenic was also detected in Bangladesh. Islam and colleagues (2017) performed a household survey at the district level to evaluate the geographical dispersion of arsenic in rice grain. The total arsenic content was found to vary from 0.003 to 0.680 mg/kg, with an average of 0.126 mg/kg on a dry basis, as documented in references (28,37,39)

Table 2. As content in rice (dry base)

Country/city	Variety of rice	Total Arsenic (mg/Kg)	Reference
SB 1	White	0.081 ± 0.006	In this study
SB 2	White	0.117 ± 0.008	In this study
SB 3	White	0.080 ± 0.013	In this study
Jakarta	White	0.085 ± 6.50	(15)
Spain	White	0.076 - 0.169	(26)
	Brown	0.116 - 0.229	
Italy	White	0.180 - 0.280	(27)
India	basmati extra long	0.074 ± 0.026	(31)
Jamaica	Red	0.050 - 0.490	(33)
Kazakhstan		0.240	(28)
Portugal		0.360	(28)
Belgium	White	0.216	(29)
	Basmati rice	0.061	
	Wild rice	0.108	
Qatar		0.0413 - 0.169	(34)
India		0.092	(7)
India		0.283	(30)
Japan	Brown	0.239	(35)
Taiwan	White	0.117	(36)
Himalaya	White basmati extra long	0.048 ± 0.010	(31)
Bangladesh		0.126	(37)
Korea		0.247	(38)

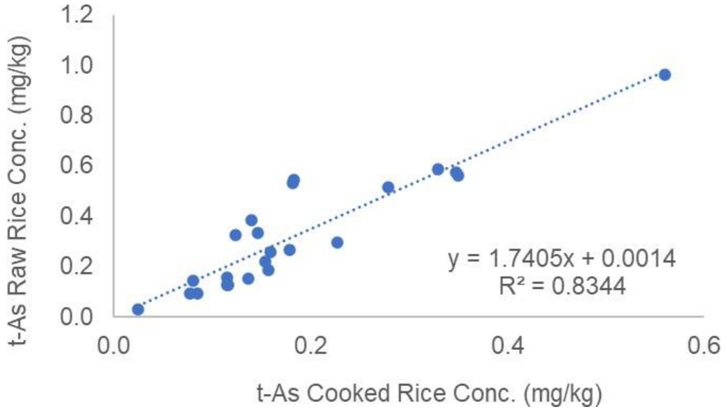

Figure 5. Correlation of (t-As) Levels of Raw and Cooked Rice (30)

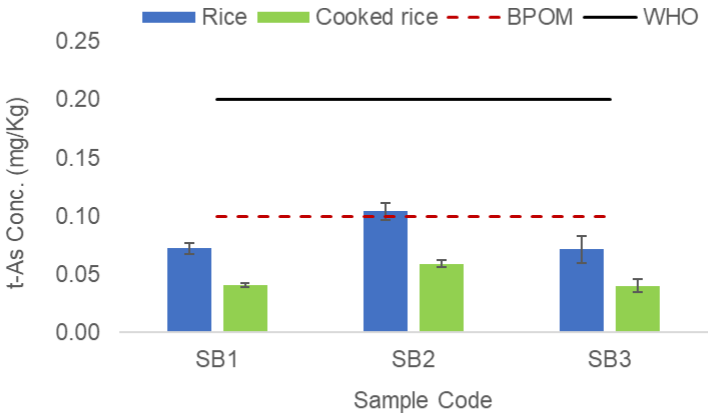

Figure 6. Total Arsenic Content in Rice and Cooked Rice With SB1-3 Label by TXRF (Wet Case) and Maximum Level of Arsenic in Rice and Rice Products by BPOM and WHO (40,41)

Arsenic in raw and cooked rice

Figure 5 shows the t-As concentration in paired raw and cooked rice samples (n = 23) was found to be 0.028–0.961 mg/kg (mean 0.295 mg/kg) in the raw samples and 0.025–0.560 (mean 0.172 mg/kg) in cooked rice, respectively. The t-As content in rice has a reasonable correlation with the t-As content in cooked rice, with $R^2 = 0.8344$. Therefore, it can be estimated to determine the t-As content in the analyzed rice sample using the equation $y = 1.7405x + 0.0014$. Based on the literature, there was up to a 34.6% decrease in cooked rice (30). The data aligns with the explanation provided by Altobiti et al. (2018), indicating a reduction of 3–43% (1). It has been reported that washing rice before preparing or boiling it in copious amounts of water could reduce the arsenic content by as much as 60% (26). However,the impact of the cooking method on arsenic concentrations in rice requires further investigation.

Figure 6 shows a 44% reduction in the estimated concentration of t-As in cooked rice. In general, the levels of arsenic present in rice and boiled rice are deemed safe for human consumption as they do not exceed the maximum limits set by BPOM and WHO. Nevertheless, the consumption of rice is substantial in Indonesia. It is imperative to monitor the consumption of heavy metals due to the adverse effects of arsenic exposure on almost all human body organs, leading to a range of health complications such as skin lesions, cancer, diabetes, and lung disorders (42).

