JJBS

Jordan Journal of Biological Sciences

Volume 16, Number 3,September 2023
ISSN 1995-6673
Pages 467 – 475
https://doi.org/10.54319/jjbs/160310

Heavy metal contamination and potential health risk assessment associated with selected farmed fish in Rajshahi, Bangladesh

Jibon Kumar Ghosh, Md. Shahidul Islam, Md. Tariqul Islam, Md. Mahedul Islam Murad and Md. Mahabubur Rahman*

Department of Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh

Received: November 10, 2022; Revised: January 29, 2023; Accepted: February 11, 2023

Abstract

The present investigation was conducted with a view to evaluating heavy metal contamination of four selected fish species (*Labeo rohita, Catla catla, Cirrhinus cirrhosus*, and *Hypophthalmichthys molitrix*) and the potential health risks of its consumers in Rajshahi, Bangladesh. Twenty-one feed samples and eighty-four fish specimens were collected from seven different farms scattered across the Rajshahi district and subsequent screening of heavy metal was conducted through atomic absorption spectrophotometer (AAS). The heavy metal concentration of Pb, Co, Cr, Cd and Ni was found beyond the permissible limits in fish feed and raw fish with a strong correlative association of heavy metals between them. Metal pollution index revealed that *C. cirrhosus* was the most polluted species while *L. rohita* was the least. Health risk assessment was conducted by evaluating estimated daily intake (EDI), health risk index (HRI), target hazard quotient (THQ), hazard index (HI) and target carcinogenic risk (TR). EDI of metal was higher in children, and according to HRI, they were at two to three times more risk compared to the adults. THQ of all the heavy metals across the selected species was found to be less than 1 demonstrating the absence of non-carcinogenic hazard for individual heavy metal intake. However, HI values indicated that the cumulative effect of those heavy metals might impart some degree of non-carcinogenic effect over lifelong exposure. TR values suggested that the carcinogenic effect of Pb remained negligible while Cr, Cd and Ni had low to moderate risk of developing cancer with a greater probability of its occurrence in women than men.

***Keywords**:* Heavy metals, Fish Feed, Carp, Health Hazard, Carcinogenic and Non-carcinogenic risk

1. Introduction

Aquaculture has played an integral part in the recent economic development of Bangladesh. Owing to the expanding aquaculture operation in concurrent times, Bangladesh has become self-sufficient in producing fish and met its required per capita consumption rate (Shamsuzzaman *et al.,* 2020). Providing for the livelihoods of millions of people, fish is Bangladesh's second-most crucial agricultural commodity (Mannan *et al.,* 2018). It is also one of the principal sources of animal protein and micronutrients and plays an important role in assuring nationwide nutritional security (DoF, 2020). However, intense fish farming has presented a number of concerns regarding environmental impact and human health hazards for consumers (Cole *et al.,* 2009). Pollution caused by heavy metals is a widely acknowledged concern that poses serious health risks for humans and animals. Heavy metals have become more prevalent in the environment in recent years in comparison to their natural abundance (Tchounwou *et al.,* 2014). This predicament has emerged due to fast population growth, growing urbanization, increased industrialization, discovery and extraction of environmental resources, the spread of other contemporary agrarian methods, and the absence of environmental policies and regulations (Mannan *et al.,* 2018). Fishes are aquatic inhabitants who can absorb heavy metals from various sources like food, water, and bottom sediments (Ayas *et al.,* 2007). It is well-acknowledged that fish feed is the most significant source of heavy metal contamination in aquaculture systems (Anhwange *et al.,* 2012). Along with various plant-based feed ingredients, solid tannery wastes are being used during fish feed manufacturing in Bangladesh, which acts as a prominent source of harmful heavy metals (Hossain *et al.,* 2007). As a result, both naturally occurring and anthropogenic origins are exerting an impact on the surrounding aquatic systems, causing environmental destruction (Ogundele and Ayeku, 2020). The accumulation of heavy metals in the human body through diet is associated with a wide range of health complications (Jaishankar *et al.,* 2014). Metals discharged in the aquatic systems may find their way within the food web due to biomagnification and have carcinogenic and other negative implications (Malik *et al.,* 2009). Trace elements are required for humans in minute quantities, but on the contrary heavy metals are carcinogenic or poisonous, damaging the neurological and osmoregulatory system, as well as the skin, bones, and teeth (Bhattacharya *et al.,* 2007). Certain heavy metals have consistently exhibited detrimental effects on neural development of children in many scientific studies (Heng *et al.,* 2022). In the Rajshahi district, intensive to semi-intensive carp polyculture is widely practiced for its high

---

* Corresponding author. e-mail: : mratan1980@gmail.com.

profitability and market demand. The fish farms in Rajshahi largely contribute to the nationwide supply of animal protein. There is reason to be concerned over the possibility of heavy metals being concentrated in fish raised in captivity to levels that are high enough to be potentially hazardous to human health. As a result, the current investigation was carried out to measure the concentrations of potentially harmful heavy metals found in fish feed and farmed fish, as well as to determine the extent of any associated risks to human health.

2. Methodology

2.1. Study Duration, Site, and Sample Collection

The investigation was performed over the course of one year, from March 2019 to February 2020. Fish feed and raw fish specimens were obtained from seven distinct fish farms located at the Gholharia, Parila Bazar, Naohata, and Paba under Paba Upazila, Mougachi under Mohanpur Upazila and Matikata under Godagari Upazila of Rajshahi district (Figure 1) as these regions were the top contributors to fish production of the region (DoF, 2019). Available secondary data also suggested that *Labeo rohita, Catla Catla, Cirrhinus cirrhosus* and *Hypophthalmichthys molitrix* were the top most produced fish species in the studied district (DoF, 2019), which led to the selection of these species for sampling and subsequent analysis for heavy metals. For the assessment of heavy metals, a total of eighty-four fish samples and twenty-one fish feed samples were analyzed. A culture duration of at least one year from fingerling stocking and a weight range of 1.5 to 2.5 kg were established as the selection criteria for collecting fish samples from polyculture systems in which all fish species were concurrently raised. Three live specimens of each species were collected from each of the seven ponds that met the selection criteria for sampling (Table 1). All the fish farms used commercial fish feeds for rearing purposes and seven different feed samples, each with three replicates, were also collected from the respective farms to determine their levels of heavy metals.

