

Metallic Health Risk through Consumption of Different Rice Varieties Cultivated in Industrial Wastewater Irrigated Farmers' Fields of Bhaluka Area, Bangladesh

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Authors' contributions

This work was carried out in collaboration among all authors. Author HMZ designed the study, managed the literatures and supervised the work. Author FI performed the sample collection & processing, analysis, data recording and wrote the first draft of the manuscript. Authors QFQ and AR helped to design the study and manuscript preparation. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i1130650

Editor(s):

(1) Dr. Maduikie Chiehiura Onwubiko Ezeibe, Michael Okpara University of Agriculture, Nigeria.

Reviewers:

(1) Angel Ramon Sanchez Delgado, Universidade Federal Rural do Rio de Janeiro, Brazil.

(2) Rubismar Stolf, São Carlos Federal University, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/57085>

Original Research Article

Received 10 March 2020

Accepted 16 May 2020

Published 28 May 2020

ABSTRACT

A study was conducted to assess metallic health risk for population through consumption of rice grown in industrial wastewater irrigated sites of Bhaluka area, Mymensingh. Total 9 rice samples of 3 different varieties (BR-28, BR-29 and *Kalozira*) were collected directly from the farmers' fields of the area and analysed for this study. The mean concentration of Zn, Cr, Cu, Ni, Cd, Mn and Fe in rice grains, straw and husk of different rice varieties were 54.46, 12.56, 5.45, 8.01, 1.37, 14.82 and 94.1 $\mu\text{g g}^{-1}$; 74.70, 8.38, 5.11, 6.29, 1.17, 594 and 7951 $\mu\text{g g}^{-1}$, and 112.8, 0.60, 0.68, 3.98, 1.29, 214 and 6568 $\mu\text{g g}^{-1}$, respectively. Present study results revealed that Cr, Cu and Ni accumulation pattern in rice followed the sequence as grain> straw> husk; Fe and Mn exhibited the order as straw> husk> grain while Zn and Cd showed the sequence as husk> straw> grain and grain> husk> straw, respectively. As regards to varieties, Cr, Cu, Ni and Cd accumulation pattern followed the sequence as *Kalozira*> BR-29> BR-28, while Zn, Mn and Fe exhibited the order as BR-28> BR-

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29> *Kalozira*, BR-29> BR-28> *Kalozira* and *Kalozira*> BR-28> BR-29, respectively. As regards to fodder, only Cd contents in straw and husk seem to be harmful for animal health. In context of human health, the calculated target hazard quotient (THQ) values for Cr and Cd of all varieties ranged from 16.12-35.44 and 5.04-11.67, respectively for both male and female, and the same values for Mn surpassed 5.0 for female in 6 sites and for male in 4 sites indicate that the exposed populations are unsafe. Finally, the study concluded that peoples should avoid consumption of rice grown in industrial wastewater irrigated agricultural fields of Bhaluka area until proper remediation strategies along with fresh water irrigation are introduced.

Keywords: *Rice grain & straw; human & animal nutrition; fodder; THQ; metal pollution index; rice industry wastewater.*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food in Asian countries, where its production constitutes over 90% of the global production [1]. Approximately 480 million metric tons of milled rice is produced worldwide annually, and China and India alone account for about 50% of the rice grown and consumed [2]. Bangladesh is the fourth largest rice producer in the world, which has a long history of rice cultivation. Recently, concern has been raised about the possible contamination of rice by different heavy metals. The rice component of the Bangladeshi diet alone may contribute up to 46, 57, 50 and 60% of the maximum tolerable daily intake for As, Cd, Pb and Cr, respectively, making it a more important factor in the dietary intake for these elements than other food stuffs [3]. Hence, intake of different heavy metals through rice could cause an adverse impact on human health.

It is very common scenario in Bangladesh that most of the industrial units are discharged wastes and effluents directly to soil, canals and rivers without any treatment. An article reported that among 120 medium to large industries in Sreepur of Gazipur district, 43.3, 44.2 and 10.8% were in red, orange-B and orange-A category, respectively while only 1.7% were in green category. Moreover, among the surveyed industries about 33% are running without any effluent treatment plant (ETP) [4]. There are lots of reports in our country that untreated industrial wastes and effluents contain different heavy metals like Cu, Zn, Pb, Cr, Cd, Ni, Fe and Mn. Thus, soils and natural water systems as well as ground water are polluting by these metals [5-11]. Some of them are toxic to plants and some others are toxic to both plants and animals. Farmers are cultivating crops including rice around the vicinity of different industries. Furthermore, in areas where sufficient amount of

surface and groundwater are scarce, the use of industrial wastewater for irrigation by the farmers is aggravating the situation.

Bhaluka is one among the newly industrialized areas of Bangladesh. This area is highly susceptible to environmental pollution over last decades. There are various types of industrial units exist, viz. pharmaceutical, textile, dyeing, leather, cosmetic, garments, glass, ceramics, packaging and others. Industrial wastewater and effluents discharging from this area are great threat to the surrounding environment, especially to agricultural fields, as because these industries discharge wastewater to the nearby canal or agricultural lands through the pipe line or drain without any treatment. Moreover, local people unconsciously grow rice and different kinds of vegetables in such contaminated lands and they are irrigating the crops using untreated wastewater from the canal. As a consequence, heavy metals are accumulating by the crops grown in such soils and subsequently contaminating the food chain, which ultimately pose serious threat to human being [12-14]. Furthermore, our previous study for the same area already focused on heavy metal contents in industrial wastewater and their deposition status in farm soils [15]. Under this circumstances, this work was undertaken to determine heavy metal contents in rice grown in those industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh and to assess health risk for adult male and female through consumption of those rice.

2. METHODOLOGY

2.1 Collection of Rice Samples

Total 9 rice (including straw) samples of 3 different varieties viz. BR-28 (HYV), BR-29 (HYV) and *Kalozira* (local) were collected directly from the farmers' fields of some selected

industrial areas of Bhaluka Upazila, Mymensingh. Samplings were done during the month of April-May, 2018 and then processed for chemical analysis. Details of the sampling sites along with the names of nearest industries and possible sources of contamination are presented in Table 1. For obtaining a representative sample, rice samples were collected from at least 3 different points and then mixed together to make a composite sample.

