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RISK ASSESSMENT OF SELECTED HEAVY METALS CONTAMINATION IN RICE GRAINS IN THE RAJSHAHI CITY OF BANGLADESH

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ABSTRACT

The concentrations of four heavy metals in rice grains and the health risk assessments of the metals were studied. Sixteen rice samples were gathered from Rajshahi, Bangladesh. Average lead, chromium, manganese, and cadmium concentrations measured in rice samples were 6.87, 0.43, 44.58, and 1.13 mg/kg, respectively. The concentration level found in most rice samples is greater than the acceptable limit as set by WHO. The risk assessment was done by using the intended hazard ratio and target carcinogenic risk where the values for the lead, manganese, and cadmium quotient were greater than 1. The target cancer-causing risk values for a maximum number of samples are lower than 10^{-4}, which is considered safe. However, target carcinogenic risk values for cadmium are higher than 10^{-4}, which is considered a carcinogenic risk. The results of the current investigations suggest that appropriate measures should be taken to determine the cause and origin of this heavy metal contamination.

Keywords: *Cadmium, Carcinogenic, Chromium, Lead, Manganese, Non-carcinogenic, Health risk assessment.*

1. INTRODUCTION

Rice is the main crop for food cultivated worldwide. About 40% of people around the world eat it as their main staple diet. Rice is a cereal. The seed of either *Oryza glaberrima* or *Oryza sativa*, is sometimes known as Asian rice (African rice). Rice is grown on every continent except Antarctica. The *Oryza sativa* plant produces these well-liked edible seeds, which come in a variety of colours, textures, sizes, and tastes. There are more than 40000 varieties. There are countless varieties since it is undoubtedly one of the most popular dishes in the world (Jessica, 2022; Melinda, 2021).

Rice is a major contributor to the economy of the agricultural sector in Bangladesh as well as the major source of carbohydrates and staple food for almost all families (Saha et al., 2021). Bangladesh grows rice in three seasons: Boro, Aman, and Aus. After the pre-monsoon rain, Aus rice is direct sown or broadcast sown in March and April, and it is harvested in July and August. Direct seeding with Aus in March and April and transplanting between July and August are the two methods that Aman is planted. Whereas Boro is planted from December to early February and is harvested in April and June (Shelley et al., 2016).

In recent times, due to the contamination of heavy metals in the crop, it is now a matter of concern for everyone. Heavy metals are often d-block elements with high specific gravity and density and they are harmful even at a concentration level of parts per billion (ppb). Examples include arsenic (As), lead (Pb), cadmium (Cd), mercury (Hg), silver (Ag), zinc (Zn), copper (Cu), nickel (Ni), iron (Fe), chromium (Cr), platinum (Pt), palladium (Pd), manganese (Mn), etc. human sources and natural sources of these metals in the environment include automotive exhaust, industrial discharge, and mining. Unlike organic contaminants, heavy metals are nonbiodegradable and tend to accumulate in living organisms (Manavi et al., 2016). The series of toxicity for the metals in decreasing order is as follows Hg > Cd > Cu > Zn > Ni > Pb > Cr > Al > Co (Kinuthia et al., 2020). The amount, degree of emission, and length of exposure all affect how toxic heavy metals are to people. In recent times Pb, Mn, Hg and Cd have gained a lot of attention because of their widespread exposure (Khalef et al., 2022).

Pb exposure can happen when someone consumes contaminated food or water or inhales polluted dust or aerosols. The liver, kidney, heart, skeleton brain and the nervous system may damage due to Pb poisoning (Kinuthia et al., 2020). Pb may cause hypertension and affect blood vessels. Blood arteries which are blocked by Pb cause fatal heart attacks and rapid demise. Rising blood Pb levels correlate significantly with a higher risk of

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mortality and cardiovascular disease (Akil & Ahmad, 2011). Acute and chronic Pb poisoning can harm the cardiovascular system and the blood vessels to both extents (Flora et al., 2012).

The harmful industrial pollutant hexavalent chromium [Cr (VI)] is recognized as human cancer causing element by a number of non-regulatory and regulatory organizations. (Ostad-Ali-Askari, 2022). The multiorgan toxicity of compounds containing Cr (VI) is known to result in human respiratory tract cancer, allergies, asthma, and kidney impairment (Rojas-Avelizapa, 2022).

Mn's overexposure increases the risk of developing harmful health conditions, such as kidney, liver, respiratory, neurological, and cardiovascular problems as well as hyperkeratosis, diabetes, mellitus, pigmentation changes, Parkinson's disease, cardiovascular disease, and Huntington's disease (Ghosh et al., 2020; Rahman et al., 2021). Serious health issues could result from exposure to Cd compounds such as the effect on reproduction system (Zhao et al., 2017), osteotoxicity (Qing et al., 2021), kidney damage (Hernandez-Cruz et al., 2022), cardiovascular abnormality (Tellez-Plaza et al., 2013) and carcinogenesis (Liu et al., 2013). Exposures to Cd can also cause irritation, this is then accompanied by symptoms such as dryness, coughing, irritation of the throat and nose, pneumonitis, dizziness, pulmonary edema, headache an chest pain (Luevano et al., 2014; Mollah et al., 2022).