Table 1. Mean weight and sample size of the studied fish

Parameters	***L. rohita***	***C. catla***	***C. cirrhosus***	***H. molitrix***
Mean Weight (kg)	1.88±0.29	1.93±0.25	1.76±0.21	2.02±0.36
Sample Size (n)	21	21	21	21

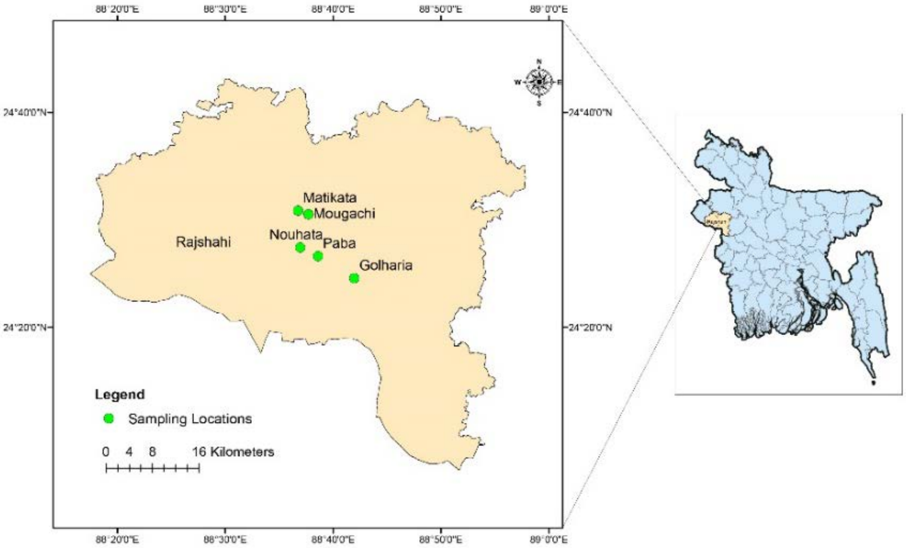

Figure 1. Locations of sample collection

2.2. Sample Preparation and Digestion

The collected feed samples were desiccated in a hot air oven and then stored at normal temperature. After the collection of fish specimens from the study ponds, they were washed with deionized water and weighed and following the removal of the fish's skin, the muscle was removed with a knife. For further laboratory analysis, the materials were fragmented, homogenized, placed in polybags, and chilled at 4°C. The preserved fish muscle was heated in an oven at 120°C for 48 hours. The samples were then cooled in a desiccator and consequently powdered and homogenized using a grinder machine. Finally, the powdered sample was placed in a dry plastic bottle that had been previously cleaned, and it was desiccated for 24 hours before further investigation. The digested feed and fish samples were analyzed quantitatively for heavy metals. Each sample was held in a separate crucible in a muffle furnace at 600°C to obtain ash. After six hours in the muffle furnace, samples were removed, cooled, and combined with 20 ml of 1N HCl acid and enough distilled water to make 100 ml of solution in a 500 ml beaker. A filter paper was used to remove any solids from this solution.

2.3. Determination of Heavy Metal Concentrations

The heavy metal concentrations of lead (Pb), cobalt (Co), chromium (Cr), cadmium (Cd), and nickel (Ni) from digested feed and raw fish samples were determined in the central lab of the University of Rajshahi. A flame atomic absorption spectrophotometer with acetylene gas and air serving as the instrument's fuel, and oxidizer, was used to determine the concentrations of heavy metals found in the collected samples. The metal concentrations in digested samples were measured using calibration curves produced from standard solutions after they were aspirated into the air acetylene flame. Each measurement was carried out using the mean of three replicate samples. The absorption wavelengths and detections thresholds were 217.0 nm and 0.001ppm for Pb, 247.7nm and 0.02 ppm for Co, 357.9 nm and 0.01 ppm for Cr, 228.8 nm and 0.002 ppm for Cd, and 232.0 nm and 0.01 ppm for Ni. Blinding of the experimenter was ensured by labelling the samples with unique codes to prevent any observation bias.

2.4. Calibration of the Instrument

Establishing a connection between the signal response and a predetermined set of standards is necessary for calibration. In atomic absorption spectrometry, the terms "standards" and "working standards" denote the creation of a set of liquid solutions with different concentrations of the target analyst. Quantifying the signals for a number of solutions with known standard concentrations yielded an appropriate measurement graph. Then, by exposing the instrument to a solution with an unknown concentration, a signal was generated that could be decoded from the graph, allowing the element concentrations in the sample solution to be calculated. The specific amount of each metal present in feed and fish samples was calculated using the following formula.

Actual concentration of metal in sample (mg/kg) = R × dilution factor
Where R = AAS Reading of digest
Dilution Factor = Volume of digest used ∕ Weight of digested sample.

2.5. Health Risk Assessment

The human health risk assessment from consuming the selected fish species in this study was conducted using the following methods.

2.5.1. Metal Pollution Index (MPI)

The metal pollution index (MPI) was used to express the cumulative heavy metal concentration in raw fish and was calculated as a geometric mean using the following equation (Usero *et al.,* 1997).

$$MPI\,\frac{mg}{kg} = -\sqrt[2]{(cf1 \times cf2 \times cf3 \times \ldots \times cfn)}$$

Here, Cfn = the quantity of each heavy metal in sample "n" in mg per kg.

2.5.2. Daily Intake of Metal (DIM)

The following equation by Islam *et al.* (2017) was implied to estimate the daily exposure to heavy metals through the consumption of the selected fish species in human individuals (adults and children).

$$DIM = \frac{CM \times FIR \times K}{B_{mean}}$$

Here, CM is the metal content in fishes (mg/kg), FIR is the average daily fish consumption rate measuring 62.58g/persons/day (DoF, 2020), B_{mean} is the average body weight measuring 52.3 kg for adults (WHO, 2011) and 19.15 kg for children of the age group 5-10 (Ferdous *et al.,* 2015). The dry weight of fish samples was converted to fresh weight using the estimated conversion factor K= 0.249 based on the average moisture levels of the fish samples.