2.2 Processing of Rice Samples

After collection, each composite sample was put into individual polythene bag with definite marking and tagging, and then the collected samples were carried to the laboratory of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh. Then the rice plant samples were separated into whole grain and straw. After separation, both samples were cleaned and air dried first. Then the samples oven dried for 72 hrs at 50°C temperature until constant weight was obtained. After drying, whole grain samples were separated again into grain (edible part) and husk. Then the samples were ground using a mechanical grinder. Each sample was then kept in a separate clean polythene bag with appropriate marking for chemical analyses. The chemical analyses of the samples were accomplished in the laboratory of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh, Bangladesh.

2.3 Preparation of Extract

Oven dried and well ground grain, straw and husk samples were used to prepare extract for the determination of different mineral nutrients and heavy metals. Extract for each sample was prepared by wet oxidation method using di-acid mixture [16]. In this method, exactly 1.00 g of finely ground each sample was taken into a 250 mL conical flask and 10 mL of di-acid mixture (HNO_3 : HClO_4 = 2:1) was added to it. Then the flask was placed on an electric hot plate for heating at 180-200°C temperature until the solid particles totally disappeared and white fumes were evolved from the flask. Then, it was cooled at room temperature, washed with distilled water and filtered into 100 mL volumetric flask through filter paper (Whatman No. 1). Finally, the volume was made up to the mark with

distilled water and preserved for the determination of total major mineral nutrients and heavy metals content in grain, straw and husk samples. To ensure the quality control purpose, a blank extract was also prepared using all reagents except sample and used for the measurements of major nutrients and heavy metals.

2.4 Determination of Major Nutrients

Among the major nutrient elements, Ca, Mg, P, S, Na and K were measured from the collected grain, straw and husk samples [16]. The amount of Ca and Mg were determined by titrimetrically against standard $\text{Na}_2\text{-EDTA}$ solution, P content in aqueous extract was measured by stannous chloride method using a spectrophotometer (660 nm absorbance wavelength; T60 UV-Visible Spectrophotometer, PG Instrument, UK), S content was determined by turbidimetrically using BaCl_2 as turbidimetric agent (425 nm absorbance wavelength; T60 UV-Visible Spectrophotometer, PG Instrument, UK), and Na and K contents in aqueous extract were estimated by flame photometrically (589 and 766 nm emission wavelengths, respectively; 0.2 ppm limit of detection; Jenway PFP7, Flame Photometer, UK) [16]. The instrumental parameters were adjusted according to the manufacturer's recommendations.

2.5 Determination of Heavy Metals

Determination of different heavy metals (Cu, Cr, Cd, Zn, Ni, Fe and Mn) in aqueous extracts of rice grain, straw and husk samples were done by an atomic absorption spectrophotometer (AAS) (SHIMADZU, AA-7000; Japan) at the Laboratory of the Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh, Bangladesh. At first the AAS was calibrated followed by the manufacturer's recommendation. Then the aqueous extract was diluted (if required) and/ or run directly in AAS for the determination of each heavy metal in different types of samples mentioned above. Hollow cathode lamp of Cu, Cr, Cd, Zn, Ni, Fe and Mn was employed as light source at wavelengths of 324.8, 357.9, 228.8, 213.9, 232.0, 248.3 and 279.5 nm, respectively for the determination of each metal. All chemicals and reagents were of analytical reagent grade quality. Before use, all glass and plastic ware were soaked in 14% HNO_3 for 24 hrs. The washing was completed with distilled water rinse.

Table 1. Details of rice sampling locations of Bhaluka, Mymensingh along with the names of nearest industries and possible sources of contamination (after Islam et al. [15])

Sample ID	Name and type of rice variety	Location	Nearest Industries and possible sources of contamination
1	BR-28 (HYV)	Khandakar para, South Habbir Bari, Seedstore union, Bhaluka, Mymensingh.	Orion Knit Textile Ltd., Noman Composite Textiles Ltd. and common discharge pipelines from other industries
2	BR-28 (HYV)	South Habbir Bari, Seedstore union, Bhaluka, Mymensingh.	Orion Knit Textile Ltd., Badsha Textile Ltd. and common discharge pipelines from other industries
3	BR-28 (HYV)	East side of Noman Composite Textile Ltd., Seedstore union, Bhaluka, Mymensingh.	Orion Knit Textile Ltd., Badsha Textile Ltd. and common discharge pipelines from other industries
4	BR-28 (HYV)	North side of Noman Composite Textile Ltd., Seedstore union, Bhaluka, Mymensingh.	Noman Composite Textiles Ltd. and common discharge pipelines from other industries
5	BR-29 (HYV)	East side of Noman Composite Textile Ltd., Seedstore union, Bhaluka, Mymensingh.	Noman Composite Textiles Ltd., Orion Knit Textile Ltd. and common discharge pipelines from other industries
6	BR-28 (HYV)	North side of the canal near SMC Enterprise Ltd., Jamirdia, Bhaluka, Mymensingh.	Wastewater from different industries discharge into a common canal
7	BR-28 (HYV)	South side of SMC Enterprise, Jamirdia, Bhaluka, Mymensingh.	Wastewater from different industries discharge into a common canal
8	<i>Kalozira</i> (Local)	9 No. Kathali ward, Kathali union, Bhaluka, Mymensingh.	Beacon Pharmaceuticals Ltd. and wastewater from different industries discharge in to a common canal
9	BR-29 (HYV)	Hazir bazar, 9 No. Kathali ward, Kathali union, Bhaluka, Mymensingh.	Consumer Knitex Ltd., Arty Composite Ltd., Dynamic Textile Ltd. and wastewater from different industries discharge into a common canal

HYV = High yielding variety

2.6 Estimation of Daily Metal Intakes (DMI)

To assess the health risk associated with heavy metal contamination in rice grain samples, the daily intake of metal was calculated with the following formula-

$$DMI = \frac{IR \times C}{BW}$$

Where, IR is the rice ingestion rate (mg person⁻¹ day⁻¹), C is the individual metal concentration in rice grain samples (mg kg⁻¹, fresh weight), BW is the body weight assuming 70 kg for adult male and 50 kg for adult female in the present study [17].

2.7 Metal Pollution Index (MPI)

To examine the overall heavy metal concentrations in rice grain samples, the metal pollution index (MPI) was computed. This index was obtained by calculating the geometrical mean of concentrations of all the metals present in rice grain samples [18].

$$MPI (mg\ kg^{-1}) = (Cf_1 \times Cf_2 \times \dots \times Cf_n)^{1/n}$$

Where, Cf_n = Concentration of nth metal in the sample and n=7.