The contamination of water, air and soil with heavy metals including Cd, Pb, Cr and Hg results from numerous industrialized activities. In rice, the pollutants can build up and spread (Huang et al., 2013). As a result of their accumulation in soil and plants due to both natural and anthropogenic sources, heavy metals are a significant indicator of serious ecological contamination issues. But one of the most serious ecological concerns is caused by issues with food safety and harmful health risks (Cui et al., 2004). The main sources of heavy metal contamination are human activities and natural resources including rapid industrial expansion, anthropogenic causes and urbanization which pose a troubling threat to the environment and the state of humanity (Krishnani and Ayyappan, 2006; Shariar et al., 2014). The maternal components appear to be the primary source of heavy metals in soils. The significance of maternal components in determining the metal content is said to be particularly important in soils formed from similar or only parental materials, such as sedimentary or igneous rocks like limestones, shales, sandstones and dolomite, or in undeveloped soils that have been battered in moderate conditions (Nael et al., 2009; Craig et al., 2007). To assess the effects of bioaccumulation and transmission, the concentrations of the toxic heavy metals in the shoots, soil, grains of rice crops and roots were measured. The potential threat to the local population's overabundance of rice, their main source of food, was evaluated for hazards (Reddy et al., 2013). In Asia, rice is known to be one of the main sources of Cd and Pb for human living, and there is abundant evidence that dysfunction of human renal system is linked to the contamination of rice with Cd in existing farmhouses (Shimbo et al., 2001; Rufus et al., 2005). Consumption of rice has been shown to be a significant source of exposure to harmful pollutants such as Cd, As, Hg, and Pb (Akther et al. 2022; Mahipal et al., 2020; Zhuang et al., 2009). Both anthropogenic and natural factors, such as weathering, erosion, lithogenesis, and other geological processes, can result in the presence of heavy metals in soil (Hua et al., 2010). Industrialized activities, Mining, traffic discharges, and staged events are examples of anthropogenic sources (mostly the usage of pesticides and chemical fertilizers) (Anju and Banarjee, 2012; Yang et al., 2014).

Water is an important factor in proper crop production, thus irrigation water plays a vital role in food safety and security. Generally, the concentration of heavy metals in water is typically low, but due to human activity, the concentrations of these metals are increase over time. Water used for irrigation in Rajshahi mainly came from the Padma River, groundwater, and surface water. However, irrigation water could be a pathway of heavy metal contamination in rice. There is also some industry are located in Rajshahi city and around Rajshahi. Also we predict that irrigation water nearby Rajshahi city may lead to metal contamination due to industrialization and different human activities. Several previous studies indicated that irrigation water could be a pathway of heavy metal contamination in crop (Bhatia et al., 2015; Malan et al., 2014; Aweng et al., 2011). Investigations are being carried out in different locations of Bangladesh by different researchers to determine the extent of heavy metals pollution in Midstream of the river, surface water, groundwater, coal mine area water, soil, and sediments (Akther et al. 2016; Ahmed et al., 2018; Bhuiyan et al., 2010; Saha and Zaman 2011; Shahriar et al., 2022). Saha and Zaman (2011) analyzed five heavy metals (Pb, Mn, Cr, Cd and As) in rice in Shibganj area of Chapai Nawabganj district of Rajshahi division of Bangladesh. They summarized that the mean toxic metal concentrations in rice are lower than the respective established safe limits for these elements, except for Pb concentration in all samples.

As heavy metals have carcinogenic and non-carcinogenic toxic effects on the human body, it is crucial to determine the pollution level of heavy metals in irrigation water. It is also essential to analyze how metal concentrations change in a study area with time. In this study, the Rajshahi City Corporation area of Bangladesh was chosen for sample collection. No previous studies were found in the literature on the heavy metal levels in this study's selected area. Hence, the current research would present baseline information of the pollution level of selected heavy metals in rice of studied areas, and it would aid in comparing the results over time and with other research. Hence, a systematic study is required in order to determine the pollution level around Rajshahi city areas.

Therefore, the present work is designed and carried out for the determination of four heavy metals (Pb, Cr, Mn and Cd) in common types of rice grown in Rajshahi City Corporation area of Bangladesh. In addition, for the assessment of potential health risks, some factors such as target hazard quotient, total target hazard quotient, target carcinogenic risk and cumulative cancer risk were also studied. This result provides the current situation of contamination of heavy metals in rice grain and is useful to take protective measures and health professionals in reducing heavy metal contamination. Furthermore, it provides a comparison to other regions in the world as well as in Bangladesh.

2. MATERIALS AND METHODS

2.1. Study area

Bangladesh is a South Asian country geographically located with a north latitude of 26°38' to 20°34' and an east longitude of 92°41' to 88°01' (Banglapedia, 2012). Rajshahi City Corporation area make up 95.56 sq km, which can be found between 88°32' and 88°40' east of the equator and between 24°20' and 24°24' north latitude. Paba upazila surrounds this city on all sides (Banglapedia, 2012). Samples of rice were gathered from several Rajshahi City Corporation locations (Fig. 1).

