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Bioaccumulation of heavy metals in fish and shellfish from Rupsa River, Bangladesh, and risk assessment for human

SUMIT KUMER PAUL, MD. ABDUL AHAD[1], KHAN KAMAL UDDIN AHMED[2], MD. KAMAL[1] AND MD. SHAHEED REZA[1*]

Department of Fisheries, Bangamata Sheikh Fojilatunnesa Mujib Science and Technology University, Melandaha, Jamalpur-2012

[1]Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202

[2]Shrimp Research Station, Bangladesh Fisheries Research Institute, Bagherhat-9300, Bangladesh

[*]Corresponding Author's Email: msreza@bau.edu.bd

Abstract. Spatial variation of Pb, Hg, Cr, Cd and As concentrations between up- and downstream fish (river shad *Tenualosa ilisha*, silver jewfish *Otolithoides pama*) and shellfish (black tiger shrimp *Penaeus monodon*) collected from Rupsa River of the south-west coast of Bangladesh was studied in order to assess the potential health risks in human. Samples (n=18) were collected representing 3 species from two locations of the river system and muscle analyzed for heavy metal concentration. Mean concentration of the elements was significantly higher ($p<0.05$) in the downstream samples compared to those of the upstream with a high concentration in benthic fauna and interspecific variation at a trend: black tiger shrimp > Hilsa shad > silver jewfish and distribution As>Cr>Hg>Cd>Pb. Bioaccumulation of As and Hg was correlated well with fish size although their concentrations were well below the international legislation limits set by WHO and FAO. For potential human health risk, the estimated daily intake (EDI), target hazard quotient (THQ) and total target hazard quotient (TTHQ) for non-carcinogenic risk suggest that most of the values were within the acceptable limits for all species expect As. Besides, the carcinogenic risk (CR) values indicate less prone to cancer risk yet CR values of Cd and As in both black tiger shrimp and Hilsa shad were close to the unacceptable range.

Keywords: Finfish, Shellfish, Bioaccumulation, Food Safety, Health Risk

Introduction

In the recent years, world consumption of fish has increased primarily due to their good nutritional value and therapeutic benefits. In addition to its important source of protein, fish typically have rich contents of essential minerals, vitamins and unsaturated fatty acids (Reza *et al.* 2009, Medeiros *et al.* 2012). The American Heart Association recommended eating fish at least twice per week in order to reach the daily intake of omega-3 fatty acids. However, fish also may contain heavy metals like essential heavy metals (vanadium; V, manganese; Mn, iron; Fe, cobalt; Co, copper; Cu, zinc; Zn, selenium; Se, strontium; Sr and molybdenum; Mo) and non-essential heavy metals (chromium; Cr, aluminum; Al, arsenic; As, barium; Ba, bismuth; Bi, cadmium; Cd, lead; Pb, mercury; Hg, nickel; Ni, uranium; U, and tin; Sn) (Connell and Miller 1984). Heavy metals like Cu, Fe and Zn are essential for fish metabolism while some others such as Hg, Cd, As and Pb have no known role in biological systems. These non-essential heavy metals cause toxic to human that could give negative effects for health (Connell and Miller 1984). The diet and food of animal origin are the most predominant sources (>90%) of heavy metals and other chemical contaminants to human (Svensson *et al.* 1987). However, the concentrations may be raised in coastal ecosystems due to the release of industrial waste, agricultural and mining activities. As a result, aquatic organisms were exposed to elevated levels of heavy metals (Sankar *et al.* 2006). The levels of heavy metals in fish are particularly important because fish is an important source of food for the general human population and the

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high levels of heavy metals in fish tissues from different water bodies receiving industrial effluents have been reported to be unfit for human consumption (Obasohan *et al*, 2006; Tyokumbur and Okorie 2014). In addition to this, fish and shellfish are sensitive to toxicants and regarded as an indicator organism / bio-monitor of ecosystem health (Authman 2008). That's why, it is necessary to determine contamination levels of various metal elements in mostly consumed fish and shellfish.