2.8 Target Hazard Quotients (THQ)

THQ was calculated by the general formula established by the US EPA as follows-

$$THQ = \frac{E_f \times F_D \times DMI}{R_fD \times W \times T}$$

Where, EF is exposure frequency; FD is the exposure duration; DMI is the daily metal ingestion (mg person⁻¹ day⁻¹) and RfD is the oral reference dose (mg kg⁻¹ day⁻¹); W is the average body weight (kg) and T is the average exposure time for noncarcinogens (365 days year⁻¹ × number of exposure years).

3. RESULTS AND DISCUSSION

3.1 Major Nutrient Status in Rice

3.1.1 Calcium (Ca)

Concentrations of Ca in rice grain, straw and husk varied from 0.073-0.671, 0.188-0.464, 0.037-0.193%, respectively with the average values of 0.265, 0.357 and 0.130%, respectively (Table 2). Ca content was comparatively higher

in straw than grain and husk. The recommended dietary Ca intakes for healthy men and women ranges between 800 and 1300 mg day⁻¹ [19]. So, it can be inferred from the present study that the edible part of rice (grain) provides a small amount of Ca in respects to human requirements. As regards to fodder, most non-forage feedstuffs contain only small amounts of Ca [20]. This result is at par with the present study results as rice straw and husk also contained comparatively lower amount of Ca. On the other hand, in context of rice varieties, grain Ca content was almost thrice in BR-28 and the mean Ca accumulation pattern followed the sequence as BR-28 > *Kalozira* > BR-29 (Table 3). The content of Ca in plant differs widely depending on the plant species as well as plant parts and the range of Ca contents in plant varied from 0.20-1.0% [21], and most of the straw samples contained Ca within this range.

3.1.2 Magnesium (Mg)

The contents of Mg in rice grain, straw and husk obtained from wastewater irrigated sites of Bhaluka area ranged from 0.037-0.264, 0.078-0.336, 0.077-0.169%, respectively with the mean values of 0.122, 0.222 and 0.123%, respectively (Table 2). Similar to Ca, Mg content was also higher in straw samples, and grain Mg content was higher in BR-28 and the mean Mg accumulation pattern followed the order as BR-28 > *Kalozira* > BR-29 (Table 3). However, an adult healthy body contain approximately 21-28 g (about 1 mole) of Mg, related to an average body weight of 70 kg [19]. Hence, it can be inferred from the present study that rice grown in this area provide little amount of Mg both for male and female. Cattle can excrete large amounts of Mg with the urine, and thus Mg toxicity is not a practical problem in dairy cattle. However, National Research Council set a maximum tolerable level of 0.4% Mg for dairy cattle [20], and Mg contents in rice straw and husk were also within this limit. The content of Mg also differs widely among the plant species and the range of Mg content in plant varied from 0.10-0.40% [21], and in most cases present study results were within this range.

3.1.3 Sodium (Na)

Sodium is very much necessary for humans to maintain the balance of the physical fluids system and is also required nerve and muscle functioning. Too much Na can damage our kidneys and increase the chances of high blood pressure [22]. Concentrations of Na in rice grain,

straw and husk varied from 0.001-0.039, 0.232-0.699, 0.041-0.072%, respectively with the average values of 0.023, 0.355 and 0.05%, respectively (Table 2). The result showed that Na concentration was several times higher in straw than husk and grain and the sequence was straw > husk > grain. Feedstuffs commonly used in diets for dairy cattle do not contain enough Na to meet requirements. The daily maintenance requirement for absorbed Na for growing cattle and non-lactating pregnant cows was set at 1.5 grams 100kg⁻¹ body weight per day [20]. Thus, rice straw can be used as good source of Na for dairy cattle nutrition. As regards to rice varieties, grain Na content was higher in BR-28 and the mean Na accumulation pattern followed the sequence as BR-28 > BR-29 > *Kalozira* (Table 3).

3.1.4 Potassium (K)

Potassium is a co-factor for many enzymes and it is required for insulin secretion, creatine phosphorylation, carbohydrate metabolism and protein synthesis [23]. Concentrations of K in rice grain, straw and husk varied from 0.13-0.19, 1.15-2.72, 0.247-0.457%, respectively with the mean values of 0.150, 1.94 and 0.353%, respectively (Table 2). Similar to Na, K concentration was also several times higher in straw than husk and grain, and the sequence was straw > husk > grain. Feedlot cattle require approximately 0.55 to 0.60% K [20]. Thus, it can be inferred from this study that rice straw can be used as a good source of K as fodder to meet the demand of K deficiency for cattle. On the other hand, according to EFSA [19], human dietary deficiency of K is very uncommon due to the wide spread occurrence of K in foods. But WHO stated that even though there isn't a recommended dietary intake (RDI) for K, organizations around the world have recommended consuming at least 3,500 mg K day⁻¹ through different foods [24]. As regards to rice varieties, grain K content was higher in local variety (*Kalozira*) than HYVs (BR-28 and BR-29), but the mean K accumulation pattern followed the sequence as BR-29 > BR-28 > *Kalozira* (Table 3).

3.1.5 Phosphorus (P)

The contents of P in rice grain, straw and husk varied from 0.33-0.72, 0.125-0.304, 0.128-0.253%, respectively with the average values of 0.500, 0.233 and 0.172%, respectively (Table 2) and grain P content was 2-3 times higher than straw and husk. As regards to rice varieties,

grain P content was higher in BR-28 and the mean P accumulation pattern followed the sequence as BR-28 > BR-29 > *Kalozira* (Table 3). The nationally averaged community concentration of P was 1.11 mg g⁻¹ for leaves; 0.31 mg g⁻¹ for stems and 0.47 mg g⁻¹ for roots [25], but P contents obtained by the present study were much higher than this report. A normal healthy individual can tolerate phosphate intake up to at least 3000 mg day⁻¹ without any adverse systemic effects. In some individuals mild gastrointestinal symptoms have been reported if exposed to supplemental intakes is greater than 750 mg P per day [19]. It can be inferred from this study that dietary intake of 100 g rice per day can provide us 330-720 mg P, which means rice is a good source of P for human nutrition. On the other hand, requirement of dietary P concentrations for lactating cattle ranging from 0.24 to 0.65% of dietary dry matter [20], and the average P concentration in straw was close to this range.

