INTERNATIONAL JOURNAL OF RESEARCH AND SCIENTIFIC INNOVATION (IJRSI)
ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

Proximate Composition and Human Health Risks from Heavy Metal Contents in Commonly Consumed Rice Varieties of Barishal District, Bangladesh

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DOI: https://doi.org/10.51244/IJRSI.2025.120700070

Received: 08 July 2025; Accepted: 12 July 2025; Published: 02 August 2025

ABSTRACT

A significant issue in food safety is the depletion of vital components in rice, Bangladesh's main agricultural product, due to various genetic and biological factors. A randomized sampling approach was employed to gather five popular rice varieties—Paijam, Swarna Bullet, BRRI Dhan-28, BRRI Dhan-29, and Chinigura—from different retail outlets in five upazilas within the Barishal district. This research focused on analyzing their proximate composition, heavy metal content, and related health risks. The proximate composition and heavy metal concentrations were assessed using AOAC methods and ICP-MS, respectively. The findings revealed notable differences ($P < 0.05$) in the proximate compositions among the rice types. The average TTHQ levels for both adults and children were below 10, indicating no significant non-carcinogenic health threats. However, the THQ values for Pb in the Paijam and BRRI Dhan-28 varieties exceeded 1, suggesting potential health risks for both children and adults. Conversely, in Swarna Bullet, the THQ values for Pb and Se surpassed 1, posing possible health risks for children. TR values for Pb and Cd were below 10^{-6} in all samples for both adults and children, indicating they are not carcinogenic. The TR value of Cr for children across all samples, and similarly, the TR values for adults in Pijam and BRRI Dhan-29, exceeded 10^{-6}, indicating a significant carcinogenic health risk, with children being more susceptible. This suggests that individuals who frequently consume these rice varieties may face an increased risk of developing cancer in the future.

Keywords: Rice varieties; Heavy metals; EDI; THQ; TR.

INTRODUCTION

Rice, a fundamental grain, accounts for half of the global daily caloric intake [50]. Nearly 40% of the world's population relies on it. Over 90% of rice is consumed by Asians. China, Taiwan, and India are responsible for producing half of the world's rice supply [65]. Bangladesh ranks among the highest in per capita rice consumption globally. Asian rice, derived from the Oryza sativa plant, is one of the most popular foods worldwide, with over 40,000 different varieties [44]. Referred to as the queen of grains, it is a bountiful source of carbohydrates, proteins, vitamins, minerals, and fiber [49]. In developing countries, it provides 25% of the total dietary energy, 20% of dietary protein, and 3% of dietary fat, contributing 715 kcal per day [36].

The proximate composition of rice includes moisture, carbohydrates, proteins, dietary fibers, fatty acids, ash, and dietary minerals. It is a rich source of carbohydrates, making up approximately 87% of its total weight, with a protein content of 7–8%, and offers significant nutritional and biological benefits. It has a moisture content of 10%, along with lower levels of crude fiber, fat (1-2%), and ash (1–2%) [50].

Heavy metals, which are dense metals and metalloids that are toxic even in minute concentrations, represent a major environmental hazard to agriculture worldwide [4]. These metals, originating from both natural and anthropogenic activities such as mining, industrial waste, and vehicle emissions, are toxic and resistant to biodegradation [44]. Their persistent presence in soil raises concerns about food security and poses a global public health risk [30]. Due to their widespread distribution in the environment, people are often unknowingly exposed to heavy metals [19]. Soil contamination directly exposes individuals to heavy metals like Cr, Ni, Cd, Pb, and As, which are identified as the most dangerous by the US Environmental Protection Agency (USEPA) [21]. Heavy metals can accumulate in human tissues and present a serious health threat when ingested from fruits, crops, and vegetables grown in contaminated soil [19]. Eating tainted food can be detrimental to human health, as food security is crucial for human survival [30]. Lead (Pb) and its compounds are stable and challenging to remove from soil, leading to health problems across various bodily systems. Pb exposure raises estrogen levels, which is a risk factor for breast cancer, and influences the behavior, cognitive skills, and postnatal development of newborns. The renal, cardiovascular, neurological, hematopoietic, and reproductive systems are all affected by lead exposure [25]. Cadmium (Cd) poses a significant problem in countries such as China, Bangladesh, India, and Pakistan due to its non-biodegradable nature and its presence in food chains. Cd bioaccumulation leads to oxidative stress, worsens diseases, and disrupts antioxidant defenses [47]. Among the various forms of chromium present in the environment, Cr (VI) and Cr (III) are the most stable, with hexavalent chromium being the most dangerous. Trivalent chromium can reduce the risk of diabetes and enhance insulin function. However, excessive exposure to trivalent chromium may lead to long-term toxicity and cancer risk. Cr (VI) can enter the human body through inhalation, ingestion, and skin contact, leading to pathological changes and an increased risk of cancer [64]. In Bangladesh, people are exposed to arsenic through drinking water and contaminated food, which results in skin lesions and affects multiple body systems, including the neurological, hepatic, renal, hematological, endocrine, respiratory, and reproductive systems [40]. Water, a vital natural resource rich in essential minerals, is highly esteemed for its diverse applications. Human activities are leading to an increase in heavy metal levels in water. The primary cause of heavy metal contamination in rice is irrigation. Barishal city, encircled by rivers, is susceptible to metal and human activity-related contamination, as farmers mainly rely on river water for irrigation. Other significant nutritional metals such as Fe, Zn, Ni, Co, and Cu are needed in trace amounts within a specific range, beyond which they pose a risk. In recent years, rapid development has led to a steady influx of heavy metals and pollutants into the soil [65].