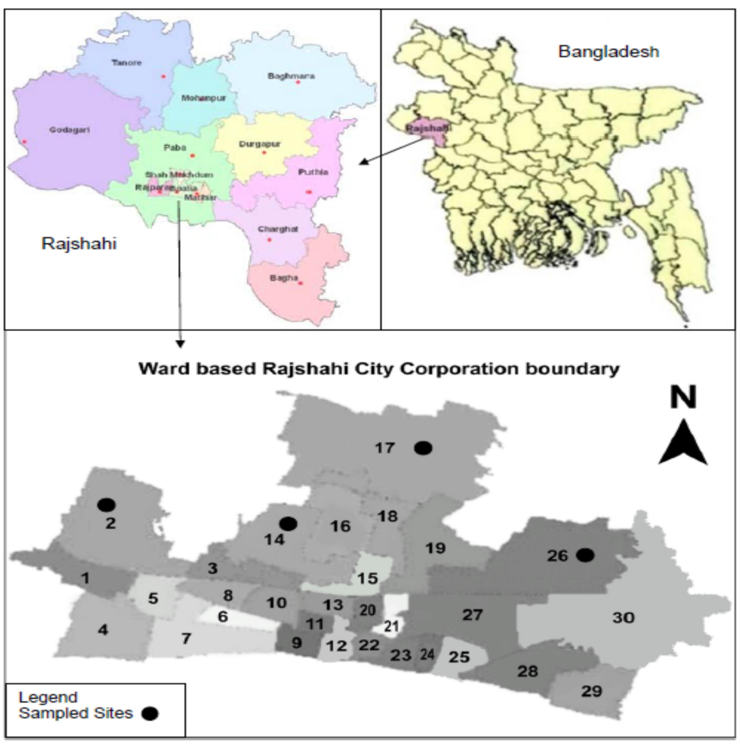

Figure 1: Location of the research area.

2.2. Date and location of the research

Between October 2021 and November 2021, samples were taken throughout Rajshahi City Corporation area of Bangladesh. The sample analysis was carried out in December 2021 at the central science laboratory of the University of Rajshahi, Rajshahi, Bangladesh.

2.3. Sample collection

Four types of rice of *Oryza sativa* species are selected for the study. The local names and sample identification numbers for the rice samples that are collected are given in Table 1. Each type of rice was collected from four locations of Rajshahi City Corporation (Fig. 1).

Table 1: The local names and sample identification numbers of the collected rice samples.

Local name of the samples	Sample Identification Nnmber
Jira rice	J_1, J_2, J_3, J_4
Atash rice	A_1, A_2, A_3, A_4
Guti swarno rice	G_1, G_2, G_3, G_4
Chikon swarno rice	C_1, C_2, C_3, C_4

Each location provided three samples (n=3) for the purpose of calculating the standard deviation. Jeera and atash rice samples were collected from agricultural storage. The farmers were used traditional storage structures such as plastic drum and gunny/plastic bags for storing rice at room temperature. Guti swarno and chikon swarno rice samples were collected from agricultural fields. The rice samples were collected (250 gm) in a clean, dry, sterilized polyethene bag. The collected samples were carefully sealed, labeled and stored at room temperature until analysis.

2.4. Sample preparation

The digestion of the rice samples was performed following the previously described methods (Kormoker, et al., 2022; Lei et al., 2015; Saha and Zaman, 2011). The moisture content was removed by overdrying at 110°C. A grinder was used to ground the samples to make fine powder. The resulting fine powder was stored in a clean, dry, sterilized polyethylene bag and sealed carefully. In a beaker, 1 g of powdered rice sample was taken and 15 mL of HNO_3 (Nitric acid) (69%) and 3 mL $HClO_4$ (perchloric acid) (70%) were added. The total volume was heated slowly to raise the temperature up to 80-90ºC till it forms a transparent solution. Then it was cooled at room temperature and filtered out through Whatman No. 42 filter paper. After filtration, the sample was transferred to a 100 mL volumetric flask and diluted to the mark with de-ionized water. The sample preparation procedures are given in Fig. 2.

Rice grain Rice powder Labeled powder Digested samples

Figure 2: Sample preparation

2.5. Analysis of heavy metals

The amount of specific heavy metals, including Cr, Cd, Mn, and Pb in the filtrate sample solutions were determined at the Central Science Laboratory at the University of Rajshahi, Bangladesh, by means of a Shimadzu AA-6800 atomic absorption spectrophotometer (AAS). The operating conditions and limit of detection (LOD) of Shimadzu AA-6800 AAS are given in Table 2. Manually generated standard solutions environmental of individual heavy metals were utilized to calibrate the AAS. All of the metals' standard stock solutions, 1000 ppm each, were purchased from Kanto Chemical Co. Inc. in Tokyo, Japan. All of the standard solutions were diluted for the required concentrations in order to calibrate the AAS. Results are shown as mean Standard Deviation (SD), and data were analyzed using IBM® SPSS® Statistics of version 17.0 for Windows®.

Table 2: Operating conditions and LOD of Shimadzu AA-6800 AAS.

Element	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Atomizer	LOD (mg/L) (mg/L)
Pb	283.3	0.5	10	Flame	0.04
Cr	357.9	0.5	10	Flame	0.01
Mn	285.2	0.5	8	Flame	0.02
Cd	228.8	0.5	8	Flame	0.006

2.6. Health risk assessment (HRA)

The process of estimating the type and likelihood of unfavourable health consequences in people who may be exposed to chemicals or other hazardous compounds in the environment is known as a human HRA. A health questionnaire called an HRA is used to assess a person's quality of life and health risks (Baker et al., 2007).