3.1.6 Sulphur (S)

Concentrations of S in rice grain, straw and husk ranged from 0.07-0.23, 0.149-0.473, 0.025-0.723%, respectively with the mean values of 0.130, 0.266 and 0.202%, respectively (Table 2). Similar to other mineral nutrients, straw contained more S than grain and husk, and the sequence of S in rice was straw > husk > grain. Sulphur is present in glutathione, one of the most important antioxidants. The sulfur-containing amino acids are methionine, cysteine, cystine, homocysteine, homocystine, and taurine [26]. Thus, S is very important for human as well as animal nutrition. The S requirement for cattle is set at 0.20% of dietary dry matter [20], and the mean contents of S in rice straw and husk was above this limit. Similar to K, grain S content was also higher in local variety (*Kalozira*) than HYVs (BR-28 and BR-29), but the mean K accumulation pattern followed the sequence as BR-28 > BR-29 > *Kalozira* (Table 3).

3.2 Heavy Metal Status in Rice

3.2.1 Zinc (Zn)

Zinc is the second most abundant metal present in the human body, after Fe but before Cu. It is found throughout the whole body system, with half in the muscle tissues [27]. Concentrations of Zn in rice grain, straw and husk ranged from 26.66-94.34, 37.24-129.8, 29.2-685.1 µg g⁻¹, respectively with the mean values of 54.46, 74.70 and 112.8 µg g⁻¹, respectively (Table 4).

Table 2. Concentration of major nutrient elements in different parts of rice samples collected from industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh

Sample ID	Minerals in rice grain (%)						Minerals in rice straw (%)						Minerals in rice husk (%)					
	Ca	Mg	Na	K	P	S	Ca	Mg	Na	K	P	S	Ca	Mg	Na	K	P	S
1	0.271	0.255	0.037	0.19	0.62	0.15	0.438	0.204	0.232	1.74	0.219	0.149	0.059	0.168	0.056	0.324	0.253	0.056
2	0.333	0.094	0.036	0.14	0.53	0.19	0.385	0.273	0.255	1.72	0.261	0.222	0.160	0.078	0.046	0.299	0.128	0.026
3	0.671	0.038	0.039	0.13	0.71	0.14	0.410	0.283	0.699	1.43	0.304	0.366	0.193	0.098	0.072	0.412	0.197	0.723
4	0.152	0.097	0.033	0.15	0.54	0.11	0.264	0.287	0.294	1.69	0.226	0.154	0.152	0.154	0.044	0.247	0.136	0.026
5	0.073	0.111	0.017	0.14	0.33	0.08	0.188	0.336	0.318	1.83	0.286	0.354	0.154	0.097	0.053	0.355	0.174	0.258
6	0.478	0.264	0.022	0.18	0.72	0.23	0.464	0.157	0.302	2.52	0.213	0.473	0.112	0.133	0.041	0.377	0.188	0.574
7	0.152	0.073	0.014	0.15	0.34	0.08	0.346	0.136	0.521	2.72	0.232	0.258	0.037	0.169	0.043	0.457	0.195	0.071
8	0.073	0.129	0.013	0.17	0.46	0.17	0.336	0.246	0.288	1.15	0.125	0.152	0.191	0.077	0.051	0.428	0.140	0.055
9	0.181	0.037	0.001	0.14	0.35	0.07	0.384	0.078	0.299	2.67	0.202	0.254	0.111	0.131	0.043	0.274	0.134	0.025
Mean	0.265	0.122	0.024	0.15	0.51	0.14	0.357	0.222	0.356	1.94	0.230	0.265	0.130	0.123	0.050	0.353	0.172	0.202
Maximum	0.671	0.264	0.039	0.19	0.72	0.23	0.464	0.336	0.699	2.72	0.304	0.473	0.193	0.169	0.072	0.457	0.253	0.723
Minimum	0.073	0.037	0.001	0.13	0.33	0.07	0.188	0.078	0.232	1.15	0.125	0.149	0.037	0.077	0.041	0.247	0.128	0.025
Std. Dev.	0.200	0.084	0.013	0.02	0.16	0.07	0.087	0.084	0.150	0.56	0.055	0.121	0.055	0.037	0.010	0.072	0.041	0.266

Table 3. Average concentration of major nutrient elements in different parts and varieties of rice samples collected from industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh

Type of sample	Rice varieties	Average concentration of different mineral elements (%)					
		Ca	Mg	Na	K	P	S
Grain	BR-28	0.343	0.137	0.030	0.157	0.577	0.150
	BR-29	0.127	0.074	0.009	0.140	0.340	0.075
	<i>Kalozira</i>	0.073	0.129	0.013	0.170	0.460	0.170
Straw	BR-28	0.385	0.223	0.384	1.970	0.243	0.270
	BR-29	0.286	0.207	0.309	2.250	0.244	0.304
	<i>Kalozira</i>	0.336	0.246	0.288	1.150	0.125	0.152
Husk	BR-28	0.119	0.133	0.050	0.353	0.183	0.246
	BR-29	0.133	0.114	0.048	0.315	0.154	0.142
	<i>Kalozira</i>	0.191	0.077	0.051	0.428	0.140	0.055
Mean total by BR-28		0.282	0.165	0.155	0.826	0.334	0.222
Mean total by BR-29		0.182	0.132	0.122	0.902	0.246	0.174
Mean total by <i>Kalozira</i>		0.200	0.151	0.117	0.583	0.242	0.126

Rice husk contained more Zn than straw and grain, and the sequence of Zn in rice samples was husk > straw > grain. However, the recommended daily allowance (RDA) for Zn is 8.0 mg day⁻¹ for women and 11.0 mg day⁻¹ for men [28]. So, it can be inferred from this results that 100 g rice grain contained about 5.45 mg Zn, which is within the RDA and hence rice grains grown in the study area can be assumed as a good source of Zn nutrition in human. Zinc content in polished rice of Bangladesh ranged from 7.0-23.9 mg kg⁻¹ with a mean value of 1.29 mg kg⁻¹ [29], which is comparatively lower than the present study result. Soil samples collected from the same wastewater irrigated farmers' fields of Bhaluka area contained higher amount of Zn [15], which might contribute in enhanced Zn accumulation in different parts of rice. The maximal tolerable level of dietary Zn for cattle is suggested to be 300 to 1000 mg kg⁻¹ diet [20], and both straw and husk contained Zn within this range (Table 4). Thus, it can be inferred from this study that both straw and husk seem to be safe as fodder in context of Zn content. As regards to rice varieties, BR-28 accumulated higher amount of Zn in all parts and the mean Zn accumulation pattern followed the sequence as BR-28 > BR-29 > *Kalozira* (Table 5).