This research involved sampling various upazilas in Barisal. There is no previous research on heavy metal levels in this area. The current study aims to establish baseline data to enable future comparisons over time and with other studies. The objective was to determine the proximate composition and levels of four heavy metals (Pb, Cd, Cr, Se) in rice varieties commonly consumed by residents of the Barisal district in Bangladesh. Additionally, the study examined factors such as estimated daily intake, target hazard quotient, total target hazard quotient, and target carcinogenic risk to evaluate potential health risks. The results are valuable for assessing heavy metal contamination in rice grains and associated health risks, as well as for evaluating preventive measures. It also aids in comparing Bangladesh with other countries in this domain.

MATERIALS AND METHODS

Study area

Bangladesh is situated in South Asia, with its geographical coordinates ranging from 26°38' to 20°34' north latitude and 92°41' to 88°01' east longitude [5]. The Barishal district spans an area of 2,797 square kilometers, located at Latitude: 22° 42' 17.89" N and Longitude: 90° 22' 12.47" E. For the purpose of collecting rice samples, five upazilas within the Barishal district—Barishal Sadar, Gournadi, Wazirpur, Babugonj, and Bakergonj—were chosen due to their dense populations (Fig. 1).

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

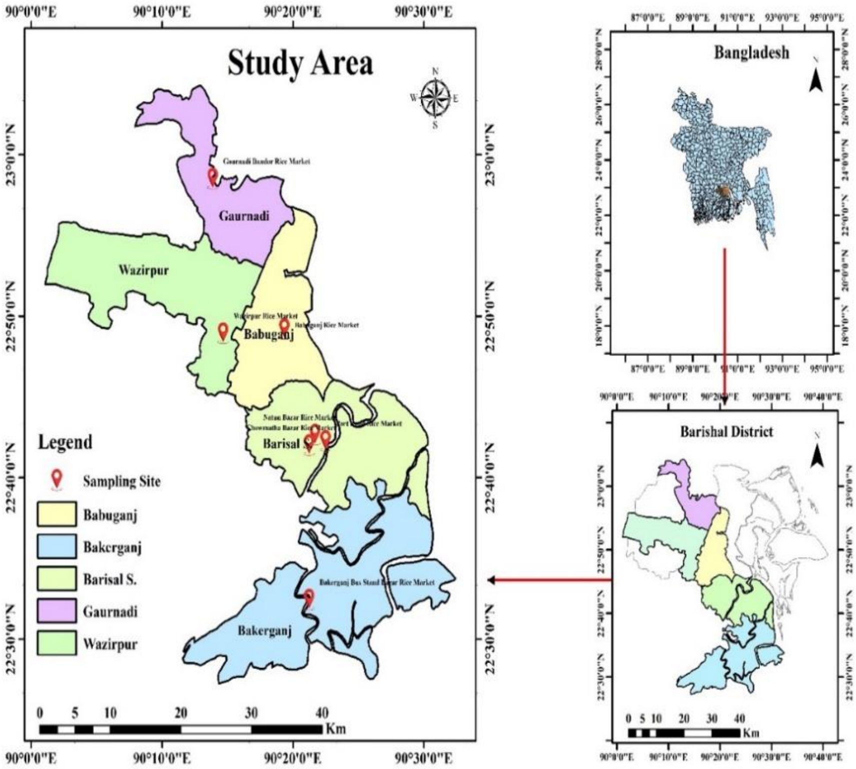

Fig 1. Sampling sites (different wholesale markets of selected upazila) of the Barisal district.

Sample collection

In the selected upazilas of Barishal district, Bangladesh, the most frequently consumed rice varieties were identified through direct interviews conducted at wholesale markets. This study utilized a structured questionnaire to select five rice varieties that were popular in the chosen upazilas. Approximately 250 grams of each variety were bought from three different shops within a single market. These samples were then mixed uniformly to create a single sample and stored in separate zipper bags before undergoing the labeling process.

Sample preparation

The composite sample for each type of rice was blended using a food processor. A 100 g portion of the ground samples was kept in the Laboratory of Biochemistry and Food Analysis at Patuakhali Science and Technology University, stored at -20°C in a plastic zip-lock bag to prevent contamination from external metals/metalloids and to preserve internal concentrations.

Proximate composition analysis

The biochemical components of the composite samples were assessed using the standard AOAC, (2010) [2] method with the procedure of Chakma et al., (2024) [8]. Moisture content was measured using a hot air oven (model: HAS/50/TDIG/SS, brand: Genlab, UK) set at a constant temperature of 105 °C for 24 hours. Ash content was determined with a muffle furnace (model: HM-9MP, brand: Raypa, Spain) at 550 °C for 6 hours. The crude protein content was analyzed using the Kjeldahl apparatus (model: Bloc Digest 12, brand: JP Selecta, Spain), and the resulting nitrogen (N_2) was converted to crude protein using a conversion factor of 5.85. The total lipid fractions in the various rice variety samples were examined using a chloroform and methanol mixture (2:1, v/v) following the method Folch et al., (1957) [15]. The carbohydrate or nitrogen-free extract (NFE) value was calculated using the method by James, 2013 [23]: Carbohydrate = 100 – [moisture + ash + crude protein + fat + fiber]. The energy value was estimated using the approach of Crisan & Sands (1978): Energy (Kcal/100g) = (2.62 × % protein) + (8.37 × % lipid) + (4.2 × % carbohydrate).