The suggested daily intake for t-As was determined after considering the amounts that adults and children consumed. Figure 7 shows the EDI t-As values of the SB1-SB3 rice samples for children aged 13–24 months and adults were 5.0×10^{-5}–7.4×10^{-5}, 1.4×10^{-4}–2.2×10^{-4} mg/kg BW/day, respectively. Children's and adults' daily intake of arsenic is still within safe limits for arsenic exposure to food. The European Food Safety Authority (EFSA) concluded that it would be appropriate to identify a range of possible total exposure benchmark dose lower limits (BMDL) for 3×10^{-4}-8×10^{-3} mg/kg BW/day) (43,44).

Toxicity level

The contamination of rice with arsenic presents a significant hazard to human health, particularly in areas where rice constitutes the principal dietary staple. The consumption of rice daily has led to substantial health concerns regarding the intake of arsenic. Arsenic species can be determined to assess the potential health hazards posed by their toxic properties. Scientists have developed measures such as the hazard quotient (HQ) to evaluate the possible non-carcinogenic effects of arsenic exposure (39). The HQ value is determined using Reference Dose (RfD) values established by the Environmental Protection Agency (EPA). The provided figures represent a calculated approximation of the amount of daily exposure that the entirety of the human population, including those particularly vulnerable, can endure throughout their lifespan without incurring a substantial probability of adverse consequences (45).

The assessment of non-carcinogenic health risks (as shown in Figure 8) revealed that the HQ values associated with the exposure of both adults and children to trace elements and heavy metals were all found to be below 1 (HQ<1). As per the study's findings, the values of adult HQ were observed to vary between 0.44 and 0.69. By comparison, children's HQ values were the highest, ranging from 0.16 to 0.24. The results of this investigation suggest that there are no discernible non-carcinogenic health risks for adults and children due to arsenic exposure in an individual basis. The characterization of overall health risks for adults and children cannot be adequately determined solely based on identifying specific risks associated with arsenic exposure. Aggregating exposure to trace elements and heavy metals may pose more significant health risks than singular exposure to these substances. Nevertheless, it has been observed that adults exhibit a greater propensity to present non-carcinogenic health hazards owing to a comparatively elevated total hazard quotient in contrast to minors (46).

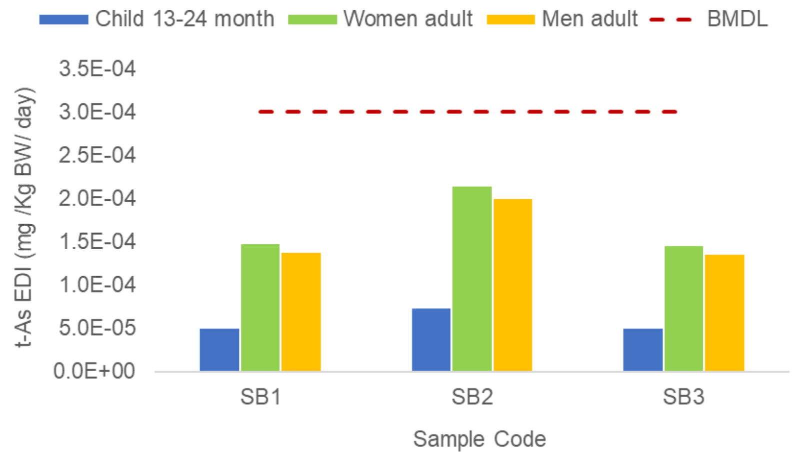

Figure 7. Intake t-As from Cooked Rice

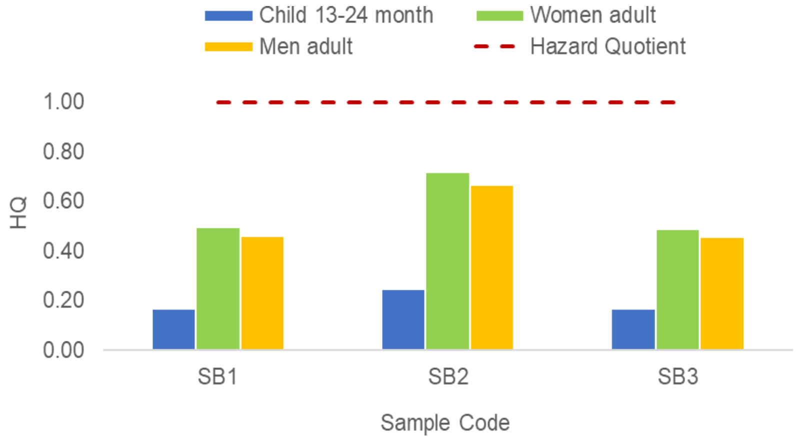

Figure 8. Hazard quotient (HQ) values for adults and children in cooked rice

CONCLUSION

The results showed that total arsenic levels (wet base) in rice samples from three regions ranged from 0.071 to 0.104 mg/kg, and the estimation of inorganic arsenic levels from 0.050 to 0.073 mg/kg within the BPOM and WHO maximum limits and in line with research in other areas. Washing and cooking rice can reduce the arsenic content by up to 44%. There is no non-carcinogenic risk with an HQ<1. Hopefully, this research can be more than comprehensive and serve as a scientific-based reference in formulating regulations to minimize the potential for arsenic pollution.

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