3.2.2 Chromium (Cr)

Chemically, trivalent Cr is non-toxic and trace amounts require for humans, while the hexavalent form is very toxic. Reference dietary intakes for Cr is 35 mg day⁻¹ for adult males and 25 mg day⁻¹ for adult females [30]. Concentrations of Cr in rice grain, straw and husk ranged from 10.02-15.74, 6.92-9.86, trace-1.81 µg g⁻¹, respectively with the mean values of

12.56, 8.38 and 0.60 µg g⁻¹, respectively (Table 4). It is evident from the above mentioned data that rice grain contained more Cr than straw and husk, and the sequence of Cr in rice was grain > straw > husk. Concentrations of Cr in 3 domestic and 7 imported rice brands varied from 0.33-0.55 µg g⁻¹ [31], which is too lower than the present study results. Such a high variation in rice grain Cr content might be due to higher amount of available Cr in soils of wastewater irrigated farmers' fields of Bhaluka area [15]. On the other hand, for livestock the maximum tolerable concentration of Cr in the diet is set at 3000 mg kg⁻¹ for the oxide form and 1000 mg kg⁻¹ for the chloride form of the trivalent forms of Cr, and hexavalent forms of Cr are at least five times more toxic for cattle [20]. So, it can be said from this study that both straw and husk seem to be safe as fodder in context of Cr content. As regards to rice varieties, higher amount of Cr was accumulated in grains of *Kalozira* followed by BR-28 and BR-29, but the mean Cr accumulation pattern in different parts of rice plant showed the sequence as *Kalozira* > BR-29 > BR-28 (Table 5).

3.2.3 Copper (Cu)

Copper is a part of many enzymes but usually occur in very low levels in different foods. Concentrations of Cu in rice grain, straw and husk ranged from 3.84-7.35, 2.89-6.89, trace-2.61 µg g⁻¹, respectively with the mean values of 5.45, 5.11 and 0.68 µg g⁻¹, respectively (Table 4). Between the two cultivars of rice, the mean Cu concentration under system of rice intensification and conventional transplanting varied between 3.17-3.42 mg kg⁻¹ [32], and the present study result was little bit higher than this range. Similar to Cr content, rice grain samples contained more

Cu than straw and husk, and the sequence of Cu in rice was grain > straw > husk. The human body only contains about 150 mg of this vital mineral and the established RDA for Cu in normal healthy adults is 2 mg day⁻¹ [33]. Thus, it can be inferred from this study that 100 g rice grain contained about 0.55 mg Cu, hence rice grown in industrial wastewater irrigated farmers' fields of Bhaluka area will not adversely affect on human health through the food chain. In the same way, only between 1 and 5% of dietary Cu is absorbed by adult cattle [20], thus both straw and husk will also not harmful as fodder in context of Cu content. As regards to rice varieties, higher amount of Cu was accumulated in grains of *Kalozira* followed by BR-29 and BR-28, and the mean Cu accumulation pattern in different parts of rice plant also showed the same sequence as *Kalozira* > BR-29 > BR-28 (Table 5).

3.2.4 Nickel (Ni)

Concentrations of Ni in rice grain, straw and husk ranged from 5.87-8.78, 5.31-7.41, 2.60-5.90 µg g⁻¹, respectively with an average values of 8.01, 6.29 and 3.98 µg g⁻¹, respectively (Table 4). The upper tolerable intake level for Ni is set at 1.0 mg day⁻¹ person⁻¹ for adult human being [30]. So, it can be inferred from this study that 100 g rice grain contained about 0.80 mg Ni, and the amount was lower than the upper tolerable intake level, hence rice grown in the study area will not adversely affect on human health through the food chain. It is evident from Table 4 that the rice grain contained more Ni than straw and husk, and the sequence of Ni in rice was grain > straw > husk. On the other hand, concentrations of Ni in 3 domestic and 7 imported rice brands reported to be 0.65-0.90 µg g⁻¹ [31], which was about 10 times lower than the present study results. Such an excessive difference in rice grain Ni content might be due to higher accumulation of Ni from wastewater irrigated soils of Bhaluka area, which contained 22.93-43.86 µg g⁻¹ Ni [15]. Nickel is relatively nontoxic with maximal tolerable dietary concentration of 50 mg kg⁻¹ for cattle [20]. So, it can be said from this study that both straw and husk seem to be safe as fodder in context of Ni content. As regards to rice varieties, higher amount of Ni was accumulated in grains of *Kalozira* followed by BR-29 and BR-28, and the mean Ni accumulation pattern in different parts of rice plant was also same and the sequence was *Kalozira* > BR-29 > BR-28 (Table 5).

3.2.5 Cadmium (Cd)

Cadmium is a heavy metal with high toxicity, which has acute and chronic effects on health

and environment. In human and animal, this metal accumulates within the body, particularly in the kidney, to cause renal damage [34]. Concentrations of Cd in rice grain, straw and husk ranged from 0.93-1.73, 0.69-1.64, 1.06-1.42 µg g⁻¹, respectively with an average values of 1.37, 1.17 and 1.29 µg g⁻¹, respectively (Table 4). Cd concentration in rice grain and husk was almost similar but rice straw contained comparatively lower amount of Cd. The contents of Cd in 3 domestic and 7 imported rice brands varied from 0.27-0.48 µg g⁻¹ [31], which was 3-4 times lower than the present study results. Hence, grain Cd concentration may be hazardous for human health, as because the World Health Organization has established a provisional tolerable weekly intake of cadmium 7.0 µg kg⁻¹ body weight [35]. Similarly, the Joint FAO/WHO Expert Committee on Food Additives [36] established a provisional maximum tolerable weekly intake of Cd at 400-500 µg per individual, i.e. 57 to 71 µg day⁻¹ per individual. Soil samples collected from the same wastewater irrigated farmers' fields of Bhaluka area contained higher amount of Cd [15], which might contribute in greater Cd accumulation in grain, straw and husk of rice. A similar observation was reported that moderate Cd contamination of arable soils can result in considerable Cd accumulation in edible parts of rice [37]. The maximal tolerable Cd in the diet of cattle was set at 0.5 mg kg⁻¹ [20]. So, it can be inferred that both straw and husk seem to be animal health hazardous as fodder in context of Cd content. As regards to rice varieties, grain Cd content was almost same and the range was 1.36-1.41 µg g⁻¹. However, the mean Cd accumulation pattern in different parts of rice plant followed the sequence as *Kalozira* > BR-28 > BR-29 (Table 5).