2.5.3. Health Risk Index (HRI)

The health risk index (HRI) is an indicator that compares the daily intake of a particular substance to its recommended daily dose (Rf_D) and is expressed as their ratio. Rf_D is an estimate of the daily oral exposure to the human population that is anticipated to not exert any substantial risk of adverse effects over the course of their lifetimes (USEPA-IRIS, 2006). The values of Rf_D for Pb, Co, Cr, Cd and Ni are presented in Table 2. The HRI for human adults and children due to consumption of the studied fish containing heavy metals was calculated using the following equation (Cui *et al.,* 2004).

$$HRI = \frac{DIM}{Rf_D}$$

An HRI > 1 indicates the possible detrimental effect of that component on human health.

2.5.4. Non-carcinogenic Health Effect

Using the following equation provided by USEPA (2011), the target hazard quotient (THQ) was derived to estimate the potential non-carcinogenic health risks associated with the consumption of heavy metal contaminated fish in adult men and women.

$$THQ = \frac{EF \times ED \times FIR \times CF \times CM}{WAB \times ATn \times Rf_d} \times 10^{-3}$$

Here, EF = frequency of exposure in days (365 days), ED = the exposure duration, which is the estimated life expectancy of 71.2 years for men and 74.5 years for women in Bangladesh (BBS, 2020), WAB = the average body weight which is 55.2 kg for adult men and 49.8 kg for adult women in Bangladesh (WHO, 2011). ATn = the average exposure duration for non-carcinogens (EF×ED) as used in depicting non-carcinogenic risk (USEPA, 2011). The remaining parameters are described earlier.

Food substances are usually polluted with more than one heavy metal and estimation of hazard index (HI) is a holistic method to quantify the degree of non-carcinogenic risk. It is expressed through the following equation as the summation of THQ of all heavy metals (n) present in a fish sample (USEPA, 2011).

$$\sum_{i=1}^{n} THQ$$

The maximum acceptable limit for both THQ and HI index is 1, and values beyond this limit are considered to be unsafe for humans (USEPA, 2011).

2.5.5. Carcinogenic Health Effect

Target cancer risk (TR) was determined in order to assess the carcinogenic risk to adult men and women posed by the lifelong ingestion of the studied fish species in accordance with the model proposed by the USEPA (2011).

$$TR = \frac{EF \times ED \times FIR \times CF \times CM \times CPSo}{WAB \times ATc} \times 10^{-3}$$

Here, ATc = the average exposure time for carcinogens (EF×ED), and CPSo is the carcinogenic potency slope. TR categories are described as if TR ≤ 10^{-6} = Low; 10^{-4} to 10^{-3} = moderate; 10^{-3} to 10^{-1} = high; ≥10^{-1} = very high (NYSDOH, 2007). Additionally, a TR value within the range of 10^{-4} to 10^{-6} is considered acceptable (USEPA, 2011). USEPA (2011) also regards cobalt as non-carcinogenic for humans; therefore, TR was estimated for the rest of the heavy metals. The CPSo values for the studied heavy metals are presented in Table 2.

Table 2. RfD and CPSo values of different heavy metals regarding human exposure (mg/kg body weight/day)

Heavy Metals	Rf_D	CPSo	Reference
Pb	0.0035	0.0085	USEPA (2010); USEPA (2011); USEPA IRIS (2006); Aendo *et al.*, (2022)
Co	0.003	-	
Cr	0.003	0.5	
Cd	0.001	0.5	
Ni	0.02	1.7	

2.6. Statistical Analysis

Statistical analysis of the collected data and development of graphs was carried out using Microsoft Excel 2016. Mean, standard deviation, and standard error of data were carried out and presented in tables where necessary. After confirming the homogeneity of the data using Levene's test, a one-way ANOVA was performed to assess if there was any significant difference ($P<0.05$) among the studied fish species for each particular heavy metal. The Correlation matrix of heavy metal levels in fish and fish feed was constructed in SPSS 21, and the measured correlations were highlighted with two distinct levels of significance ($P<0.01$ and $P<0.05$).

3. Results

3.1. Heavy Metal Concentration in Fish Feed

The heavy metal content in the collected fish feeds was estimated to determine any possible correlation it may display with the metal deposition in fish muscle. The collected feed samples exhibited a wide range of variation in heavy metal concentration in most cases which is apparent from the minimum and maximum values (Table 3). The concentration of Pb was recorded to be the highest among the tested heavy metals, followed by Co, Ni, Cr and Cd, respectively. Although heavy metals in a few feed samples were within the acceptable level, the overall mean values of the heavy metals were well beyond the maximum permissible limit (MPL) proposed by FAO and EU.

Table 3. Heavy metal concentrations in fish feed (mg/kg) in dry weight

Heavy Metals	Heavy metal concentrations in fish feed (mg/kg) in dry weight					MPL	
	Min.	Mean	Max.	SE	SD	FAO/WHO (1984)	EU (2003)
Pb	1.04	9.36	13.96	1.02	3.82	2.0	5.0
Co	1.06	5.74	11.77	1.06	3.95	1.0	1.0-1.5
Cr	1.90	2.79	4.23	0.18	0.69	1.0-2.0	1.0
Cd	0.99	2.60	3.65	0.24	0.89	2.2	2.0
Ni	2.17	4.00	7.35	0.42	1.56	2.0	0.1-8.0

Abbreviation: Min. = Minimum, Max. = Maximum, SE = Standard Error, SD = Standard Deviation

3.2. Heavy Metal Concentration in Fish

Heavy metal levels in the selected four carp species were estimated in order to quantify their impact on human health due to fish consumption. The measured concentration of heavy metals is shown in Table 4. Similar to the feed samples, Pb was the most abundant heavy metal in fish muscle, followed by Co, Ni, Cr, and Cd. The highest amount of Pb, Co, and Ni were found to be deposited in *C. cirrhosus*, whereas the highest amount of Cr and Cd deposition was recorded in *C. catla.* Alarmingly, every heavy metal concentration present in the studied fish species was found to be exceeding the MPL suggested by FAO and EU in this investigation. Additionally, no statistically significant difference ($P<0.05$) was observed when comparing particular heavy metal levels across the studied fish species.