The Rupsa River is one of the most important and largest estuarine river systems in Khulna and in the south-west coast of Bangladesh. It flows by the side of Khulna district and connects to the Bay of Bengal through Poshur River at Mongla Channel. Due to lack of water treatment facilities, the domestic wastes and industrial effluents are disposed of in this river. This increases the concentration of different kinds of pollutants. The trace elements in the surface and groundwater in the region often exceed the recommended values of WHO. A recent review on water pollution situation in Bangladesh revealed that both surface and groundwater sources of the country were contaminated with different contaminants like toxic trace elements, coliforms as well as other organic and inorganic pollutants (Hasan *et al.* 2019). As contamination showed a sharp rise in Khulna distinct in recent years (Alam 2019). So, there is a chance of contamination of fish, shellfish and plant-based food materials by trace elements. An estimated 3,346 mt of fish and shellfish are harvested from rivers, 133 mt from the low-lying watersheds (locally called *beel*) and 19,192 mt from floodplain of this district (DoF 2021). They are consumed locally, and also transported to different parts of the country for domestic consumption. Their consumption may also result deleterious effects on human health.

There is insufficient information on the contamination status of fish and shellfish obtained from Rupsa river. Therefore, this study aims to determine the level of Pb, Cd, Cr, As and Hg in commonly consumed fish and shellfish caught along the river as it may represent risk for human health through bioaccumulation and magnify them up in the food chain. The carcinogenic and non-carcinogenic health risk for humans through fish consumption was also evaluated.

Materials and Methods

***Study area*:** The study area is located from 22.781934 N and 89.58415 E to 22.736661 N and 89.522009 E (Fig. 1). Geographical data of these locations were recorded using GPS Device (Model Mobile Mapper, Thales, UK). Two locations, upstream (UPS) and downstream (DWS) river, were carefully chosen near Rupsa Ferry Ghat and Batiaghata Ghat of Rupsa River, respectively. The UPS was relatively less polluted portion of river, and the DWS mostly polluted because it was a major dumping site of Khulna city sewerage and also the water of this point received effluents of different industries and garbage of markets.

***Survey to identify possible sources of contamination*:** Data was collected using questionnaire through interview, personal contact, market survey and participatory rural appraisal like focus group discussion (FGD). A set of preliminary questionnaires was prepared for different stakeholders including fishers, traders, processors, community leaders, government officials of the Department of Fisheries (DoF) and scientists of Bangladesh Fisheries Research Institute (BFRI). It was pre-tested by checking if the questionnaire fulfilled the objectives of the study. Then the pre-tested questionnaires were used to collect information from respondents with assistance from the DoF officials. Major aspects of recorded data included location of fishing, number and amount of fish and shellfish species caught, changes in the amount caught, disease occurrence in fish, possible causes of water pollution etc. Similarly, the District Fisheries Officer (DFO), Upazila Fisheries Officer (UFO) of the DoF and scientists of BFRI were surveyed using prescribed questionnaire to collect information like current production of fish from the river, any deviation of fish production over time, name of fish species extinct, causes of extinction, sources of contamination etc.