2.6.1. Estimated daily intake (EDI)

To assess health risk, the United States Environmental Protection Agency (USEPA) guidelines were followed (USEPA 1989). The daily intake of metals is influenced by both the amount of metal present in the diet and how much food is eaten each day. The human's body weight can also affect how well they tolerate pollutants. To account for these factors, the EDI idea was established. According to the literature, the EDI is calculated by the equation (Eq.) 1 (USEPA 1989; UNEP/FAO/WHO,1992; Islam et al., 2014).

$$EDI = \frac{C \times FIR}{Bw} \tag{1}$$

Where C is the level of heavy metal contamination in the rice, FIR is the rate of rice consumption (g/person/day) in the study area, and Bw stands for body weight. In Bangladesh, rice is the primary food consumed everyday, and adults in the area consume an average of 425 g of rice daily (AOAC, 1984; Yunus et al., 2019). The weight of each individual in this study was set to 60 kg. Furthermore, the average quantity of rice consumed by a child was chosen as 200 g/day and the weight was set to 25 kg.

2.6.2. Target hazard quotient (THQ)

As the greatest level at which no unfavourable health effects are anticipated, the reference dosage, or THQ, is defined as the ratio of exposure to the hazardous element. The method for THQ for individual metal/loid is provided in the United States EPA, Region III risk-based concentration table (Smith, 1995). The earlier described non-carcinogenic health risks related to food consumption by the locals were calculated (Eq. 2) in this study (USEPA 1989; Islam et al., 2014).

$$THQ = \frac{EFr \times ED \times FIR \times C}{RfD \times Bw \times AT} \tag{2}$$

Where THQ is the target hazard quotient, EFr is the exposure frequency (365 days/year), ED is the exposure duration (70 years), equivalent to the average human lifetime, FIR is the rate of rice ingestion (g/person/day) in the study region, C is the metal concentration in foods (mg/kg fw), RfD is the oral reference dose (mg/kg/day), Bw is the average body weight, AT is the averaging time for non-carcinogens (365 days/year × number of exposure years, assuming 70 years). The oral reference doses were based on 0.002, 1.5, 0.14 and 0.0001 mg/kg/day for Pb, Cr, Mn and Cd, respectively (USEPA, 2022; EFSA, 2010; Zeng et al., 2015; Robert, 1991). The exposed population is unlikely to incur overtly harmful consequences if THQ is less than 1. When THQ is equal to or more than 1, there may be a possible health risk, and appropriate preventative measures and actions should be performed (USEPA 1989; Islam et al., 2014).

2.6.3. Total target hazard quotient (TTHQ)

The total heavy metals' THQs are added to create the TTHQ. The total of THQ (Eq. 3) was used to determine TTHQ (USEPA 1989; Fakhri et al., 2017).

$$TTHQ = THQPb + THQCr + THQMn + THQCd \tag{3}$$

When TTHQ 1.0, adverse consequences are improbable, but when TTHQ 1.0 indicates the likelihood of unfavorable effects; when TTHQ>10, the risk of chronic adverse health impacts is significant (USEPA 1989; Lei et al., 2015).

2.6.4. Target carcinogenic risk (TR)

The TR derived from the intake of heavy metals was calculated using the Eq. 4 provided in USEPA Region III risk-based concentration table (USEPA, 1989; USEPA, 2020; Islam et al., 2014).

$$TR = \frac{EFr \times ED \times FIR \times C \times CSFo}{Bw \times AT} \times 10^{-3} \tag{4}$$

Whereas AT is for the average time for carcinogens (365 days/year ED), TR stands for the goal cancer risk or lifetime cancer risk, and CSFo stands for the oral carcinogenic slope factor, which is, respectively, 8.5×10^{-3}, 0.420, and 15 $(mg/kg/day)^{-1}$ for Pb, Cr and Cd (USEPA, 1989; Zeng et al., 2015). In this study for estimating carcinogenic risk, the heavy metals of Pb, Cr and Cd with CSFo were considered.

2.6.5. Cumulative cancer risk (TCR)

Then, using data from metal(oid) consumption, the TCR, which, depending on the exposure level, may induce carcinogenic effects, was estimated for Pb, Cr and Cd by using Eq. 5 (USEPA, 1989; USEPA, 2020; Alsafran et al., 2021).

$$TCR = \sum_{n=1}^{i} TR_n \quad (5)$$

Here, i = 1, 2, 3, ……. n, TR represents target cancer risk and n represents the heavy metals number considered for risk calculation of cancer. Since Cd, Pb and Cr may induce both non-carcinogenic and carcinogenic effects depending on the exposure level, the TR generated from their intake was computed. When TR and/or TCR is greater than 10^{-4} value, the exposed population are at a significant carcinogenic risk, but if TR and/or TCR is lower than 10^{-6} value, the exposed population are not at a considerable carcinogenic risk (USEPA, 2020). Also, if TR and/or TCR are between 10^{-4} to 10^{-6} value, the exposed population are at threshold cancer risk (USEPA, 2020).

3. RESULTS AND DISCUSSION

3.1. Heavy metals

The level of Cd, Cr, Pb and Mn in rice samples and their maximum values (Max), minimum values (Min), average values, percentage relative standard deviations (%RSD) and Standard Deviations (SD) are shown in Table 3. The amounts were measured in milligrams per kilogram of the rice samples.