Table 4. Heavy metal concentrations in fish (mg/kg) in dry weight

Heavy Metals	Heavy metal concentrations in fish (mg/kg) in dry weight				MPL	
	L. rohita	*C. catla*	*C. cirrhosus*	*H. molitrix*	FAO (1983)	EU(2001)
Pb	5.63±1.02^a	5.74±1.14^a	6.94±1.58^a	5.97±1.12^a	2.5	0.1
Co	3.67±0.81^a	2.38±0.29^a	3.71±0.42^a	3.66±0.74^a	-	-
Cr	1.83±0.24^a	2.09±0.57^a	1.80±0.21^a	1.66±0.39^a	1.0	1.0
Cd	1.84±0.58^a	2.01±0.17^a	1.82±0.36^a	1.75±0.41^a	0.2	0.05
Ni	1.88±0.33^a	2.31±0.22^a	2.31±0.49^a	2.26±0.20^a	0.2	-

3.3. Correlation of Heavy Metals Between Fish Feed and Fish

To estimate the degree of correlative association of heavy metals within and between fish feed and fish, a Pearson correlation matrix was constructed and presented in Table 5**.** A statistically significant positive correlation was found between each of the tested heavy metals in the collected feeds and their corresponding levels in the fish samples. This finding led to the assumption that fish feed acted as a potential source for heavy metal accumulation in studied fish species. Within the feed samples, Ni showed a significant positive correlation with Co, Cr, and Cd suggesting that these elements might be originating from a similar biochemical source in the feeds. However, Pb and Co were the only two metals which demonstrated a significant negative correlation both within the feed and the fish samples. In case of fish samples, a significant positive correlation was also found among Co, Cr and Cd, whereas Pb, Cr, and Cd also showed a significant positive correlation with Ni.

Table 5. Pearson correlation matrix of heavy metals in fish (f) and fish feed (ff) samples

	Pb (f)	**Co (f)**	**Cr (f)**	**Cd (f)**	**Ni (f)**	**Pb (ff)**	**Co (ff)**	**Cr (ff)**	**Cd (ff)**	**Ni (ff)**
Pb (f)	1									
Co (f)	-0.435**	1								
Cr (f)	0.213	0.356**	1							
Cd (f)	0.149	0.404**	0.787**	1						
Ni (f)	0.402**	0.216	0.528**	0.508**	1					
Pb (ff)	0.850**	-0.599**	0.113	0.038	0.215	1				
Co (ff)	-0.435**	0.850**	0.360**	0.477**	0.267*	-0.696**	1			
Cr (ff)	0.207	0.399**	0.775**	0.684**	0.399**	0.090	0.317*	1		
Cd (ff)	0.182	0.456**	0.580**	0.649**	0.595**	0.113	0.479**	0.354**	1	
Ni (ff)	0.037	0.625**	0.687**	0.764**	0.412**	-0.089	0.589**	0.740**	0.685**	1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

3.4. MPI of the Selected Fish Species

MPI index was calculated to measure the degree of contamination of the studied fish species owing to the presence of different heavy metals and is illustrated in figure 2. The higher the MPI value is, the more health risk is expected to be associated with it. The highest MPI value was found in *C. cirrhosus* (2.43 mg/kg) while, the lowest was recorded in *L. rohita* (2.29 mg/kg). MPI values for *C. catla* and *H. molitrix* were 2.40 and 2.31 mg/kg, respectively.

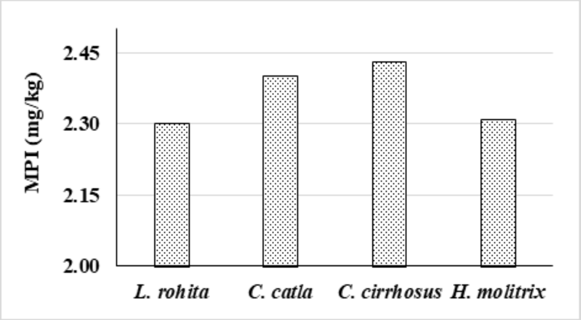

Figure 2. Estimated metal pollution index (MPI) of the selected fish species (mg/kg)

3.5. DIM in Adults and Children due to fish consumption

In this study, the daily intake of heavy metals was estimated for adults and children to assess the potential health risk to its consumers. In all cases, DIM for children were found to be higher than the adults (Table 6). The highest daily intake of Pb and Co was associated with the consumption of *C. cirrhosus*, whereas the corresponding levels for Cr and Cd were found in *C. catla.* Furthermore, both *C. cirrhosus* and *C. catla* were found to be the leading source of Ni when consumed by adults and children. In contrast, the lowest daily intake of Pb and Ni was associated with the consumption of *L. rohita* whereas the lowest Cr and Cd intake was linked with consuming *H. molitrix.*

Table 6. Estimated DIM in adults and children due to fish consumption (mg/kg body weight/day)

Heavy Metals	*L. rohita*		*C. catla*		*C. cirrhosus*		*H. molitrix*	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Pb	16.8×10^{-04}	45.8×10^{-04}	17.1×10^{-04}	46.7×10^{-04}	20.7×10^{-04}	56.5×10^{-04}	17.8×10^{-04}	48.6×10^{-04}
Co	10.9×10^{-04}	29.9×10^{-04}	7.09×10^{-04}	19.4×10^{-04}	11.1×10^{-04}	30.2×10^{-04}	10.9×10^{-04}	29.8×10^{-04}
Cr	5.45×10^{-04}	14.9×10^{-04}	6.24×10^{-04}	17.0×10^{-04}	5.37×10^{-04}	14.7×10^{-04}	4.96×10^{-04}	13.5×10^{-04}
Cd	5.47×10^{-04}	14.9×10^{-04}	6.01×10^{-04}	16.4×10^{-04}	5.45×10^{-04}	14.9×10^{-04}	5.24×10^{-04}	14.3×10^{-04}
Ni	5.60×10^{-04}	15.3×10^{-04}	6.89×10^{-04}	18.8×10^{-04}	6.89×10^{-04}	18.8×10^{-04}	6.74×10^{-04}	18.4×10^{-04}

3.6. HRI Associated with Fish Consumption in Adults and Children

HRI in this investigation was estimated to quantify the potential health risks in humans caused by daily exposure to hazardous heavy metals. The HRI for adults and children due to the consumption of the studied fish is shown in Table 7. HRI value associated with individual heavy metals was found to be less than 1 for each of the experimental fish when consumed by adults. However, in case of children, the HRI values for the heavy metals were two to three folds greater than those of adults. Additionally, in children, the HRI for Pb and Cd exceeds the acceptable limit in all the studied fish species suggesting they were at a greater health risk than adults owing to the toxic effects of these two metals.