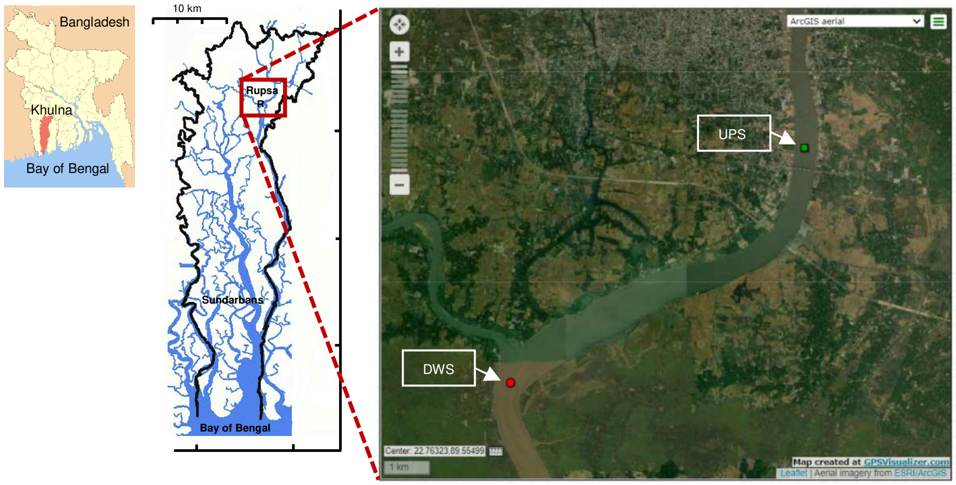

Fig. 1. Map showing the sampling locations; upstream (UPS, green circular dot) near Rupsa Ferry Ghat and downstream (DWS, red circular dot) near Batiaghata Ghat of Rupsa River (Google Maps).

Collection and chemical analysis of water samples: A total of 20 water samples were collected from upstream and downstream locations of Rupsa River during November-December 2013. First, 1 L glass bottle was rinsed with distilled water twice and water sample was collected and stored in an ice box. Then they were transported to the temporary laboratory enclosure set by the side of the river bank, and measurements of temperature, pH, dissolved oxygen (DO), total alkalinity, ammonia and hardness using HANNA Test kit, Hanna Instruments Ltd., Germany as described previously (Ferdous *et al.* 2013).

***Collection of fish samples*:** Three species from 3 trophic levels, viz., one pelagic finfish (river shad, *T. ilisha*) (500-650g), one benthopelagic finfish (silver jewfish, *O. pama*) (100-120g) and one benthic shellfish (black tiger shrimp, *P. monodon*) (10-15g) were collected for UPS and DWS locations of the Rupsa from the fishermen during November-December 2013. A total of 18 samples representing 3 species from each individual location were collected and kept in Ziploc plastic bags, stored in ice box and transported to the laboratory. The fish samples were washed with clean tap water, chopped into small pieces and muscle samples cleaned with deionized water. They were air dried to remove the extra water and homogenized in a food processor. All tissue samples were stored at −20°C for subsequent analyses.

***Determination of heavy metals*:** The concentration of five heavy metals (Pb, Cd, Cr, As, Hg) in fish and shrimp samples determined at the Central Laboratory, Société Générale de Surveillance (SGS), Dhaka, Bangladesh using Inductively Coupled Plasma-Optical Emission

Spectroscopy (ICP-OES) technique on an Agilent ICP-OES; 700 Series System (Agilent Technologies, Inc., Australia) after digestion with HNO_3–H_2O_2. All analyses were replicated 3 times to confirm reproducibility of the measurement. Metal concentrations were determined as ppm (mg kg^{-1}).

Potential human health risk assessment

***Estimated daily intake (EDI)*:** Estimated daily intake (EDI) was calculated by the following equation (Formula 1) (Bo *et al.*, 2009):

$$EDI = \frac{MC \times IR}{Bwt}$$

where, ***MC*** is the concentration level of metal in the selected fish and shellfish tissues (mg/kg); ***IR*** is the acceptable ingestion rate, which is 62.58 g/day for adults (DoF, 2021); ***Bwt*** is the body weight: average 70 kg for adults (USEPA, 2010).

***Target hazard quotient (THQ) and total target hazard quotient (TTHQ) for non-carcinogenic risk assessment*:** THQ was estimated by the ratio of *EDI* and oral reference dose (*RfD*). *RfD*s of the different metals for example Pb, Cd, Cr, As and Hg are 0.01, 0.005, 0.25, 0.015 and 0.015 mg $kg^{-1}day^{-1}$ respectively (USEPA, 2016). The THQ equation (Formula 2) is expressed as follows (Chien *et al.,* 2002):

$$THQ = \frac{E_d \times E_f \times EDI \times C_f}{A_t \times RfD} \times 10^{-3}$$

Where E_d is exposure duration (30 years for non-cancer risk, as used by (USEPA, 2011), E_f is exposure frequency (350 days/ year) (Chien *et al.* 2002); C_f is the conversion factor (0.208) to convert fresh wet weight to dry weight given that 79% is the moisture content in fish (Baki *et al.,* 2018), A_t is the average time for the non-carcinogenic element (365 days $year^{-1}$ for 65 years (i.e. A_t =23,725 days) (USEPA 2011).