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Heavy metal analysis and quality control

The composite rice grain samples were ground into a fine powder using a grinding machine, then weighed with an analytical balance (EK 300H), and oven-dried (approximately 15 g at 110 °C) for 24 hours. Desiccators and crucible tongs were employed to handle empty beakers and samples, facilitating the cooling of heated items and moisture absorption in humid conditions. Once dried, the samples were placed in a muffle furnace (model: WISD) at 150 °C, with the temperature gradually increased to 600 °C over 4 hours, and maintained at this level for an additional 10 hours. The remaining ash was digested with 10 ml of HNO_3, 2.5 ml of H_2O_2, and deionized water on a hotplate at 150 °C, then filtered into a 25 ml volumetric flask using Whatman filter paper, topped up with DI water, and stored until analysis. Heavy metals (Se, Pb, Cr, and Cd) were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). Proper quality assurance measures and safety precautions were implemented to ensure the accuracy of the results. The entire analysis was conducted with deionized water. The reagents were of analytical grade, and the glassware used for metal quantification was soaked in 20% HNO_3 overnight. To check for contamination, 5 samples were mixed with a blank sample. Standards from each metal's stock solution were prepared to calibrate the instrument. Repeated analyses were conducted to confirm the precision and accuracy of the analysis, with an accuracy level between 1 and 2%. Certified reference materials for rice grains were supplied by the BCSIR, Dhaka, Bangladesh, to ensure quality.

Heavy metal risk assessment

Estimated daily intake of heavy metals

The calculation of the daily intake of heavy metals from rice was performed using this equation [54,17].

$$EDI = \frac{FIR \times C}{BW} \quad (1)$$

The FIR represents the rate at which food is consumed (mg person^{-1} day^{-1}). According to the Household Income and Expenditure Survey of Bangladesh [20], the food consumption rate is 367.19 g per person per day for adults and 200 g for children [20]. The variable C stands for the concentration (mg kg^{-1}, fresh weight), while BW refers to the average body weight, which is 60 kg for adults and 25 kg for children [39,44].

Target hazard quotient (THQ)

The reference dosage, or THQ, is identified as the highest level at which no negative health effects are expected, according to the (USEPA, 1989) [56]. It is calculated as the ratio of exposure to the hazardous element. The procedure for determining THQ for individual metals or metalloids is outlined in the United States EPA, Region III risk-based concentration table [46]. This study calculated the previously mentioned non-carcinogenic health risks associated with local food consumption [21].

$$THQ = \frac{EFr \times ED \times FIR \times C}{RfD \times BW \times AT} \times 10^{-3} \quad (2)$$

The target hazard quotient (THQ) is calculated using several factors: EFr, the exposure frequency, is set at 365 days per year [60, 41], and the exposure duration (ED) is 70 years, which corresponds to the average human lifespan [41]. FIR represents the daily rice consumption rate in grams per person in the study area, while C denotes the concentration of metals in food, measured in mg/kg fresh weight. RfD is the oral reference dose, expressed in mg/kg/day, and Bw stands for the average body weight. AT is the averaging time for non-carcinogens, calculated as 365 days per year multiplied by the number of exposure years, assuming a 70-year lifespan [41]. The oral reference doses are specified as 0.0035, 1.5, 0.005, and 0.001 mg/kg/day for Pb, Cr, Se, and Cd, respectively [51,12,67]. If THQ is less than 1, the exposed population is unlikely to experience significant adverse effects. However, if THQ is equal to or greater than 1, there may be potential health risks, necessitating the implementation of preventive measures and actions [56,18,21].

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Combined risk of multiple metals

The TTHQ is calculated by summing the THQs of all heavy metals. To find the TTHQ, the total THQ (Eq. 3) was utilized [56,13].

TTHQ (individual foodstuff) = THQ (toxicant 1) + THQ (toxicant 2) + ... + THQ (toxicant n) (3)

When TTHQ is at 1.0, the chances of negative outcomes are unlikely. However, if TTHQ exceeds 1.0, it suggests a potential for adverse effects. When TTHQ is greater than 10, there is a considerable risk of long-term negative health consequences [56,26].

Target carcinogenic risk (TR)

The TR resulting from the consumption of heavy metals was determined using Eq. 4 from the USEPA Region III risk-based concentration table [56,21].

$$TR = \frac{EFr \times ED \times FIR \times C \times CsFo}{BW \times AT} \times 10^{-3} \qquad (4)$$

While AT represents the average time for carcinogens (365 days/year ED), TR denotes the target cancer risk or lifetime cancer risk, and CSFo refers to the oral carcinogenic slope factor, which are 8.5×10^{-3}, 0.50, and 15 (mg/kg/day) for Pb, Cr, and Cd, respectively [56, 67]. In this research, the carcinogenic risk was assessed by considering the heavy metals Pb, Cr, and Cd along with their CSFo values.

Statistical analysis

The statistical software MINITAB 18 (Minitab Inc., State College, PA, USA) was utilized for the analysis of the data. We calculated the mean and standard deviation for the proximate composition and heavy metal content across five different rice varieties. To assess the data, a one-way analysis of variance (ANOVA) was conducted. Tukey's pairwise comparison tests were used to determine statistical significance at a P-value of 0.05.