Table 7. HRI values for adult and children due to fish consumption

Heavy Metals	*L. rohita*		*C. catla*		*C. cirrhosus*		*H. molitrix*	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
Pb	0.48	1.31	0.49	1.33	0.59	1.62	0.51	1.39
Co	0.36	1.00	0.24	0.65	0.37	1.00	0.36	0.99
Cr	0.18	0.50	0.21	0.57	0.18	0.49	0.17	0.45
Cd	0.55	1.49	0.60	1.64	0.54	1.49	0.52	1.43
Ni	0.03	0.08	0.03	0.09	0.03	0.09	0.03	0.09

3.7. Non-carcinogenic Health Effect of the Studied Fish on Men and Women

THQ and HI indices were estimated in this study to evaluate the non-carcinogenic effect to human health due to prolonged consumption of toxic heavy metals**.** THQ for the consumption of each fish species in adult men and women are shown in Table 8. All the heavy metals in this study exhibited THQ values of less than 1 for both men and women indicating no potential health hazard for individual heavy metal intake.

Table 8. THQ values for men and women due to fish consumption

Heavy Metals	*L. rohita*		*C. catla*		*C. cirrhosus*		*H. molitrix*	
	Men	Women	Men	Women	Men	Women	Men	Women
Pb	0.45	0.50	0.46	0.51	0.56	0.62	0.48	0.53
Co	0.35	0.38	0.22	0.25	0.35	0.39	0.34	0.38
Cr	0.17	0.19	0.20	0.22	0.17	0.19	0.16	0.17
Cd	0.52	0.57	0.57	0.63	0.52	0.57	0.50	0.55
Ni	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.04

However, fish contains more than one heavy metal in its edible parts and, therefore, a cumulative THQ value for all the heavy metals known as hazard index (HI) should be taken into account while estimating non-carcinogenic health risks. The HI value shown in figure 3 indicates that the consumers were at risk of a health hazard as the aggregated THQ values for each of the five heavy metals in all four species exceeded 1. The highest HI for men and women were found to be associated with the consumption of *C. cirrhosus* (1.63 and 1.81) and the lowest with *C. catla* (1.49 and 1.65).

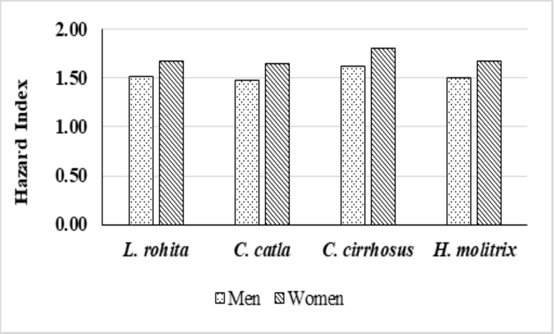

Figure 3. Estimated values of hazard index **(**HI) in men and women due to fish consumption

3.8. Carcinogenic Health Effect of the Studied Fish on Men and Women

In order to estimate the degree of carcinogenic risk due to consumption of heavy metal polluted fish, the TR levels were determined and are presented in Table 9. The findings showed that the estimated cancer risk of Pb in this study was very low and considered to be negligible across all four fish species. However, TR for Cr, Cd, and Ni were within the low to moderate range and indicated some degree of risk of contracting cancer for the consumers. The TR values also indicated that Ni had the highest probability of being carcinogenic in the studied fish followed by Cd and Cr. Similar to the non-carcinogenic health hazards, women were more likely to develop cancer than men.

Table 9. TR values for adult men and women due to fish consumption

Heavy Metals	***L. rohita***		***C. catla***		***C. cirrhosus***		***H. molitrix***	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
Pb	1.35×10^{-05}	1.50×10^{-05}	1.38×10^{-05}	1.53×10^{-05}	1.67×10^{-05}	1.85×10^{-05}	1.43×10^{-05}	1.59×10^{-05}
Cr	2.58×10^{-04}	2.86×10^{-04}	2.96×10^{-04}	3.28×10^{-04}	2.55×10^{-04}	2.82×10^{-04}	2.35×10^{-04}	2.60×10^{-04}
Cd	2.59×10^{-04}	2.87×10^{-04}	2.85×10^{-04}	3.15×10^{-04}	2.58×10^{-04}	2.86×10^{-04}	2.48×10^{-04}	2.75×10^{-04}
Ni	9.02×10^{-04}	1.00×10^{-03}	1.11×10^{-03}	1.23×10^{-03}	1.11×10^{-03}	1.23×10^{-03}	1.09×10^{-03}	1.20×10^{-03}

4. Discussion

Heavy metal pollution is one of Bangladesh's top environmental concerns, where most farmland, field crops, and freshwater outflows are heavily contaminated (Islam *et al.,* 2018). Fish feeds are regarded to be the most significant reason for heavy metal pollution in aqua farms where no other anthropogenic source of contamination is in action (Ali and Khan, 2018; Sabbir *et al.,* 2018). The mean heavy metal (Pb, Co, Cr, Cd and Ni) concentration in fish feed was found to be exceeding the maximum permissible limit of FAO and EU, thus raising concerns regarding its effect on fish biology and ultimately human health. An unacceptable limit of Pb and Cd in numerous commercial fish feeds has been reported by Kundu *et al.* (2017) in tilapia farms in Bangladesh. An extensive feed evaluation study by Sarkar *et al.* (2021) also confirmed the presence of Cd and Cr in several types of fish feeds (starter, grower, finisher, and mixed) beyond the permissible limit with respect to national and international standards. The findings of this study are also in accordance with Saha *et al.* (2018) who reported that concentrations of Pb, Co and Cr are exceeding the maximum permissible limit in different brands of fish feed. An unsafe level of Ni is also documented by Fatema *et al.* (2019) in different commercial feeds used in the culture of *Anabas testudineus* in Bangladesh. Heavy metal pollution in fish feed can be attributed to the use of trace metal feed additives like tannery waste without performing a proper decontamination process (Sarker *et al.,* 2017; Sarker *et al.,* 2022). The strong correlative association among Co, Cr, Cd, and Ni in the studied feeds found in this study might be an indication of utilizing such toxic components as feed additives as these metals are the major pollutant in tannery wastes (Islam *et al.,* 2013).