Since humans might have exposed to two or more contaminants with associated combined or interactive effects at the same time (Huang *et al.*, 2013), In this study, the total THQ (TTHQ) was treated as the arithmetic sum of the individual metal THQ values (Formula 3), derived by the method of (Chien *et al.,* 2002):

$$TTHQ = THQ\text{-}Pb + THQ\text{-}Cd + THQ\text{-}Cr + THQ\text{-}As + THQ\text{-}Hg$$

Where, THQ-Pb is the target hazard quotient for Pb intake and so on.

***Carcinogenic risk (CR)*:** Carcinogenic risk (CR) is estimated by the following equation (Formula 4) (Vieira *et al.,* 2011):

$$CR = \frac{E_d \times E_f \times EDI \times C_f \times CPS_O}{A_t} \times 10^{-3}$$

Where, *CPSo* is the carcinogenic potency slope for oral route. Available *CPSo* values (mg kg^{-1} day^{-1}) are: As (1.5), Pb (0.0085) and Cd (6.3) (USEPA, 2011).

***Statistical analysis*:** Mean, standard deviation average, and two-way analysis of variance (ANOVA) were used to indicate significant differences in metal levels between fish and shellfish species and between different locations. All analyses were conducted using MSExcel 2013 (Microsoft Corp., Redmond, USA)

Results

***Physico-chemical parameters of water*:** Water quality parameters such as pH, DO, total alkalinity, ammonia, hardness and temperature were measured on site during sample collection and the results are presented in Table I. Temperature and pH in both sampling sites were found very similar that showed no significant difference between DSW and UPS locations. Highest salinity was found in DWS 10±0.1 ppt while in UPS was 9.8±0.1 ppt. In case of alkalinity, hardness and DO of DWS location, the values were 320±20 ppm, 750±20 and 2.4±0.5 ppm respectively which were also higher than UPS location. Besides, the amount of ammonia was found to be slightly higher in UPS (0.04±0.01 ppm) compared to DWS (0.02±0.01 ppm). Among these parameters, the mean values of salinity, alkalinity, hardness and DO (Table I) were found significantly different ($p<0.05$) between the sampling sites.

Table I. Water Quality parameters of the river Rupsa

Parameters	Sampling location	
	UPS	DWS
Temperature (°C)	29.0±0.7	29.7±0.7
pH	8.25±0.01	8.23±0.01
Salinity (ppt)	9.8±0.1	10±0.1
Alkalinity (ppm)	220±28	320±20
Hardness	650±50	750±20
DO (ppm)	1.9±0.5	2.4±0.5
Ammonia (ppm)	0.04±0.01	0.02±0.01