RESULTS

Proximate composition of rice varieties

Table 1 displays the results of the moisture content analysis for various rice varieties. To determine moisture levels, samples were dried in an oven overnight at temperatures between 100-110 ºC and subsequently cooled in a desiccator. The weight reduction is considered the moisture content. Notable differences in moisture content were observed among the selected rice varieties. The moisture content varied from 10.92% to 12.39%, with BRRI Dhan-28 having the highest moisture content at 12.39%, and Swarna Bullet having the lowest at 10.92%. The moisture content for Paijam, BRRI Dhan-29, and Chinigura was 12.14%, 11.69%, and 11.44%, respectively. In a Soxhlet apparatus, petroleum ether (60–80 ºC) was employed to extract lipids from rice samples, uncovering notable differences among the chosen varieties. Chinigura (0.58%) and Paijam (0.53%) exhibited high lipid levels, whereas other varieties such as BRRI Dhan-28 (0.27%) and Swarna Bullet (0.30%) demonstrated lower lipid content (Table 1). The protein content in rice, which can constitute up to 8% of the grain, plays a crucial role in its nutritional value due to its distinct amino acid profile. The research revealed notable variations in crude protein levels across five rice varieties. BRRI Dhan-28 exhibited the highest protein content at 7.74%, while Paijam had the lowest at 6.21%. The remaining varieties showed protein contents of 7.37%, 6.86%, and 6.43%, respectively (Table 1). The samples were subjected to a temperature of 550 ºC in a muffle furnace, resulting in the formation of white or greyish-white ash, which was then weighed directly. As shown in Table 1, the ash content percentage varied between 5.66% and 7.00%. Among the rice varieties, Swarna Bullet exhibited the highest ash content, whereas Paijam had the lowest, with Chinigura following closely. To determine crude fiber, samples were treated sequentially with 1.25% H_2SO_4, 1.25% NaOH, and 1% HNO_3, followed by filtration and rinsing with hot water after each treatment. The remaining material was then dried in an oven at 130ºC and subsequently ashed in a furnace at 550ºC. The loss in weight upon ignition was used to calculate the crude fiber content. The experiment showed crude fiber levels ranging from 2.14% to 2.45%, with BRRI Dhan-29 exhibiting the highest content, followed by Swarna Bullet and Chinigura, while BRRI Dhan-28 had the lowest content (Table 1). The carbohydrate content in the rice samples ranged from 71.04% to 73.50%, which is close to the ideal level of nearly 80%, indicating they are a good carbohydrate source. Among the five rice varieties, Chinigura had the highest carbohydrate content at 73.50%, while BRRI Dhan 28 had the lowest at 71.04%. There was a notable (P<0.05) variation in the energy values among the different rice varieties. The energy values spanned from 321.73 to 330.40 kcal/100g. Among the five rice varieties examined, Chinigura exhibited the highest energy value at 330.40 kcal/100g, while BRRI Dhan 29 recorded the lowest at 321.73 kcal/100g.

Table 1: Analysis of the proximate composition of five popular rice varieties in Barishal City.

Proximate Composition	Paijam	Swarna Bullet	BRRI Dhan 28	BRRI Dhan 29	Chinigura	PValue
Moisture	12.14 ± 0.13^{b}	10.92 ± 0.03^{e}	12.39 ± 0.00^{a}	11.69 ± 0.02^{c}	11.44 ± 0.00^{d}	0.001
Crude Lipid	0.53 ± 0.02^{a}	0.30 ± 0.02^{c}	0.27 ± 0.01^{c}	0.38 ± 0.01^{b}	0.58 ± 0.04^{a}	0.001
Crude Protein	6.21 ± 0.05^{e}	6.86 ± 0.09^{c}	7.74 ± 0.03^{a}	7.37 ± 0.02^{b}	6.43 ± 0.12^{d}	0.001
Ash	5.66 ± 0.23^{c}	7.00 ± 0.15^{a}	6.42 ± 0.09^{b}	6.86 ± 0.06^{ab}	5.70 ± 0.07^{c}	0.001
Crude Fiber	2.32 ± 0.06^{b}	2.39 ± 0.03^{ab}	2.14 ± 0.02^{c}	2.45 ± 0.01^{a}	2.36 ± 0.01^{ab}	0.001
Carbohydrate	73.13 ± 0.10^{a}	72.53 ± 0.18^{b}	71.04 ± 0.10^{c}	71.25 ± 0.04^{c}	73.50 ± 0.19^{a}	0.001
Energy (kcal/100g)	327.89 ± 0.6^{b}	325.08 ± 0.9^{c}	320.91 ± 0.5^{d}	321.73 ± 0.3^{d}	330.40 ± 0.3^{a}	0.001

The values are presented as means ± SD based on three measurements. Within the rows, different superscripts indicate significant differences (P<0.05).

Heavy metal levels of rice varieties

Table 2 presents the mean levels of toxic metals such as Pb, Cd, Cr, and Se in selected rice types, measured in milligrams per kilogram (mg/kg). The concentrations of these heavy metals in rice showed significant (P<0.05) differences among the different rice species. The study ranked the metal concentrations in different rice varieties in descending order as Cr>Pb>Se>Cd. The samples collected exhibited varying Pb levels, ranging from 0.2996 to 0.8384 mg/kg, with Paijam having the highest concentration and Chinigura the lowest. Other varieties, such as BRRI Dhan 28, BRRI Dhan 29, and Swarna Bullet, contained Pb concentrations of 0.6906 mg/kg, 0.4286 mg/kg, and 0.4645 mg/kg, respectively. According to the permissible Pb levels set by the World Health Organization, the Ministry of Health of the People's Republic of China (MHPRC), the United States Environmental Protection Agency (USEPA), and the Bangladesh Standards and Testing Institution (BSTI), the limits are 0.2, 0.2, 0.1, and 0.3 mg/kg, respectively [61,32,54,4].