All the selected fish species have exhibited heavy metal levels that are higher than the maximum allowable limit for food fish. Fish having unsafe levels of heavy metals pose a variety of health risks to people of all ages, including children. Pb is a very toxic substance, especially for children, as they have less renal excretion capabilities and greater gastrointestinal absorption (Azaman *et al.,* 2015). Pb also has nephrotoxic and neurotoxic potency and can induce cardiovascular disease (Umar *et al.,* 2001; García-lestón *et al.,* 2010). Toxic levels of Co can introduce cardiovascular, neurological, and endocrinal dysfunction (Leyssens *et al.,* 2017). Cr is a carcinogenic element and can cause disruption in nutrient metabolism (Akoto *et al.,* 2014). Chronic exposure to cadmium may result in respiratory discomfort, cancer of the lung and breasts, damage to blood vessels, and cardiac issues (Prozialeck *et al.,* 2008). Ni is another potential carcinogen that can cause inflammation of the respiratory system, fibrosis, and emphysema (Forti *et al.,* 2011).

Many studies have depicted the concerning scenario of heavy metal pollution in a broad spectrum of fish species and numerous toxic heavy metals in Bangladesh. Ghosh *et al.* (2021) have reported unacceptable levels of Co and Cr with an alarming level of Pb in farmed *Pangasius pangasius, Oreochromis niloticus, Heteropneustes fossilis, A. testudineus,* and *Clarias batrachus,* Mannan *et al.* (2018) have also concluded that commercial fish feed can contribute to unsafe bioaccumulation of Pb, Cd, and Ni in *L. rohita* and raise potential health risks upon consumption of such fish. Nowadays, heavy metal pollution in fish is not exclusively limited to aquaculture farms and can be detected in open-water fish species as well. Ahmed *et al.* (2019) have reported bioaccumulation of Pb, Cr, and Cd exceeding the recommended limits in five estuarine fish species. Kumar *et al.* (2020) also validated the presence of hazardous levels of Pb and Cd in *L. rohita* from Mahananda river, India. The MPI of our studied fish species was found to be ranging from 2.29 to 2.43 mg/kg. Ghosh *et al.* (2021) also evaluated MPI values in the five fish species and reported relatively higher MPI ranging from 4.85 to 6.65 mg/kg. The MPI value estimated by Ahmed *et al.* (2019) in estuarine fish species ranging from 3.65 to 4.70 mg/kg also surpassed our current findings. In both studies, demersal and bottom dwelling fish exhibited higher MPI which is in accordance with the result of this investigation where *C. cirrhosus* demonstrated the highest MPI value.

The DIM values of heavy metals in this study showed that *C. cirrhosus* and *C. catla* are the top contributors to heavy metals in daily diets. The findings also indicate Pb as the most and Cd as the least consumed among the studied heavy metals which agree with the findings of Ullah *et al.* (2017) and Saha and Zaman (2013). Consistent with the results of Ghosh *et al.,* (2021) the HRI in this study implies that children are more at risk than adults from consuming heavy metal contaminated fish. The THQ value indicates the potential non-carcinogenic risk of heavy metal consumption, and it is desired to be less than 1. The THQ values for Pb, Co, Cr, Cd, and Ni in this study were within this acceptable limit and posed no serious non-carcinogenic health upon consumption of individual heavy metals and in accordance with earlier studies (Ahmed *et al.,* 2019; Akter *et al.,* 2021). However, a number of heavy metals are simultaneously contained within the fish muscle; therefore, HI values should be taken into consideration while estimating non-carcinogenic risk as it is more representative of the actual scenario of heavy metal consumption. The HI values for all fish species for each heavy metal surpassed the suggested threshold of 1, indicating possible non-carcinogenic health risks similar to previously reported findings (Ahmed *et al.,* 2019; Ullah *et al.,* 2017; Saha and Zaman, 2013). The findings of this study also indicated that in comparison to men, women were more susceptible to the non-carcinogenic impacts of heavy metals.

The TR values in this investigation imply that Cr, Cd, and Ni from the lifelong consumption of the studied fish species may have a carcinogenic impact in both men and women at some stage. It should be noted that TR is not certain estimation for the occurrence of cancer but rather an expression of the probability that the exposed individuals may develop cancer at a stage of life. Evidence of potential carcinogenic risk owing to the intake of both cultured and captured fish species has been presented in prior studies (Kundu *et al.,* 2017; Kawser *et al.,* 2016; Wahiduzzaman *et al.,* 2021). The data also suggest that women are more likely to develop cancer compared to the men population which agrees with the findings of Javed and Usmani (2016) and Vahter *et al.* (2002).

5. Conclusion

This investigation was accomplished to determine the levels of five heavy metals in four demandable fish species (*L. rohita, C. catla, C. cirrhosus,* and *H. molitrix*) of Rajshahi region and their possible implication on human health. Heavy metals from all the selected fish species exceeded the recommended international threshold. Correlative association implies that fish feed is the primary source of heavy metal accumulation in the studied fish. MPI values flagged *C. cirrhosus* as the most contaminated species and *L. rohita* as the least. HRI values indicated greater health risks to children compared to adults. THQ values showed no substantial health risk for individual heavy metal consumption, while TR values suggested possible carcinogenic impact of Cr, Cd, and Ni on consumers. It is recommended that necessary regulations are strictly implied by the respective authorities while feed manufacturers and farmers become more careful in eliminating or controlling the sources of heavy metal in aquaculture practice.

Acknowledgement

We would like to express our gratitude to the BANBEIS, Ministry of Education (under GARE), the Government of the People's Republic of Bangladesh for providing financial support for this project. We would also like to express their gratitude to the Center Laboratory authorities at the University of Rajshahi in Rajshahi, Rajshahi, for their cooperation and laboratory support.

Ethics Approval

In compliance with the National Wildlife Protection and Conservation Act of 2012, the Rajshahi University Research Ethics Committee has exempted this investigation from the need for ethical approval on the grounds that the fish species utilized in this study are farmed and not wild or endangered. Nonetheless, all procedures in this study were conducted in conformity with the ethical criteria given by The International Council for Laboratory Animal Science (ICLAS) for researchers.