Values are mean ± SD

***Heavy metal concentration in fish and shrimp*:** The concentration of Pb, Cd, Cr, As and Hg in different fish species from Rupsa River are shown in Fig. 2. Metal content found in silver jewfish collected from upstream location was 0.018±0.002 mg kg^{-1} Pb, 0.00 mg kg^{-1} Cd, 0.012±0.004 mg kg^{-1} Cr, 0.243±0.004 mg kg^{-1} As and 0.020±0.017 mg kg^{-1} Hg. The mean concentration of As was highest followed by Hg, Pb, Cr and Cd. These values exhibited slight increase in silver jewfish muscle collected from the downstream where significant increase was found for Pb (0.025±0.004 mg kg^{-1}) and Cr (0.031±0.002 mg kg^{-1}), but rest of the three metals viz., Cd (0.010±0.010 mg kg^{-1}), As (0.247±0.008 mg kg^{-1}) and Hg (0.048±0.013 mg kg^{-1}) did not show any significant increase. The order of metal bioaccumulation in muscle tissue was As > Hg > Pb > Cr > Cd. In river shad, metal content collected in the upstream was 0.00 mg kg^{-1} Pb, 0.018±0.003 mg kg^{-1} Cd, 0.025±0.012 mg kg^{-1} Cr, 0.214±0.012 mg kg^{-1} As and 0.039±0.001 mg kg^{-1} Hg. The order of metal bioaccumulation in this fish was As > Cr > Hg > Cd > Pb. Interestingly for Hilsa shad, all five metals exhibited significant increase in the muscle that were collected from the river downstream. Their concentration was 0.011±0.001 mg kg^{-1} Pb, 0.047±0.006 mg kg^{-1} Cd, 0.043±0.006 mg kg^{-1} Cr, 0.273±0.006 mg kg^{-1} As and 0.027±0.001 mg kg^{-1} Hg. Finally, the levels of heavy metals in black tiger prawn were 0.033±0.003 mg kg^{-1} Pb, 0.020±0.001 mg kg^{-1} Cd, 0.130±0.035 mg kg^{-1} Cr, 0.252±0.023 mg kg^{-1} As and 0.016±0.004 mg kg^{-1} Hg. Except for Pb (0.042±0.007 mg kg^{-1}), rest of the four metal components exhibited significant rise in their content in shrimp muscle corresponding to 0.048±0.004 mg kg^{-1}, 0.401±0.074 mg kg^{-1}, 0.441±0.016 mg kg^{-1} and 0.027±0.001 mg kg^{-1} for Cd, Cr, As and Hg respectively. The order of metal bioaccumulation in these tissue was As>Cr>Pb>Cd>Hg. All these values were well below the permissible limit according to the guidelines of FAO, WHO and Japan standard (Table II).

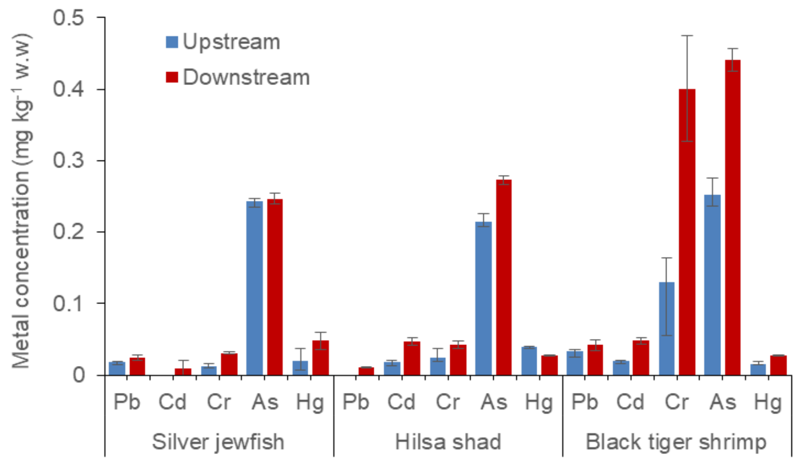

Fig. 2. Concentration of heavy metals in fish and shrimp muscle collected from up- and downstream locations of River Rupsa.

Table II. Guideline for acceptable limit for heavy metal concentration in fish

Guideline	Acceptable limit (mg kg^{-1})				
	Pb	Cd	Cr	As	Hg
FAO*	2.5	0.2	1.0	1.0	-
WHO**	2.0	1.0	0.15	0.01	-
Japan***	-	-	-	-	0.4

* (FAO, 1983); ** (WHO, 1985); *** (Nakagawa *et al.,* 1997)

Possible sources of heavy metal contamination: Survey was conducted among the fishermen, DoF and BFRI officials to identify the possible sources of heavy metals contamination. The results revealed that a wide variety of industries including textile mills, oil refineries, Jute mills, garments etc. were located around the river bank. The survey results (Fig. 3) indicated that the downstream location was densely polluted by the discharge of untreated industrial effluents and dumping of Khulna city sewerage which might cause harm to the aquatic biota. However, upstream was located on the 5 km south from Rupsa upazila which is less polluted and mainly used for agricultural and fishing purposes with a few numbers of industries (Fig. 3). Though heavy metals are natural trace components of the aquatic environment, but available reports suggest that the natural aquatic systems have been extensively contaminated with heavy metals released from domestic, industrial, agricultural and other man- made activities (Singh *et al.,* 2007; Sprocati *et al.*, 2006; Velez and Montoro, 1998).