The Pb concentrations in the collected samples were ranged from 2.00 mg/kg to 17.99 mg/kg. The highest concentration was found in the rice sample C_4 and the lower concentration was found in samples J_3, A_2 and A_3. World Health Organization (WHO) and the Ministry of Health of the People's Republic of China (MHPRC) specified the allowable level of Pb in rice grain as 0.2 mg/kg (FAO/WHO, 2002; WHO, 2011; MHPRC, 2005), whereas the United States Environmental Protection Agency (USEPA) set the value 0.1 mg/kg (USEPA, 2021). Bangladesh Standards and Testing Institution (BSTI) set the permissible limit of Pb as 0.3 mg/kg (BSTI, 2018). It was found that, the concentration of Pb in the selected rice samples was greater than the allowable concentration limit (Kormoker et al. 2022) collected rice from Tangail district of Bangladesh and found Pb in a range from 0.35 to 18.05 mg/kg that is greater than the WHO-permitted limit. Similarly, Pb concentration was found 5.67 mg/kg (Avijit and Anindya, 2018), which also exceeds the permissible limit. The excessive amount of Pb has a toxic effect in human health. Chronic exposure to heavy metals from eating rice can have a major impact on mental disorders and may cause permanent harm to human health.

The concentration of Cr in rice samples were varied from 0.10 mg/kg to 0.70 mg/kg. The higher amount of Cr was found in the rice samples J_1, A_4, G_1, C_2 and the lower concentration was found in the samples J_3, A_1 and G_2. The International Agency for Research on Cancer categorizes Cr(VI) as 'carcinogenic to humans' and Cr(III) as 'not classifiable' (IARC, 1987). However, the USEPA lists total Cr in drinking water as having 'inadequate or no human and animal evidence of carcinogenicity' (USEPA, 1996). WHO states that 0.05 mg/1 drinking water guideline for total Cr is unlikely to cause significant health risks (WHO, 1996). The maximum permissible limit for Cr in rice grain is $1.0\,mg/kg^{-1}$ as found in several studies (FAO/WHO, 2002; WHO, 2011; MHPRC, 2005; Qu et al., 2016). It was found that the concentration of chromium in the selected rice samples is lower than the allowable concentration limit. Guo et al., (2020) collected rice from the Jin-Qu Basin, China to conduct a regional field survey and determined the concentration of Cr in rice grain. They found maximum concentration is 1.34 mg/kg and the minimum concentration is 0.05 mg/kg.

The concentration of Mn in rice samples was found between 36.80 mg/kg to 55.55 mg/kg. The higher level of Mn was found in the rice samples C_3, C_4 and the lower level was found in samples J_1 and J_4. In Australia, Rahman et al. (2014) examined the Mn content of rice cultivated in Australia, Bangladesh, India, Italy, Pakistan, Thailand, and Vietnam. According to Rahman et al., the Mn content of rice farmed in Australia was higher than that of rice imported from India, Italy, Pakistan, Thailand, and Vietnam but lower than Bangladesh. The Australian rice with the greatest Mn content was organic brown rice, followed by brown rice, white rice, and sushi rice. According to Antoine et al. (2012), in Jamaican, the Mn concentration in white rice was reported from 5.02 to 17.1 mg/kg which have 10.5 mg/kg as the mean while brown rice was 5.19 to 43.5 mg/kg and mean reported at 26.5 mg/kg. The Mn concentration in rice samples of white rice from seven Asian countries which includes Korea, Malaysia, Indonesia, Thailand, Japan, Philippines and China was studied by Moon et al. (2012). According to their research, rice was consumed on average in seven different countries at 300 g per day, with a mean Mn concentration of 8.46 mg/kg. The findings showed that there was no significant heavy metal pollution in the rice samples from any of the participating nations. Manganese is necessary for human survival. It can act as an enzyme activator for a variety of enzymes as well as a component of some metalloenzymes. It is also necessary for bone development, carbohydrate and lipid metabolism. Deficiency is unlikely in an adequate diet due to its ubiquity in foods. On the other hand, its toxicity is well documented, but dietary exposure is doubtful except in exceptional circumstances (McDowell, 1992; Crossgrove and Zheng, 2004). Despite the fact that there is no defined recommended daily amount for manganese, adult males and girls are thought to need 2.3 and 1.8 mg per day, respectively. The National Institutes of Health has established a maximum of 11 mg daily for adults (Antoine et al., 2012).

Table 3: The concentration of selected heavy Metals (mean ± SD) in rice samples (mg/kg).