References

Aendo P, Netvichian R, Thiendedsakul P, Khaodhiar S and Tulayakul P. 2022. Carcinogenic risk of Pb, Cd, Ni, and Cr and critical ecological risk of Cd and Cu in soil and groundwater around the municipal solid waste open dump in central Thailand. *J Environ Public Health.*, **2022**: 1-12.

Ahmed A, Sultana S, Habib A, Ullah H, Musa N, Hossain M, Rahman M and Sarker M. 2019. Bioaccumulation of heavy metals in some commercially important fishes from a tropical river estuary suggests higher potential health risk in children than adults. *PLoS One.*, **14**: e0219336.

Akoto O, Bismark Eshun F, Darko G and Adei E. 2014. Concentrations and health risk assessments of heavy metals in fish from the Fosu lagoon. *Int J Enviro. Res.*, **8(2)**: 403-410.

Akter A, Hosen A, Hossain M, Khalil F and Mustafa T. 2021. Heavy metal concentrations and human health risk assessment of selected wild and cultured fishes of Bangladesh. *Bangladesh J. Zool.*, **49**: 189-203.

Ali H and Khan E. 2018. Bioaccumulation of non-essential hazardous heavy metals and metalloids in freshwater fish. Risk to human health. *Environ Chem Lett.*, **16**: 903-917.

Anhwange BA, Asemave K, Kim BC and Nyiaatagher DT. 2012. Heavy metals contents of some synthetic fish feeds found within Makurdi metropolis. *Int J Food Nutr Safe.*, **2:** 55-61.

Ayas Z, Ekmekci G, Yerli SV and Ozmen M. 2007. Heavy metal accumulation in water, sediments and fishes of Nallihan Bird Paradise, Turkey. *J Environ Biol*., **28(3)**: 545-549.

Azaman F, Juahir H, Yunus K, Azid A, Kamarudin M, Toriman M, Mustafa A, Amran M, CheHasnam C and Mohd Saudi A. 2015. Heavy metal in fish: analysis and human health-a review. *J Teknol.*, **77**(1).

BBS. 2020. **Bangladesh sample vital statistics 2020**. Bangladesh Bureau of Statistics. Ministry of Planning, Dhaka, Bangladesh.

Bhattacharya P, Welch AH, Stollenwerk KG, McLaughlin MJ, Bundschuh J and Panaullah G. 2007. Arsenic in the environment: biology and chemistry. *Sci Total Environ.,* **379(2-3)**: 109-120.

Cole DW, Cole R, Gaydos SJ, Gray J, Hyland G, Jacques ML, Powell-Dunford N, Sawhney C and Au WW. 2009. Aquaculture: Environmental, toxicological, and health issues. *Int J Hyg Environ Health.,* **212**: 369–377.

Cui YJ, Zhu YG, Zhai RH, Chen DY, Huang YZ, Qiu Y and Liang JZ. 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. *Environ Int.,* **30(6)**: 785-791.

DoF. 2019. **Yearbook of Fisheries Statistics of Bangladesh, 2018-19**. Fisheries Resources Survey System (FRSS), Department of Fisheries, Bangladesh: Ministry of Fisheries and Livestock.

DoF. 2020. **Yearbook of Fisheries Statistics of Bangladesh, 2019-20**. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries and Livestock.

EU. 2001. **Commission Regulation as Regards Heavy metals**, Directive, 2001/22/EC, No: 466.

EU. 2003. **Opinion of the scientific committee on animal nutrition on undesirable substances in feed**. https://food.ec.europa.eu/system/files/2020-12/sci-com_scan-old_report_out126_bis.pdf. Accessed 13 October 2022.

FAO. 1983. **Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products**. FAO Fishery Circular No. 464, Food and Agriculture Organization.

FAO/WHO. 1984. **List of maximum levels recommended for contaminants by the Joint FAO/WHO**. Codex Alimentarius Commission. Second Series. CAC/FAL, Rome.

Fatema K, Sakib MN, Zahid MA, Sultana N and Hasan MR. 2019. Growth performances and bioaccumulation of heavy metals in *Anabas testudineus* (Bloch, 1792) cultured using different market feeds. *Bangladesh J Zool*., **47(1)**: 77-88.

Ferdous MJ, Sumon MAA, Mahamud AMN, Ara MG, Fatema J and Fatema MK. 2015. A comparative study on fish intake and nutritional status of children in different areas of Sylhet district, Bangladesh. *Int J Nat Soc.,* **2(4)**: 113-117.

Forti E, Salovaara S, Cetin Y, Bulgheroni A, Tessadri R, Jennings P and Prieto P. 2011. In vitro evaluation of the toxicity induced by nickel soluble and particulate forms in human airway epithelial cells. *Toxicol in Vitro.*, **25(2)**: 454-461.

García-lestón J, Mendez J, Pásaro E and Laffon B. 2010. Genotoxic effects of lead: An updated review. *Environ Int.,* **36**: 623-636.

Ghosh P, Ahmed Z, Alam R, Begum B, Akter S and Jolly Y. 2021. Bioaccumulation of metals in selected cultured fish species and human health risk assessment: a study in Mymensingh Sadar Upazila, Bangladesh. *Stoch Environ Res Risk Assess.,* **35**: 2287-2301.

Heng YY, Asad I, Coleman B, Menard L, Benki-Nugent S, Hussein WF, Karr CJ, McHenry MS. Heavy metals and neurodevelopment of children in low and middle-income countries: A systematic review. *PLoS One*., **17(3):** e0265536.

Hossain AMMM, Monir T, Haque AMRU, Kazi ALM, Islam SM and Elahi SF. 2007. Heavy metal concentration in tannery solid wastes used as poultry feed and ecotoxicological consequences. *Bangladesh J Sci Ind Res*., **42**: 397-426.

Islam GMR, Habib MR, Waid JL, Rahman MS, Kabir J, Akter S and Jolly YN. 2017. Heavy metal contamination of freshwater prawn (*Macrobrachium rosenbergii*) and prawn feed in Bangladesh: A market-based study to highlight probable health risks. *Chemosphere.*, **170**: 282-289.

Islam MM, Karim MR, Zheng X and Li X. 2018. Heavy metal and metalloid pollution of soil, water and foods in Bangladesh: a critical review. *Int J Environ Res Public Health.*, **15(12)**:2825.