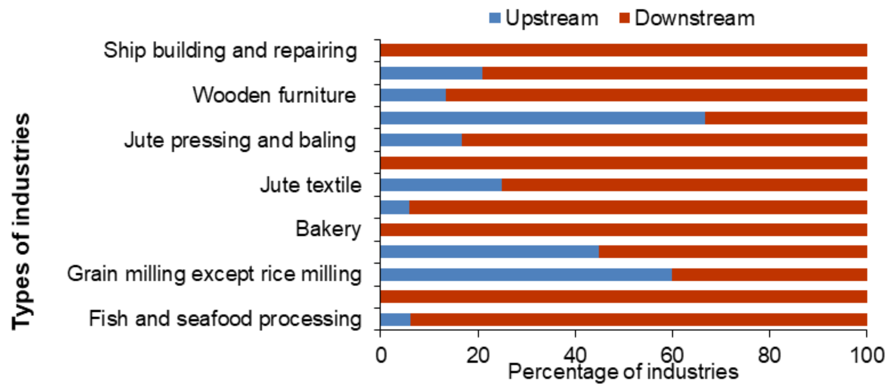

Fig. 3. Factories and industries established at upstream and downstream locations of Rupsa River.

***Inter-species variation in metal accumulation*:** In this study, two pelagic finfish viz., silver jewfish and Hilsa shad, and an omnivorous benthic organism black tiger shrimp were analyzed for heavy metal content to check trophic level variation. It was observed that metal content varied among the three species based on their trophic level and their mode of feeding (Weber *et al.*, 2013). Bioaccumulation of As and Cr was the highest in black tiger shrimp collected from the river downstream with 0.401±0.074 mg kg^{-1} for Cr and 0.441±0.016 mg kg^{-1} for As.

***Relationship between metal concentration and fish size*:** A strong significant positive relationship found between bioaccumulation of heavy metals with fish length and weight from downstream of Rupsa River. There was a strong significant positive relationship found between bioaccumulation of As and fish length, r= 0.999, $p<0.05$. On the other hand, strong significant positive relationship also found between bioaccumulation of As and Hg with fish weight (Table III).

Table III. Correlation coefficients between heavy metal concentration and fish size

Elements	Fish in upstream	Fish in downstream
Length		
Pb	-	-
Cd	-	-
Cr	-	-
As	-	0.999 (0.020)
Hg	-	-
Weight		
Pb	-	-
Cd	-	-
Cr	-	-
As	-	0.998 (0.042)
Hg	-	0.999 (0.000)

Values in the parentheses indicate level of significance

***Estimated daily intake (EDI) of fish and shellfish for health risk assessment*:** One of the most important aspects of assessing the pollutant contents in aquatic organism is to estimate the human dietary exposure. Among all the metals highest EDI of As was found for black tiger shrimp (0.309) while lowest was Pb for silver jewfish (0.019) (Table IV).

Table IV. Estimated daily intake (EDI), Target hazard quotient (THQ), Total Target hazard quotient (TTHQ) and Carcinogenic risk (CR) for adult consumer of fish and shellfish of Rupsa river

Species	Estimated daily intake					Target hazard quotient					TTHQ	Carcinogenic risk		
	EDI-Pb	EDI-Cd	EDI-Cr	EDI-As	EDI-Hg	THQ-Pb	THQ-Cd	THQ-Cr	THQ-As	THQ-Hg		CR-Pb	CR-Cd	CR-As
Black tiger shrimp	0.033	0.03	0.237	0.309	0.019	0.116	0.21	0.032	0.715	0.044	1.12	2.85E-07	1.91E-04	4.65E-04
Hilsa shad	0.004	0.029	0.03	0.217	0.029	0.017	0.201	0.004	0.503	0.068	0.794	4.18E-08	1.83E-04	3.27E-04
Silver jewfish	0.019	0.004	0.019	0.219	0.031	0.059	0.027	0.002	0.452	0.063	0.605	3.40E-08	5.86E-06	6.83E-05