Table 2: Concentrations of heavy metals (mg/kg) in different rice varieties from Barishal city.

Rice Varieties	Pb	Cd	Cr	Se	References
Paijam	$0.8384^{a} \pm 0.0047$	$0.0075^{a} \pm 0.0017$	$3.4264^{b} \pm 0.0294$	$0.5845^{b} \pm 0.0163$	
Swarna Bullet	$0.4645^{c} \pm 0.0163$	$0.0073^{a} \pm 0.0017$	$2.3830^{d} \pm 0.0531$	$0.6858^{a} \pm 0.0125$	
BRRI Dhan 28	$0.6906^{b} \pm 0.0216$	$0.0066^{a} \pm 0.0017$	$2.4204^{d} \pm 0.0492$	$0.3313^{e} \pm 0.0125$	

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

BRRI Dhan 29	0.4286[c]±0.0047	0.0055[a]±0.0009	3.6566[a]±0.0368	0.4326[c]±0.0082	
Chinigura	0.2996[d]±0.0294	0.0080[a]±0.0017	3.2319[c]±0.0262	0.3820[d]±0.0170	
Average	**0.5443±0.0153**	**0.0070±0.0015**	**3.0237±0.0389**	**0.4832±0.0133**	**This Study**
Guidelines values					
	0.2	0.1	0.1	-	[14, 61]
	0.1	0.4	-	-	[52]
	0.2	0.2	0.1	-	[32]
	0.3	0.2	-	-	[6]
Comparison with recent literature					
Bangladesh					
Rice Cultivars of Bangladesh	0.24±0.06	0.07±0.02	0.91±0.14	-	[42]
Rice Cultivars of Bangladesh	0.49±0.0049	0.10±0.0012	0.99±0.0091	-	[21]
Rice Cultivars of Bangladesh	0.07−1.3	0.001−0.073	0.26−4.2		[21]
International					
Pigmented and non-pigmented Rice varieties	-	0.05 ± 0.07	0.23 ± 0.08	0.03 ± 0.02	[27]
Rice varieties	0.1–0.82	0.11–0.56	0.23–1.09	-	[10]
Rice varieties	0.009–1.959	0.013–2.066	0.019–4.583	-	[68]
Rice varieties	0.003–0.089	0.020–1.456	ND–4.226	-	[58]
Cooked Rice	0.11	0.16	2.7	-	[37]
Rice varieties	-	0.0100–0.6400	-	-	[17]
Rice varieties	0.003–0.103	0.005–2.089	0.029–0.508	-	[67]

The values are presented as means ± SD based on three measurements. Within the rows, different superscripts indicate significant differences ($P<0.05$).

The levels of Cadmium (Cd) detected in rice samples varied between 0.0055 mg/kg and 0.0080 mg/kg, with Chinigura showing the highest concentration and BRRI Dhan-29 the lowest. According to the WHO, the permissible limit for Cd in rice is 0.1 mg/kg, whereas the USEPA allows up to 0.4 mg/kg, and both MHPRC

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

and BSTI set the limit at 0.2 mg/kg. Rice samples exhibited different levels of Cr, ranging from 2.3830 to 3.6566 mg/kg. Among them, BRRI Dhan 29 contained the highest amount, followed by Paijam, Chinigura, BRRI Dhan 28, and Swarna Bullet. The study also identified varying Se concentrations, from 0.3313 to 0.6858 mg/kg, with Swarna Bullet having the highest and BRRI Dhan-28 the lowest. Other rice samples showed Se levels as follows: Paijam at 0.5845 mg/kg, BRRI Dhan-29 at 0.4326 mg/kg, and Chinigura at 0.3820 mg/kg.

Estimated daily intake of metals

Table 3 forecasts the dietary intake of heavy metals through rice consumption, emphasizing potential exposure and levels that might surpass safety limits. The daily intake and Estimated Daily Intake (EDI) figures for Pb, Cd, Cr, and Se from Paijam rice, Swarna Bullet rice, BRRI Dhan-28 rice, BRRI Dhan-29 rice, and Chinigura rice differed for adults and children in the examined region. Our research indicated the estimated daily intake range of these metals from various rice types in milligrams per Kg per day ($mg.Kg^{-1}.d^{-1}$) for both demographics: Pb (0.0051-0.0.0018), (0.0067-0.0024); Cd (0.0000-0.0000), (0.0001-0.0001); Cr (0.0224-0.0146), (0.0293-0.0191); and Se (0.0042-0.0020), (0.0055-0.0027). These findings indicate that, for both adults and children, the sequence of EDI values for these four trace metals from rice grains is: Cd < Se < Pb < Cr. Importantly, the EDI values for these metals from rice grains were below the Maximum Tolerable Daily Intake (MTDI) values recommended by WHO, except for Pb from Paijam and BRRI Dhan-28, suggesting that metal intake from rice does not exceed safe limits.

Table 3: Estimated Daily Intake (EDI; exposure per day) of heavy metals in various rice varieties.