Islam S, Islam F, Bakar MA, Das S and Bhuiyan HR. 2013. Heavy metals concentration at different tannery wastewater canal of Chittagong city in Bangladesh. *Int J Agric Environ Biotechnol*., **6(3)**: 355-361.

Jaishankar M, Tseten T, Anbalagan N, Mathew BB and Beeregowda KN. 2014. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol*., **7**: 60-72.

Javed M and Usmani N. 2016. Accumulation of heavy metals and human health risk assessment via the consumption of freshwater fish *Mastacembelus armatus* inhabiting, thermal power plant effluent loaded canal. *Springerplus.*, **5**: 776.

Kawser AM, Baki M, Kundu G, Saiful Islam M, Monirul Islam M and Muzammel Hossain M. 2016. Human health risks from heavy metals in fish of Buriganga river, Bangladesh. *Springerplus.*, **5**:1697.

Kumar A, Kumar A and Jha S. 2020. Human health risk assessment of heavy metals in major carp (*Labeo rohita*) of Mahananda river in Northern India. *Emergent Life Sci Res.,* **06**: 34-49.

Kundu GM, Alauddin M, Akter MS, Khan MS, Islam MM, Mondal G, Islam D, Mohanta LC and Haque A. 2017. Metal contamination of commercial fish feed and quality aspects of farmed tilapia (*Oreochromi sniloticus*) in Bangladesh. *Biores Commun.*, **3(1)**: 345-353.

Leyssens L, Vinck B, Van Der Straeten C, Wuyts F and Maes L. 2017. Cobalt toxicity in humans-A review of the potential sources and systemic health effects. *Toxicol.*, **387**: 43-56.

Malik N, Biswas AK, Qureshi TA, Borana K and Virha R. 2009. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. *Environ Monit Assess.,* **160(1-4)**:267-276.

Mannan M, Hossain M, Sarker M, Hossain M, Chandra L, Haque A and Zahan M. 2018. Bioaccumulation of toxic heavy metals in fish after feeding with synthetic feed: A potential health risk in Bangladesh. *J Nutr Food Sci.*, **8(5)**: 100728.

NYSDOH. 2007. **Hopewell precision area contamination: appendix C-NYS DOH**. Procedure for evaluating potential health risks for contaminants of concern. https://www.health.ny.gov/environmental/investigations/hopewell/appendc.htm. Accessed 12 October 2022.

Ogundele LT and Ayeku PO. 2020. Source apportionment and associated potential ecological risk assessment of heavy metals in coastal marine sediments samples in Ondo, Southwest, Nigeria. *Stoch Env Res Risk Assess.,* **34**: 2013-2022.

Prozialeck WC, Edwards JR, Nebert DW, Woods JM, Barchowsky A and Atchison WD. 2008. The vascular system as a target of metal toxicity. *Toxicol Sci.,* **102**: 207-218.

Sabbir W, Rahman Z and Halder T. 2018. Assessment of heavy metal contamination in fish feed available in three districts of South Western region of Bangladesh. *Int J Fish Aquatic Stud.,* **26(2)**:100-104.

Saha B, Mottalib MA and Al-Razee ANM. 2018. Assessment of selected heavy metals concentration in different brands of fish feed available in Bangladesh. *J Bangladesh Acad*., **42(2)**: 207-210.

Saha N and Zaman M. 2013. Evaluation of possible health risks of heavy metals by consumption of foodstuffs available in the central market of Rajshahi City, Bangladesh. *Environ Monit Assess.*, **185**: 3867-3878.

Sarkar M, Rohani M, Hossain M and Shahjahan M. 2021. Evaluation of heavy metal contamination in some selected commercial fish feeds used in Bangladesh. *Biol Trace Elem Res.*, **200**: 844-854.

Sarker A, Kim J, Islam A, Bilal M, Rakib M, Nandi R, Rahman M and Islam T. 2022. Heavy metals contamination and associated health risks in food webs - A review focuses on food safety and environmental sustainability in Bangladesh. *Environ Sci Pollut Res.*, **29**: 3230-3245.

Sarker MS, Quadir QF, Zakir HM, Nazneen T and Rahman A. 2017. Evaluation of commonly used fertilizers, fish and poultry feeds as potential sources of heavy metals contamination in food. *Asian Aus J Food Safe Sec.,* **1(1)**: 74-81.

Shamsuzzaman MM, Hoque Mozumder MM, Mitu SJ, Ahamad AF and Bhyuian MS. 2020. The economic contribution of fish and fish trade in Bangladesh. *Aquac Fish*., **5**:174–181.

Tchounwou PB, Yedjou CG, Patlolla AK and Sutton DJ. 2012. Heavy metal toxicity and the environment. *Exp Suppl*., **101**: 133-164.

Ullah A, Maksud M, Khan S, Lutfa L and Quraishi S. 2017. Dietary intake of heavy metals from eight highly consumed species of cultured fish and possible human health risk implications in Bangladesh. *Toxicol Rep.,* **4**: 574-579.

Umar A, Umar R and Ahmad MS. 2001. Hydrogeological and hydrochemical frame works of regional aquifer system in kali-ganga sub-basin. *India Envir Geol.,* **40(4-5)**: 602611.

USEPA. 2010. **Risk-based concentration table**, Washington, DC, USA: United States Environment Protection Agency.

USEPA. 2011. **Regional screening level (RSL) summary table: November 2011**. United States Environment Protection Agency, Washington, DC, USA.

USEPA-IRIS. 2006. **Integrated Risk Information System, Washington, DC, USA.** United States Environmental Protection Agency. http://www.epa.gov/iris. Accessed 23 September 2019.

Usero J, González-Regalado E, Gracia I. 1997. Trace metals in the bivalve molluscs *Ruditapes decussatus* and *Ruditapes philippinarum* from the Atlantic Coast of Southern Spain. *Environ Int*., **23(3)**:291-298.

Vahter M, Berglund M, Åkesson A and Lidén C. 2002. Metals and women's health. *Environ Res.*, **88**: 145-155.

Wahiduzzaman M, Islam M, Sikder A and Parveen Z. 2021. Bioaccumulation and heavy metal contamination in fish species of the Dhaleswari river of Bangladesh and related human health implications. *Biol Trace Elem Res.,* **200**: 3854-3866.

WHO. 2011. **Non-Communicable Disease Risk Factor Survey Bangladesh 2010**. Dhaka: World Health Organization.