THQ and TTHQ for non-carcinogenic risk assessment**:** Estimated THQ for individual heavy metal through the consumption of different fish and shellfish species were presented in Table 5. The average THQs values of Pb, Cd, Cr, As and Hg were 0.064, 0.146, 0.013, 0.557 and 0.058 respectively. It was observed that these values were all below 1 for all heavy metals in all three species. On the other hand, TTHQ value was found higher than 1 in benthic black tiger shrimp (1.12) while less than 1 was found in two finfish under investigation. The values were 0.794 and 0.605 for Hilsa shad and silver jewfish respectively (Table IV).

Carcinogenic risk (CR) assessment**:** Exposure of CR was estimated for a particular element and summarized in Table 5. The measured CR values for silver jewfish were 3.40E-08 Pb; 5.86E-06 Cd and 6.83E-05 As and for Hilsa shad were 4.18E-08 Pb; 1.83E-04 Cd and 3.27E-04 As. However, CR values for the black tiger shrimp were 2.85E-07 Pb; 1.91E-04 Cd and 4.65E-04 As.

Discussion

This paper reports the results from analysis of five highly toxic heavy metal viz., Pb, Cd, Cr, As and Hg in up- and downstream locations of Rupsa River located at the south-west coast of Bangladesh. They were included in the study as they rank among the priority metals of public health significance, and for their ability to induce multiple organ damage even at low levels of exposure (Bo *et al.* 2009).

Although muscle/flesh accumulates lower concentration of metals in fish and shellfish, they were investigated in the present study since muscle/flesh is principal edible part of aquatic animals. There were marked variations in the level of heavy metals in fish and shellfish species of the two sampling areas (UPS and DWS). Generally, the levels of heavy metal in UPS were lower compared to DWS. This is because in River Rupsa DWS is densely polluted than UPS that evidently shows increased bioaccumulation of different heavy metals in fish and shellfish have been connected with urban runoff, sewage treatment plants, industrial effluents and wastes, mining operations, boating activities, domestic garbage dumps and agricultural fungicide runoff (AlemdaroĞLU *et al.* 2013, Islam *et al.* 2014). Still the values of heavy metal content were below the permissible limit suggested by different international organizations. These lower values compared to other river systems of Bangladesh is not well understood, but may be linked to physico-chemical parameters of water and sediments of the sampling sites. It was reported that particle size of sediment and the vicinity to mangrove forests are some factors that helps reduce heavy metal content is the surrounding environment and the organisms living in that area (Enuneku *et al.* 2018). Although our study did not investigate particle size of the sediment of the sampling locations, they were clearly located in the Sundarbans mangrove zone. Significant difference was observed in heavy metal content for silver jewfish and black tiger shrimp collected from UPS and DWS locations, but not much for Hilsa shad. This may be explained by the nature of movement of Hilsa shad across its habitat. This anadromous fish migrates into freshwater from the sea during their adult stage for spawning while their young rear in the river channels and estuaries before descending to the sea for further feeding and growth (Bhaumik, 2015). Since the Hilsa shad samples were collected from waters with a salinity ranging from 9.8 ± 0.1 ppt in the upstream to 10.0 ± 0.1 ppt in the downstream, these are clearly for temporary nature of their habitation that is also reflected by their relatively lower levels of heavy metal content in muscle tissue.