3.2.6 Manganese (Mn)

Manganese is an essential trace element for plants, domestic animals, and humans. The contents of Mn in rice grain, straw and husk ranged from 3.82-21.63, 201.0-964.0, 74.0-351.0 µg g⁻¹, respectively with an average values of 14.82, 594.0 and 214.0 µg g⁻¹, respectively (Table 4). Thus, it can be inferred from this data that rice straw could be treated as a huge source for Mn, and the sequence of Mn in rice was straw > husk > grain. Manganese helps the body to form connective tissue, bones, blood-clotting factors and sex hormones [38]. The RDA for Mn is set at 2.3 mg day⁻¹ for adult males and 1.8 mg day⁻¹ for adult females [30]. Among the two cultivars of rice grain, the mean Mn concentrations varied between 39.0-41.2 mg kg⁻¹

[32], which is twice than the present study result. On the other hand, according to Dobermann and Fairhurst [39], Mn toxicity is relatively rare, especially in irrigated rice systems and rarely occurs in lowland rice if soil pH <5.5. Thus, higher amount of soil Mn don't show any toxicity symptom in rice but it is mainly accumulated in rice straw and husk from the wastewater irrigated agricultural soils of Bhaluka. However, there is no precise data on maintenance requirements for Mn in dairy cattle, but its deficiency has been reported when diets contained 16 to 17 mg of dietary Mn kg⁻¹ of dry matter [20]. In context of rice varieties, higher amount of Mn was accumulated in grains BR-29 followed by BR-28 and *Kalozira*, and the mean Mn accumulation pattern in different parts of rice plant was also same, where the sequence was BR-29> BR-28>*Kalozira* (Table 5).

3.2.7 Iron (Fe)

The contents of Fe in rice grain, straw and husk ranged from 66.5-113.6, 4225-9927 and 2530-10301 µg g⁻¹, respectively with an average values of 94.1, 7951 and 6568 µg g⁻¹, respectively (Table 4). It can be inferred from this data that similar to Mn, Fe in rice is also mainly accumulated in straw and husk and the sequence of Fe content in different part of rice plant was straw> husk> grain. The mean Fe concentrations in two different cultivars of rice grain varied between 40.3-47.7 mg kg⁻¹ [32], and among 3 different genotypes of rice ranged between 11.95-14.87 µg g⁻¹ [40], which were lower than that of the present study results. Higher amount of Fe in rice grain is good for nutrition as because Fe deficiency is the most widespread micronutrient deficiency in humans. But Fe deficiency in adult cattle is very rare. The absorbed Fe requirement for growth of cattle has been set at 34 mg Fe kg⁻¹ average daily gain [20]. Thus, it can be inferred that both straw and husk seem to be good source of Fe for cattle nutrition as fodder. As regards to rice varieties, higher amount of Fe was accumulated in grains of *Kalozira* followed by BR-28 and BR-29, and the mean Fe accumulation pattern in different parts of rice plant was also same and the sequence was *Kalozira*> BR-28> BR-29 (Table 5).

3.3 Estimation of Daily Metal Intake (DMI)

To estimate metallic health risk through consumption of rice grown in wastewater irrigated agricultural fields of Bhaluka, the daily

intakes of metals were calculated. Among the possible pathways of exposure of metals to humans, food chain is the most important. The DMI was calculated according to the average rice consumption for both adults male and female. Rice ingestion rate in Bangladesh is 367.19 g person⁻¹ day⁻¹ [41], which was used to calculate DMI. Then the DMI of Fe, Mn, Zn, Cu, Cr, Cd and Ni from rice grain samples were calculated by multiplying the amount of daily intake by each metal concentration determined in this study. The DMI were compared with the upper tolerable daily intakes for metals. It is evident from Table 6 that daily metal intake for all studied metals for both male and female were higher than that of upper tolerable intake levels, which indicates serious adverse effects have been associated with dietary intake of rice grown in industrial wastewater irrigated agricultural fields of Bhaluka area, Mymensingh.

3.4 Target Hazard Quotients (THQ)

The estimation of potential health risks associated with long term exposure to chemical pollutants using THQ is a common practice worldwide [45-48]. The THQ < 1 means that the exposed population is assumed to be safe; 1 < THQ < 5 means that the exposed population is in a level of concern interval, and THQ > 5 means exposed population is unsafe. In this study, THQ was calculated considering the obtained DMI, average body weight (male: 70 kg and female: 50 kg) and average life expectancy (male: 70.6 and female: 73.1) [17] of people of Bangladesh. Values of THQ due to consumption of rice grown in wastewater irrigated agricultural fields of Bhaluka for all studied metals are presented in Fig. 1. THQ values for Cr and Cd in all samples surpassed 5.0 (THQ values ranged from 16.12-35.44 and 5.04-11.67, respectively) for both male and female, and the same values for Mn also surpassed 5.0 for female in 6 sites and for male in 4 sites indicate that the exposed populations are unsafe. Such high values of THQ are mainly contributed by the metal concentration as well as pattern of Bangladeshi diet where contribution of rice is always higher (367.19 g person⁻¹ day⁻¹ [41]). It is also worth mentioning from the Fig. 1 that all individual THQ values for Ni for both male and female surpassed 1 (ranged between 1.42 to 2.97) i.e. in context Ni both male and female are in level of concern interval. Similarly, THQ values for Zn also surpassed 1 for female in 6 sites and for male in 4 sites indicate exposed populations are also in level of concern interval. So, it can be concluded from the present study results that Cr,

Cd and Mn concentrations in rice grown in industrial wastewater irrigated agricultural fields of Bhaluka area are unsafe for human consumption. On the other hand, Ni and Zn content in rice grains are in level of concern interval for both male and female. Thus, peoples should refrain from consumption of rice grown in industrial wastewater irrigated agricultural fields of Bhaluka area until proper remediation

strategies are introduced along with fresh water irrigation. However, a similar report also stated that consumption of different food stuffs grown in wastewater irrigated site of north east Varanasi in India presented a significant threat of negative impact on human health [48]. They also made similar observation that health risk through consumption of rice was greater due to higher contribution of it in the Indian diet.