Sample Identification No.	Samples collection location in Rajshahi city corporation area	Pb	Cr	Mn	Cd
J_1	Ward no. 14 (n=3)	4.00 ± 0.089	0.70 ± 0.002	36.80 ± 0.042	0.96 ± 0.001
J_2	Ward no. 26 (n=3)	12.00 ± 0.001	0.40 ± 0.001	37.27 ± 0.464	1.01 ± 0.002
J_3	Ward no. 17 (n=3)	2.00 ± 0.003	0.10 ± 0.001	39.61 ± 0.464	1.30 ± 0.001
J_4	Ward no. 2 (n=3)	10.00 ± 0.034	0.60 ± 0.002	36.80 ± 0.051	0.45 ± 0.001
A_1	Ward no. 14 (n=3)	4.00 ± 0.023	0.10 ± 0.001	39.85 ± 0.552	2.02 ± 0.006
A_2	Ward no. 26 (n=3)	2.00 ± 0.001	0.30 ± 0.002	41.49 ± 0.231	1.05 ± 0.004
A_3	Ward no. 17 (n=3)	2.00 ± 0.052	0.60 ± 0.003	42.43 ± 0.411	1.05 ± 0.002
A_4	Ward no. 2 (n=3)	4.00 ± 0.041	0.70 ± 0.007	44.07 ± 0.023	1.48 ± 0.002
G_1	Ward no. 14 (n=3)	8.00 ± 0.002	0.70 ± 0.002	43.36 ± 0.342	1.59 ± 0.006
G_2	Ward no. 26 (n=3)	4.00 ± 0.005	0.10 ± 0.003	45.71 ± 0.423	1.21 ± 0.007
G_3	Ward no. 17 (n=3)	12.00 ± 0.031	0.20 ± 0.003	46.18 ± 0.018	2.06 ± 0.002
G_4	Ward no. 2 (n=3)	6.00 ± 0.021	0.60 ± 0.001	47.35 ± 0.061	0.92 ± 0.003
C_1	Ward no. 14 (n=3)	12.00 ± 0.002	0.60 ± 0.005	52.50 ± 0.041	0.31 ± 0.003
C_2	Ward no. 26 (n=3)	4.00 ± 0.077	0.70 ± 0.002	50.40 ± 0.231	1.66 ± 0.001
C_3	Ward no. 17 (n=3)	6.00 ± 0.068	0.30 ± 0.001	53.91 ± 0.052	0.56 ± 0.001
C_4	Ward no. 2 (n=3)	17.99 ± 0.002	0.20 ± 0.002	55.55 ± 0.021	0.45 ± 0.001
Min	-	2.00	0.10	36.80	0.31
Max	-	17.99	0.70	55.55	2.06
Average	-	6.87	0.43	44.58	1.13
SD	-	4.673	0.241	6.072	0.537
%RSD	-	67.974	55.985	13.620	47. 5444
AL^a	-	0.2	1.0	-	0.1
AL^b	-	0.1	-	-	0.4
AL^c	-	0.2	1.0	-	0.2
AL^d	-	0.3	-	-	0.2

AL: Allowable Limit of heavy metals in rice.
[a]WHO (FAO/WHO, 2002; WHO, 2011).
[b]USEPA (USEPA, 2021)
[c]Ministry of Health of the People's Republic of China (MHPRC, 2005).
[d]Bangladesh Standards and Testing Institution (BSTI, 2018)

The level of Cd in rice samples were ranged from 0.314 mg/kg to 2.062 mg/kg. The higher amount of Cd was found in the rice samples A_1, G_3 and the lowest amount was found in sample C_1. WHO gives the allowable limit of cadmium in rice grain as 0.1 mg/kg (FAO/WHO, 2002; WHO, 2011), whereas USEPA set the value as 0.4 mg/kg. MHPRC and BSTI set the allowable limit of cadmium in rice grain as 0.2 mg/kg (BSTI, 2018; MHPRC, 2005). It was revealed that, the concentration of Cd in the selected rice samples is higher than the allowable concentration limit. Kormoker et al., (2022) collected rice from Tangail district of Bangladesh and found higher cadmium concentration than the permissible limit set by WHO. Guo et al. (2020) collected rice from the Jin-Qu Basin, China to conduct a regional field survey and determined the concentration of Cd in rice grain. They found maximum concentration is 1.094 mg/kg and minimum concentration is 0.028 mg/kg.

3.2. Health Risk Assessment

Table 4 shows the EDI amounts of observed heavy metals in different types of rice at each area. The estimated daily intake of Pb, Cr, Mn and Cd from rice varied from 0.0142 to 0.1274 $mg.kg^{-1}.d^{-1}$, 0.0007 to 0.005 $mg.kg^{-1}.d^{-1}$, 0.2607 to 0.3935 $mg.kg^{-1}.d^{-1}$ and 0.0022 to 0.0146 $mg.kg^{-1}.d^{-1}$, respectively, for an adult in the investigated area. For child EDI amount for Pb, Cr, Mn and Cd varied from 0.016 to 0.1439 $mg.kg^{-1}.d^{-1}$, 0.0008 to 0.0056 $mg.kg^{-1}.d^{-1}$, 0.2944 to 0.4444 $mg.kg^{-1}.d^{-1}$and 0.0025 to 0.0165 $mg.kg^{-1}.d^{-1}$,respectively, Tolerable daily intake for Pb, Cr, Mn and Cd is .0036, 1.5, 0.14 and 0.001 $mg.kg^{-1}.d^{-1}$ for adult recommended by WHO (WHO, 1993). It was found that the EDI value observed for Pn, Mn and Cd is far above than the recommended tolerable daily intake (TDI).

Table 4: EDI of heavy metals of Rice (Exposure per Day).