EDI ($mg.kg^{-1}.d^{-1}$)								
Rice varieties	**Pb**		**Cd**		**Cr**		**Se**	
	Adult	**Child**	**Adult**	**Child**	**Adult**	**Child**	**Adult**	**Child**
Paijam	0.0051	0.0067	0.0000	0.0001	0.0210	0.0274	0.0036	0.0047
Swarna Bullet	0.0028	0.0037	0.0000	0.0001	0.0146	0.0191	0.0042	0.0055
BRRI Dhan-28	0.0042	0.0055	0.0000	0.0001	0.0148	0.0194	0.0020	0.0027
BRRI Dhan-29	0.0026	0.0034	0.0000	0.0001	0.0224	0.0293	0.0026	0.0035
Chinigura	0.0018	0.0024	0.0000	0.0001	0.0198	0.0259	0.0023	0.0031
TDI [62]	**0.0036**	**-**	**0.001**	**-**	**1.5**	**-**	**0.05**	**-**

Health risk assessment

Non-carcinogenic health risk

Target hazard quotient (THQ)

In our research, we employed the THQ to evaluate the potential health risks to humans from consuming rice grains contaminated with metals, focusing on both adult residents and children. Table 4 displays the THQ values for adults and children. The target hazard quotient (THQ) serves as an indicator of health risk based on non-carcinogenic effects. In terms of heavy metal exposure, THQ values under 1.0 are deemed “safe,” those between 1.0 and 5.0 suggest “a potential risk of adverse effects,” and values over 5.0 indicate “an unsafe level of exposure.” [68].

The findings of our study indicate that the THQ values for heavy metals in rice samples are below 1 for each metal, with the exception of Pb in Paijam (adult 1.4652; child 1.9163), BRRI Dhan-28 (adult 1.2069; child 1.5785), Swarna Bullet (child 1.0617), and Se in Swarna Bullet (child 1.0973). The TTHQ is an indicator used to assess the cumulative non-carcinogenic impacts of various metals. As shown in Table 4, the TTHQ values associated with rice consumption consistently surpassed 1 across all rice types. It is noteworthy that nearly all rice varieties exceeded the acceptable thresholds, with children being significantly more susceptible than adults.

Table 4: Assessment of target hazard quotient (THQ) and cumulative target hazard quotient (TTHQ) for heavy metals across various rice types.

THQ									TTHQ	
Rice varieties	**Pb**		**Cd**		**Cr**		**Se**			
	Adult	**Child**	**Adult**	**Child**	**Adult**	**Child**	**Adult**	**Child**	**Adult**	**Child**
Paijam	1.4652	1.9163	0.0459	0.0600	0.0140	0.0183	0.7150	0.9352	2.2401	2.9298
Swarna Bullet	0.8118	1.0617	0.0447	0.0584	0.0097	0.0127	0.8390	1.0973	1.7051	2.2301
BRRI Dhan-28	1.2069	1.5785	0.0404	0.0528	0.0099	0.0129	0.4053	0.5301	1.6624	2.1743
BRRI Dhan-29	0.7490	0.9797	0.0336	0.0440	0.0149	0.0195	0.5292	0.6922	1.3268	1.7353
Chinigura	0.5236	0.6848	0.0489	0.0640	0.0132	0.0172	0.4673	0.6112	1.0530	1.3772

Carcinogenic health risk

In this research, our primary aim was to evaluate the cancer risk associated with human exposure to certain metals, specifically Pb, Cd, and Cr. As per the USEPA (1989) [56], a cancer risk of less than 1×10^{-6} exposure (equivalent to a 1 in 1,000,000 lifetime chance) is considered negligible. However, a cancer risk exceeding 1×10^{-4} (equating to a 1 in 10,000 lifetime exposure chance) is deemed to have significant negative implications, and decisions regarding risk management are made within this range [55]. In this research, the levels of Pb, Cd, and Cr in adults were found to range from 1.5×10^{-8} to 4.3×10^{-8}, 5.0×10^{-7} to 7.3×10^{-7}, and 1.05×10^{-5} to 9.9×10^{-6}, respectively. For children, the concentrations of Pb, Cd, and Cr varied from 2.0×10^{-8} to 5.7×10^{-8}, 6.60×10^{-7} to 9.60×10^{-7}, and 1.0×10^{-5} to 1.5×10^{-5}. Our results reveal that Cr (1.12×10^{-5} for adults and 1.5×10^{-5} for children) poses the greatest cancer risk. The hierarchy of cancer risk, from highest to lowest, is Cr > Cd > Pb. It is clear that Cr among these metals represents a considerable health threat to both adults and children across different rice varieties, while Pb and Cd do not present a carcinogenic risk.

Table 5: The Target Carcinogenic Risk (TR) of heavy metals in rice in adults and children.

Target Carcinogenic Risk (Tr)						
Rice Varieties	**Pb**		**Cd**		**Cr**	
	Adult	**Child**	**Adult**	**Child**	**Adult**	**Child**
Paijam	4.3×10^{-8}	5.7×10^{-8}	6.9×10^{-7}	9.00×10^{-7}	1.05×10^{-5}	1.4×10^{-5}
Swarna Bullet	2.4×10^{-8}	3.1×10^{-8}	6.7×10^{-7}	8.76×10^{-7}	7.3×10^{-6}	1.0×10^{-5}
BRRI Dhan-28	3.5×10^{-8}	4.6×10^{-8}	6.1×10^{-7}	7.92×10^{-7}	7.4×10^{-6}	1.0×10^{-5}
BRRI Dhan-29	2.2×10^{-8}	2.9×10^{-8}	5.0×10^{-7}	6.60×10^{-7}	1.12×10^{-5}	1.5×10^{-5}
Chinigura	1.5×10^{-8}	2.0×10^{-8}	7.3×10^{-7}	9.60×10^{-7}	9.9×10^{-6}	1.3×10^{-5}