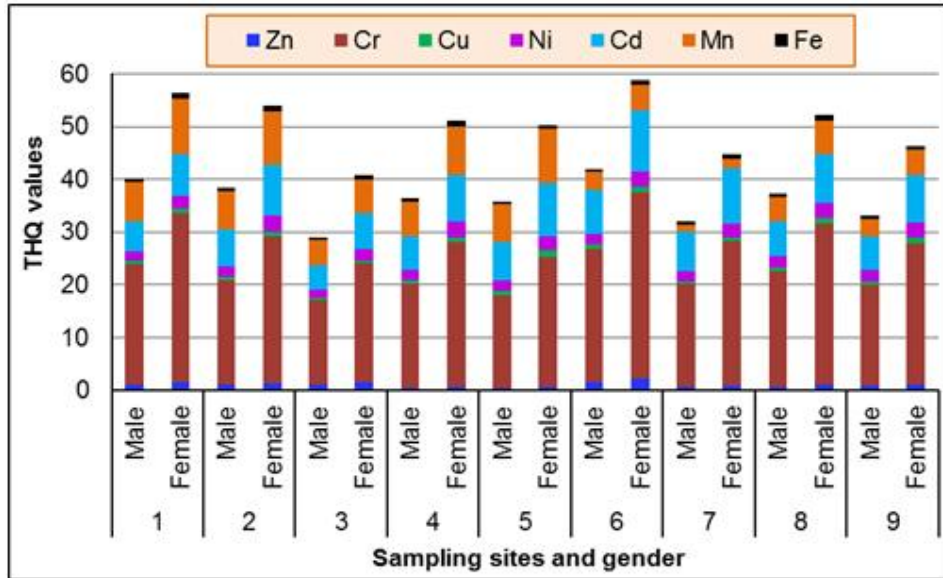


Fig. 1. Target hazard quotient (THQ) for adult male and female due to intake of rice grown in industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh

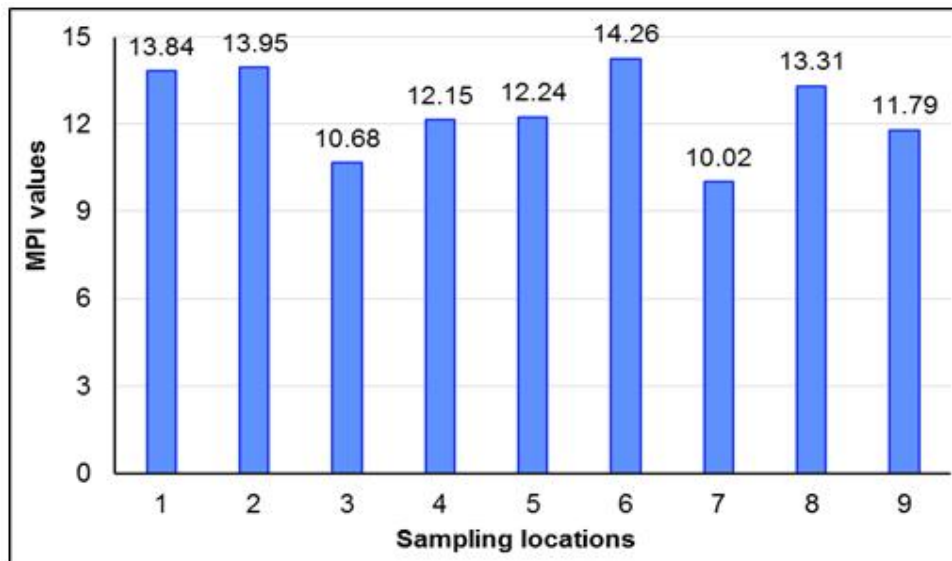


Fig. 2. Metal pollution index (MPI) values of rice grain samples collected from industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh

Table 4. Concentration of heavy metals in different parts of rice samples collected from industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh

Sample ID	Heavy metals in rice grain ($\mu\text{g g}^{-1}$)							Heavy metals in rice straw ($\mu\text{g g}^{-1}$)							Heavy metals in rice husk ($\mu\text{g g}^{-1}$)						
	Zn	Cr	Cu	Ni	Cd	Mn	Fe	Zn	Cr	Cu	Ni	Cd	Mn	Fe	Zn	Cr	Cu	Ni	Cd	Mn	Fe
1	69.59	14.23	4.59	7.51	1.16	21.63	113.6	86.1	6.92	4.61	7.41	1.64	823	9792	53.6	trace	trace	4.58	1.39	229	9476
2	64.28	12.37	5.03	8.57	1.45	20.93	98.8	56.3	6.92	5.52	5.52	1.04	751	8574	49.0	trace	trace	5.36	1.27	319	4724
3	65.82	10.02	3.84	5.87	0.93	13.41	80.9	46.9	7.38	3.30	5.31	0.86	793	9609	54.7	trace	trace	3.41	1.18	259	8002
4	26.66	12.23	5.46	8.49	1.31	19.16	103.2	129.8	8.47	4.55	6.25	1.31	675	9280	685.1	trace	trace	2.60	1.06	245	7323
5	30.65	10.91	7.35	7.90	1.49	21.46	66.5	49.5	8.78	6.75	5.63	0.69	964	5770	39.6	trace	2.61	3.17	1.30	351	3288
6	94.34	15.74	6.17	8.29	1.73	10.07	90.6	124.4	8.62	6.75	6.78	0.90	424	6363	29.2	0.57	0.84	2.87	1.41	74	10301
7	41.73	12.12	4.53	7.99	1.53	3.82	95.2	37.2	9.55	4.75	6.36	1.08	201	8016	31.6	1.50	0.69	3.48	1.33	94	4828
8	47.41	13.53	6.59	8.72	1.37	13.10	111.8	42.1	9.86	6.89	6.15	1.56	405	9927	29.8	1.55	0.86	4.46	1.42	179	8638
9	49.64	11.92	5.46	8.78	1.33	9.79	86.1	100.0	8.93	2.89	7.20	1.44	311	4225	42.2	1.81	1.10	5.90	1.23	172	2530
Mean	54.46	12.56	5.45	8.01	1.37	14.82	94.1	74.70	8.38	5.11	6.29	1.17	594	7951	112.8	0.60	0.68	3.98	1.29	214	6568
Maximum	94.34	15.74	7.35	8.78	1.73	21.63	113.6	129.8	9.86	6.89	7.41	1.64	964	9927	685.1	1.81	2.61	5.90	1.42	351	10301
Minimum	26.66	10.02	3.84	5.87	0.93	3.82	66.5	37.24	6.92	2.89	5.31	0.69	201	4225	29.2	trace	trace	2.60	1.06	74.3	2530
Std. Dev.	21.27	1.72	1.11	0.90	0.23	6.33	15.1	36.21	1.08	1.49	0.74	0.33	264	2042	214.9	0.79	0.85	1.15	0.12	93.5	2802