Sample Id No.	EDI ($mg.kg^{-1}.d^{-1}$)							
	Pb		Cr		Mn		Cd	
	Adult	Child	Adult	Child	Adult	Child	Adult	Child
J_1	0.0283	0.0320	0.0050	0.0056	0.2607	0.2944	0.0068	0.0077
J_2	0.0850	0.0960	0.0028	0.0032	0.2640	0.2982	0.0072	0.0081
J_3	0.0142	0.0160	0.0007	0.0008	0.2806	0.3169	0.0092	0.0104
J_4	0.0708	0.0800	0.0043	0.0048	0.2607	0.2944	0.0032	0.0036
A_1	0.0283	0.0320	0.0007	0.0008	0.2823	0.3188	0.0143	0.0162
A_2	0.0142	0.0160	0.0021	0.0024	0.2939	0.3319	0.0074	0.0084
A_3	0.0142	0.0160	0.0043	0.0048	0.3005	0.3394	0.0074	0.0084
A_4	0.0283	0.0320	0.0050	0.0056	0.3122	0.3526	0.0105	0.0118
G_1	0.0567	0.0640	0.0050	0.0056	0.3071	0.3469	0.0113	0.0127
G_2	0.0283	0.0320	0.0007	0.0008	0.3238	0.3657	0.0086	0.0097
G_3	0.0850	0.0960	0.0014	0.0016	0.3271	0.3694	0.0146	0.0165
G_4	0.0425	0.0480	0.0043	0.0048	0.3354	0.3788	0.0065	0.0074
C_1	0.0850	0.0960	0.0043	0.0048	0.3719	0.4200	0.0022	0.0025
C_2	0.0283	0.0320	0.0050	0.0056	0.3570	0.4032	0.0118	0.0133
C_3	0.0425	0.0480	0.0021	0.0024	0.3819	0.4313	0.0040	0.0045
C_4	0.1274	0.1439	0.0014	0.0016	0.3935	0.4444	0.0032	0.0036
TDI (WHO, 1993)	0.0036		1.5		0.14		0.001	

Table 5 shows the THQ of observed heavy metals in rice of different types at each area. The value of THQ for each metal, with the exception of Cr, from each sample of rice was greater than one, which may result in dangers that are not related to cancer. While the THQ value for Cr is not more than 1, which indicates that it might not cause any non-cancer causing risks. Observed TTHQ is shown in Table 5. The TTHQ for adults and children was greater than 10, indicating a high risk for chronic or even acute adverse health effects (Lei et al., 2015).

Table 5: THQ and TTHQ of heavy metals in Rice.

Sample Id No.	THQ								TTHQ	
	Pb		Cr		Mn		Cd		Adult	Child
	Adult	Child	Adult	Child	Adult	Child	Adult	Child		
J_1	7.075	8	0.0033	0.0037	1.862	2.103	6.8	7.7	15.7	17.81
J_2	21.25	24	0.0019	0.0021	1.886	2.130	7.2	8.1	30.3	34.23
J_3	3.55	4	0.0005	0.0005	2.004	2.264	9.2	10.4	14.8	16.66
J_4	17.70	20	0.0029	0.0032	1.862	2.103	3.2	3.6	22.8	25.71
A_1	7.08	8	0.0005	0.0005	2.016	2.277	14.3	16.2	23.4	26.48
A_2	3.55	4	0.0014	0.0016	2.099	2.371	7.4	8.4	13.1	14.77
A_3	3.55	4	0.0029	0.0032	2.146	2.424	7.4	8.4	13.1	14.83
A_4	7.08	8	0.0033	0.0037	2.230	2.519	10.5	11.8	19.8	22.32
G_1	14.18	16	0.0033	0.0037	2.194	2.478	11.3	12.7	27.7	31.18
G_2	7.08	8	0.0005	0.0005	2.313	2.612	8.6	9.7	18.0	20.31
G_3	21.25	24	0.0009	0.0011	2.336	2.639	14.6	16.5	38.2	43.14
G_4	10.63	12	0.0029	0.0032	2.396	2.706	6.5	7.4	19.5	22.11
C_1	21.25	24	0.0029	0.0032	2.656	3.000	2.2	2.5	26.1	29.50
C_2	7.075	8	0.0033	0.0037	2.550	2.880	11.8	13.3	21.4	24.18
C_3	10.63	12	0.0014	0.0016	2.728	3.081	4.0	4.5	17.4	19.58
C_4	31.85	35.98	0.0009	0.0011	2.811	3.174	3.2	3.6	37.9	42.76

The estimated TR values of Pb, Cr and Cd due to exposure from rice samples collected from Rajshahi city are shown in Table 6. The TR values of Pb for rice were 0.1×10^{-6} to 1.1×10^{-6} for adults and 0.1×10^{-6} to 1.2×10^{-6} for children, for Cr were 0.3×10^{-6} to 2.1×10^{-6} for adult and 0.3×10^{-6} to 2.4×10^{-6} for children and for Cd were 0.33×10^{-4} to 2.19×10^{-4} for adults and 0.38×10^{-4} to 2.48×10^{-6} for children. It was found that, TR values for Pb and Cr are lower than 10^{-6} for some samples which are considered as non-carcinogenic, rest of the values is between 10^{-4} to 10^{-6} values, which is considered as a threshold cancer risk. But most of the TR values for Cd are higher than 10^{-4} which is carcinogenic, rest are between 10^{-4} to 10^{-6}. Most of the TCR values are also higher than 10^{-4}. Table 6 also shows that maximum TCR values are higher than 10^{-4}, which is considered as carcinogenic risk (USEPA, 2020).

Table 6: TR and TCR of heavy metals in rice.