DISCUSSION

Proximate composition of rice varieties

The moisture content of rice grains plays a crucial role in determining their quality and taste, as well as their shelf life [1]. The elevated moisture level in BRRI Dhan-28 could pose challenges for extended storage. Nonetheless, the moisture content in each sample was close to the acceptable threshold of 12% for preserving rice over the long term [31]. The elevated moisture level in BRRI Dhan-28 could pose challenges for extended storage. Differences in moisture levels among rice varieties might result from laboratory handling, packaging, post-harvest processing methods, or the natural moisture content of the paddy. The findings of this study are within the 3.67–18.00% moisture range observed in Indian rice [50]. Rice is a source of linoleic and other essential fatty acids, but it does not contain cholesterol. Increasing the fat content in cooked rice can reduce starch levels and enhance its flavor [49]. The rice varieties examined in this study have lipid contents ranging from 0.27% to 0.58% (Table 1), with Chinigura having the highest lipid content at 0.58%. These results are consistent with the findings of Oko & Ugwu, (2011) [35] who reported lipid contents between 0.50% and 3.5% in five rice varieties. In Nigeria's Ebonyi state, five lowland rice varieties showed lipid contents ranging from 1.5% to 3.5% [34]. Most of the fat in rice grains is unsaturated, making it susceptible to rapid oxidation by ambient oxygen, which may explain the variability in fat content among rice samples [57]. Differences in fat content among rice accessions can be attributed to factors such as the milling process, removal of the aleurone layer, or oxidation of unsaturated fatty acids [33]. Rice is a fundamental food source worldwide, and its protein content plays a crucial role. The high protein levels in rice help address protein shortages in ecologically disadvantaged regions. While rice protein is of higher quality compared to other cereal grains, it is not as high as that found in pulses and oilseeds [33]. In our research, the protein content of five rice varieties ranged from 7.74% to 6.21%, aligning with earlier studies. Oko et al., (2012) [34] reported protein levels in five rice cultivars ranging from 1.58% to 7.94%. Variations in protein content among different rice types can be attributed to factors such as water availability, handling practices, fertilizer use, environmental stressors, growing conditions, and timing, all of which can enhance grain protein [57]. Ash, an important biological component, is rich in vital minerals necessary for bodily functions and plays a key role in revealing the mineral content and significant mineral levels in food samples [24]. The ash content in 20 different rice varieties was found to range between 0.5% and 2.0% [34]. Similarly, Oko et al., (2012) [35] reported that five rice varieties exhibited ash content ranging from 0.50% to 2.0%. In our study, the ash content varied from 5.66% to 7.00%, which is higher than previously reported values. The differences in ash content across all rice samples could be attributed to the mineral composition of the soil and irrigation water [57]. The digestibility of rice diminishes as its crude fiber content increases. Well-milled rice typically contains between 0.5% and 1.0% fiber [57]. Oko & Ugwu, (2011) [35] reported fiber levels of 1.5% to 2% in five different rice samples. noted that 20 rice varieties from Ebonyi State, Nigeria, have crude fiber content ranging from 1% to 2.5%. Our study results, showing fiber content between 2.14% and 2.45%, align with these previous findings. The research identified that all rice varieties contained high carbohydrate levels, ranging from 71.04% to 73.50%. These findings align with those of Oko & Ugwu, (2011) [34], who observed carbohydrate content between 76.92% and 85.09% in five rice samples. Similarly, Oko et al., (2012) [34] reported carbohydrate levels between 51.53% and 86.92% across 20 rice varieties. The significant carbohydrate content in the rice varieties studied supports their role as staple foods (or calorie sources) in the regions examined.

Heavy metal levels of rice varieties

Lead (Pb), the most hazardous of the heavy metals, is taken into the body through breathing and the intake of food and beverages, leading to harm to the central nervous system, kidneys, joints, reproductive systems, and digestive tract, while also adversely affecting the brain development of children’s [11]. In this study, the highest concentration of Pb detected is 0.8384 mg/kg in the Paijam rice variety, which exceeds the levels reported by Islam et al., (2014) [21] at 0.734 mg/kg and 0.019 mg/kg [38]. These variations in heavy metal content can be attributed to several factors, such as differences in the growth environment (including the presence of heavy metals in the soil and the application of synthetic fertilizers or pesticides), losses during mechanical processing, or the addition of food additives [59,7]. Cadmium (Cd) is naturally present in trace amounts and poses a risk even at minimal levels. Like air and water, food is a primary source of cadmium exposure [38]. Prolonged exposure to cadmium can result in kidney dysfunction and obstructive lung disease,

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

marked by tubular proteinuria, while high levels of exposure may lead to cadmium pneumonitis. In this research, Chinigura exhibited the highest Cd concentration at 0.0080 mg/kg, whereas BRRI Dhan-29 had the lowest at 0.0055 mg/kg. Earlier studies reported average Cd concentrations of 0.088 mg/kg [4] and 0.04 mg/kg [39] in rice from Bangladesh. Therefore, the average Cd concentration found in this study is lower than those in previous research. Chromium is a trace mineral essential for human health. In this study, the levels of Cr found in rice samples varied from 2.3830 mg/kg to 3.6566 mg/kg. These values are significantly higher than the average concentrations in earlier research, which were 0.183 mg/kg [3] and 0.01 mg/kg [41]. The current research determined that the selenium concentration in five rice varieties ranges from 0.3313 to 0.6858 mg/kg, which exceeds the 0.202 mg/kg reported by Shraim (2017) [45]. Selenium functions as a non-specific intracellular antioxidant, complementing the role of vitamin E [9]. Consequently, a deficiency in selenium leads to a reduction in the vitamin E levels within body tissues, while excessive selenium intake can cause toxicity. Considering the recommended selenium intake of 50-100 µg per day [9], the selenium levels in these rice varieties can be regarded as within the normal range.