Table 5. Average concentration of heavy metals in different parts and varieties of rice samples collected from industrial wastewater irrigated farmers' fields of Bhaluka area, Mymensingh

Type of sample	Rice varieties	Average concentration of heavy metals ($\mu\text{g g}^{-1}$)						
		Zn	Cr	Cu	Ni	Cd	Mn	Fe
Grain	BR-28	60.40	12.78	4.94	7.79	1.36	14.84	97.04
	BR-29	40.15	11.42	6.40	8.34	1.41	15.63	76.30
	<i>Kalozira</i>	47.41	13.53	6.59	8.72	1.37	13.10	111.84
Straw	BR-28	80.14	7.98	4.91	6.27	1.14	611.48	8605.77
	BR-29	74.73	8.86	4.82	6.42	1.07	637.56	4997.58
	<i>Kalozira</i>	42.07	9.86	6.89	6.15	1.56	405.13	9927.48
Husk	BR-28	150.53	0.35	0.26	3.72	1.27	203.47	7442.29
	BR-29	40.91	0.91	1.86	4.54	1.27	261.54	2908.74
	<i>Kalozira</i>	29.85	1.55	0.86	4.46	1.42	179.30	8637.83
Mean total by BR-28		97.02	7.04	3.37	5.93	1.26	276.60	5381.70
Mean total by BR-29		51.93	7.06	4.36	6.43	1.25	304.91	2660.87
Mean total by <i>Kalozira</i>		39.78	8.31	4.78	6.44	1.45	199.18	6225.72

Table 6. Calculated daily metal intake (DMI) for rice grown in industrial wastewater irrigated farmers' fields of Bhaluka area along with the upper tolerable intake level (UTIL) and oral reference doses (RfD)

	Cu	Zn	Cr	Ni	Fe	Cd	Mn
UTIL ($\text{mg day}^{-1} \text{ person}^{-1}$)	10.00 ^a	40.00 ^a	NE ^a	1.0 ^a	45.00 ^a	0.064 ^b	11.00 ^a
RfD ($\text{mg kg}^{-1} \text{ day}^{-1}$)	0.040 ^c	0.300 ^c	0.003 ^d	0.02 ^c	0.700 ^c	0.001 ^c	0.014 ^c
Average DMI ($\text{mg day}^{-1} \text{ person}^{-1}$)	Male	26.28	262.8	60.63	38.67	454.0	6.59
	Female	36.79	367.9	84.88	54.14	635.6	9.23

NE= Not established; ^a = FDA [30]; ^b = Garcia-Rico et al. [42]; ^c = US EPA [43]; ^d = IRIS [44] and ^e = Khan et al. [45]

3.5 Assessment of Metal Pollution Index (MPI)

The metal pollution index (MPI) was used to compare the total metals accumulation level in rice grain samples collected from different locations of Bhaluka area. This is a reliable and precise method for monitoring metal pollution in different food samples [48,49]. The MPIs of individual rice samples collected from the industrial wastewater irrigated agricultural fields of Bhaluka area are presented in Fig. 2. Among the locations, the MPI values for rice grain samples ranged between 10.02 and 14.26. A report stated that MPI values for different vegetables collected from five wastewater irrigated sites of Patna, India ranged from 8.61-15.66 [49]. However, higher MPI values of rice recommend that rice grown in industrial wastewater irrigated soils of Bhaluka area has potential in causing more human health risk due to the increased accumulation of different heavy metals. Singh et al. [48] was also reported higher values of MPIs for different cereals and vegetables grown in wastewater irrigated sites in India, and they concluded that wastewater irrigation led to accumulation of heavy metals in

food stuff causing potential health risks to consumers.

4. GENERAL COMMENTS AND CONCLUSIONS

Usage of industrial wastewater for irrigation is a common practice in the study area due to lack of sufficient amount of surface and groundwater. This study provides baseline data on mineral elements and heavy metal contents in rice grown in industrial wastewater irrigated farmer's fields of Bhaluka area in Mymensingh, along with their accumulation pattern in grain, husk and straw of different rice varieties. Our previous study for the same area already focused on heavy metals present in wastewater and their deposition in soil [15], and the present study tried to establish a link among these three components. Higher amount of heavy metals, particularly Cr, Cd and Mn were accumulated in rice grain, which make them unsafe for human consumption. Similarly, Cd contents in straw and husk were also found higher than the maximal tolerable limit of cattle, which means these are harmful for animal health as fodder. Thus, farmers should aware about the harmful effects of industrial wastewater on their

land and growing crops along with the possible detrimental effects after human and animal consumption. At the same time, initiative will have to take to increase awareness among the industrialists about such pollution problem, and their legal and social responsibilities to prevent it. Finally, it can be concluded that untreated industrial wastewater should be avoided to irrigate crops, and peoples also should refrain from consumption of rice grown in such farmers' fields of the study area until fresh water irrigation and proper remediation strategies are introduced.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest among the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

ACKNOWLEDGEMENTS

This work was financially supported by the Ministry of Science and Technology (MoST), Government of the People's Republic of Bangladesh, Dhaka-1000, Bangladesh for the financial year 2018-19 under the Sanction Order no. 39.00.0000.009.14.004.19/BS-61/92.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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