Sample Id No.	Target Carcinogenic Risk (TR)						TCR	
	Pb		Cr		Cd		Adult	Child
	Adult	Child	Adult	Child	Adult	Child		
J_1	0.2×10^{-6}	0.3×10^{-6}	2.1×10^{-6}	2.4×10^{-6}	1.02×10^{-4}	1.16×10^{-4}	1.04×10^{-4}	1.19×10^{-4}
J_2	0.7×10^{-6}	0.8×10^{-6}	1.2×10^{-6}	1.3×10^{-6}	1.08×10^{-4}	1.22×10^{-4}	1.10×10^{-4}	1.24×10^{-4}
J_3	0.1×10^{-6}	0.1×10^{-6}	0.3×10^{-6}	0.3×10^{-6}	1.38×10^{-4}	1.56×10^{-4}	1.38×10^{-4}	1.56×10^{-4}
J_4	0.6×10^{-6}	0.7×10^{-6}	1.8×10^{-6}	2.0×10^{-6}	0.48×10^{-4}	0.54×10^{-4}	0.50×10^{-4}	0.55×10^{-4}
A_1	0.2×10^{-6}	0.3×10^{-6}	0.3×10^{-6}	0.3×10^{-6}	2.15×10^{-4}	2.43×10^{-4}	2.16×10^{-4}	2.44×10^{-4}
A_2	0.1×10^{-6}	0.1×10^{-6}	0.9×10^{-6}	1.0×10^{-6}	1.11×10^{-4}	1.26×10^{-4}	1.12×10^{-4}	1.26×10^{-4}
A_3	0.1×10^{-6}	0.1×10^{-6}	1.8×10^{-6}	2.0×10^{-6}	1.11×10^{-4}	1.26×10^{-4}	1.13×10^{-4}	1.26×10^{-4}
A_4	0.2×10^{-6}	0.3×10^{-6}	2.1×10^{-6}	2.4×10^{-6}	1.58×10^{-4}	1.77×10^{-4}	1.60×10^{-4}	1.80×10^{-4}
G_1	0.5×10^{-6}	0.5×10^{-6}	2.1×10^{-6}	2.4×10^{-6}	1.70×10^{-4}	1.91×10^{-4}	1.73×10^{-4}	1.94×10^{-4}
G_2	0.2×10^{-6}	0.3×10^{-6}	0.3×10^{-6}	0.3×10^{-6}	1.29×10^{-4}	1.46×10^{-4}	1.30×10^{-4}	1.47×10^{-4}
G_3	0.7×10^{-6}	0.8×10^{-6}	0.6×10^{-6}	0.7×10^{-6}	2.19×10^{-4}	2.48×10^{-4}	2.20×10^{-4}	2.50×10^{-4}
G_4	0.4×10^{-6}	0.4×10^{-6}	1.8×10^{-6}	2.0×10^{-6}	0.98×10^{-4}	1.11×10^{-4}	1.00×10^{-4}	1.11×10^{-4}
C_1	0.7×10^{-6}	0.8×10^{-6}	1.8×10^{-6}	2.0×10^{-6}	0.33×10^{-4}	0.38×10^{-4}	0.36×10^{-4}	0.39×10^{-4}
C_2	0.2×10^{-6}	0.3×10^{-6}	2.1×10^{-6}	2.4×10^{-6}	1.77×10^{-4}	2.00×10^{-4}	1.79×10^{-4}	2.03×10^{-4}
C_3	0.4×10^{-6}	0.4×10^{-6}	0.9×10^{-6}	1.0×10^{-6}	0.60×10^{-4}	0.68×10^{-4}	0.61×10^{-4}	0.69×10^{-4}
C_4	1.1×10^{-6}	1.2×10^{-6}	0.6×10^{-6}	0.7×10^{-6}	0.48×10^{-4}	0.54×10^{-4}	0.50×10^{-4}	0.56×10^{-4}

4. CONCLUSION

This investigation sought to ascertain the quantities of heavy metals (Mn, Cd, Pb, and Cr) in rice grain gathered from several locations of Rajshahi City Corporation in Bangladesh as well as their health risks assessment. The baseline information on heavy metal pollution was supplied by the current study, which can also be used to estimate the danger of exposing consumers to levels of heavy metal that are expected. The order for average concentration (mg/kg) of heavy metals in rice samples were measured as Cr (0.43) < Cd (1.13) < Pb (6.87) < Mn (44.58). The concentrations of heavy metals were higher than the permissible limit recommended by WHO in maximum cases. The concentrations of Pb and Cd exceeded 100%, whereas, for Cr, none (0%) of the samples exceeded the permissible limit as set by WHO. The results clearly showed a divergence from the permissible levels by WHO. The maximum permissible limit of Mn was not found in the literature. Based on the result of THQ, the studied rice samples are at high risk with respect to Pb, Mn and Cd (except Cr) which can cause non-carcinogenic risks. Similarly, TR values of Pb, Mn and Cr indicate safe for carcinogenic risk, but Cd indicates carcinogenic risk. This study could suggest that further research is needed to find out possible sources of heavy metal contamination and concentration determination to take proper action. This study will be helpful for future studies on heavy metals of selected areas.

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LIST OF ABBREVIATIONS

Pb: lead; Cr: chromium; Mn: manganese; Cd: cadmium; AAS: atomic absorption spectroscopy; FAO: Food and Agricultural Organization; WHO: World Health Organization EDI: Estimated daily intake; THQ: Target hazard quotient; TTHQ: Total target hazard quotient; TR: Target carcinogenic risk; TCR: Cumulative cancer risk; TDI: Tolerable daily intake; Min: Minimum values; Max: Maximum values; SD: standard deviations; %RSD: percentage relative standard deviations.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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