Health Risk Assessment

Non-Carcinogenic Health Risk

The toxicity of heavy metals is influenced by the level of daily exposure. Mollah et al., (2022) [29] reported EDI values for Pb, Cd, Cr, and As in various vegetables and spices, which ranged from 0.000181-0.000328, 0.0000253-0.000202, 0.000134-0.000246, and 0.0000380-0.0000983, respectively. According to Shahriar et al., (2023a) [43] an adult living in Rajshahi city had an estimated daily intake of Pb, Cr, Mn, and Cd from rice, with values between 0.0142 to 0.1274, 0.0007 to 0.005, 0.2607 to 0.3935, and 0.0022 to 0.0146 mg/kg/day. For children, the EDIs for Pb, Cr, Mn, and Cd were 0.016 to 0.1439, 0.0008 to 0.0056, 0.2944 to 0.4444, and 0.0025 to 0.0165 mg/kg/day. The total daily intake of Cr, Ni, Cu, As, Cd, and Pb was 1.33, 1.81, 7.53, 0.333, 0.155, and 1.63 mg/day for adults, and 0.637, 0.896, 3.70, 0.167, 0.079, and 0.765 mg/day for children, as reported by Islam et al., (2015) [22]. This research observed that both adults and children had lower EDI values compared to previous studies, aligning closely with the WHO's tolerable daily intake (TDI) [62]. The IARC has classified Pb and Cd as carcinogens, indicating that prolonged exposure to low levels of As, Cd, and Pb could lead to various cancers [48]. When compared to the findings of Shahriar et al., (2023b) [44] the current study suggests there might not be cancer-related risks. However, the THQ value for Pb in Paijam, BRRI Dhan 28, exceeds 1 for both adults and children, and only for children in Swarna Bullet, indicating potential non-cancer health concerns. Similarly, the THQ value for Se in Swarna Bullet is also above 1 for children, which could be hazardous. This research identified increased TTHQ levels in Paijam. Swarna bullet and BRRI Dhan-28 showed high TTHQ values for children. According to Xingmei Liu et al., (2013) [27], both adults and children had TTHQs exceeding 10, suggesting a significant risk of health issues over the long or short term. Our findings, however, revealed much lower values, suggesting a minimal health risk.

Carcinogenic health risk

Xingmei Liu et al., (2013) [27] state that TR values for Pb and Cr under 10^{-6} are considered non-carcinogenic, while values between 10^{-4} and 10^{-6} represent the threshold for cancer risk. In this study, the TR values for Pb and Cd in each rice sample, for both adults and children, are below 10^{-6}, suggesting there is no cancer risk. In children, the TR value of Cr exceeds 10^{-6}, suggesting a carcinogenic risk. Importantly, children are more susceptible to these risks compared to adults. Therefore, it is crucial to implement effective risk management strategies, with a particular emphasis on safeguarding children's health.

CONCLUSION

This study examined the levels of nutrient elements and heavy metals in popular ready-to-cook rice grains from the Barishal district in Bangladesh, along with the potential health risks they pose, particularly differentiating between risks for adults and children. The essential composition of rice highlights its crucial role as a staple food worldwide. This research found that all rice samples, particularly Chinigura and Pijam, are abundant in carbohydrates, which are the main source of energy. Compared to other grains, rice has a lower protein content, aligning with the findings of this study. The fat content in all rice samples is minimal, which contributes to its extended shelf life. All rice varieties with average daily heavy metal intakes below the WHO thresholds do not pose any negative health effects. However, the THQ values for lead in Pijam, BRRI Dhan-28, and selenium in Swarna Bullet exceed 1, indicating notable non-carcinogenic health risks. TR values for Pd and Cd were found to be below 10^{-6}, suggesting no cancer risk for both children and adults. However, Cr posed a significant cancer risk, with TR values exceeding 10^{-6} for children across all samples, indicating their increased susceptibility. The study identified specific regions in the Barishal district of Bangladesh—namely Barishal Sadar, Gounadi, Wzirpur, Babugong, and Bakergonj—as primary sources of rice contaminated with heavy metals. Further investigation into water, soil quality, and rice production processes is necessary to pinpoint contamination sources. In summary, continuous monitoring, quality control, and effective management are crucial to ensuring food safety in Bangladesh.

ACKNOWLEDGMENTS

The authors thank the National Science and Technology (NST) of the Ministry of Science and Technology, Bangladesh, for funding this research project (Grant No.: SRG-224564).

CRediT authorship contribution statement

Conception and Design: Sourav Debnath; Analysis and Interpretation of Result: Sourav Debnath, Susmita Karmakar, Suprakash Chakma; Writing-original draft preparation: Sourav Debnath, Suprakash Chakma, M. M. Mehedi Hasan; Writing review and editing: Sourav Debnath, Mohammad Abdulla Al Noman. All authors have reviewed and given their approval to the final manuscript.

CONFLICT OF INTEREST

The authors confidently assert their impartiality, declaring that they have no conflicts of interest, which underscores the integrity and reliability of their research.

ETHICAL APPROVAL

The animal welfare and ethical committee, Patuakhali Science and Technology University, approved the experimental procedures used in this study.

DATA AVAILABILITY

This article comprehensively presents all the necessary data.

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