Inter-species and trophic level variations in heavy metal content in fish and shellfish collected from Rupsa river were also observed in this study. It was found that bioaccumulation of As and Cr was the highest in the benthic species collected from the river downstream. Ahmed *et al.* (2011) reported that Cd and Pb concentrations in some macrobenthic fauna of the Sundarbans mangrove forest ranged from 0.46 ± 0.11 to 0.859 ± 0.2 and 4.66 ± 1.17 to 6.77 ± 2.1 mg kg^{-1}. These values were slightly higher than those obtained in the present study. The level of heavy metals contamination in order of magnitude was: black tiger prawn > Hilsa shad > silver jewfish. This is because of the fact that shellfish species had a high capacity and propensity to concentrate pollutants (Agusa *et al.*, 2005) than finfish as they live on the bottom where different effluents accumulate in the sediments and highest heavy metals concentration was observed in the sediments (Li *et al.*, 2013). So, knowledge on the living and feeding habitat may be considered essential as it may represent the basis for accumulation of trace metals in fishes. Furthermore, a strong positive correlation ($r = 0.999$, $p < 0.05$) was found between bioaccumulation of heavy metals and fish size from UPS of Rupsa River that agrees with many previous literatures. It was stated the occurrence of trace mineral including Hg and others were also related to length, weight and age of fish (Agusa *et al.* 2005).

The degree of toxicity of heavy metal to humans depends upon the daily intake (Singh *et al.* 2010). Among all the metals high doses of As was exposed to the consumers through consuming benthic black tiger shrimp from Rupsa river as the food items while lowest was Pb of pelagic silver jewfish. The mean values of EDI of the metals for the consumers compared with recommended daily allowance (RDA), provided by National Academic Press; Washington, DC were still lower which eventually implies a lower possible health effect of the elements to the consumers except As (NRC, 1989). The average THQs values of the present study (Table 5) were below than observed in the fish and shellfish of Sicilian coasts (Mediterranean Sea) (Traina *et al.* 2019). The result shows that, the THQ values of all heavy metals in all species were all below 1.0, which indicates no potential non-carcinogenic health risk from the ingestion of a single metal through the consumption of these species (Abtahi *et al.*, 2017). Furthermore, in case of Total THQ (TTHQ) the combined impact of all metals under consideration was higher than the acceptable limit of 1 in shellfish (Table 5). Therefore, the continuous and excessive intake of black tiger shrimp could result in chronic non-carcinogenic effects. However, in the case of pelagic fish species, the value of TTHQ was lower than the acceptable limit. Yet, human can dramatically suffer in the long run due to multiple simultaneous pollutants (Li *et al.*, 2013). But, THQ and TTHQ do not directly measure risk because they do not define any dose-response relationship (USEPA, 1989).

To assess the probability of developing cancer over a lifetime, the carcinogenic risk was evaluated for the consequence of exposure to the substantial carcinogens. Exposure of CR was estimated for a particular element and summarized in Table 4. The measured CR values of Pb, Cd and As were ranged from 3.4E-08 to 2.85 E-07, 5.86E-06 to 1.83E-04 and 6.83E-05 to 3.27E-04 respectively in consumers for all the species. The CR values lower than 10^{-6} indicates a negligible health risk whereas values in the range of 10^{-6} to 10^{-4} are in the acceptable belt (Li *et al.*, 2012). Therefore, the present study indicates consumers were less prone to carcinogenic risk and studies carried out by several authors in similar conditions were in line with our results (Ahmed *et al.* 2019). Moreover, CRs higher than 10^{-4} are likely to increase the probability of carcinogenic risk effect (USEPA, 2010). Yet the CR values of Cd and As in both black tiger shrimp and Hilsa shad were near to the unacceptable range and for this reason carcinogenic risk should be given more attention.

Heavy metal in the muscles of three fish and shellfish species from Rupsa river up- and downstream were measured to investigate potential human health risk. High levels of As was generally observed in all samples with highest in black tiger shrimp, although the concentrations didn't exceed permissible limits. The THQ value for a single heavy metal in all species were below 1.0, which indicates no potential non-carcinogenic health risk from the ingestion of a single metal but the TTHQ value of black tiger shrimp was above 1.0, implied intake of multiple simultaneous pollutants could result in chronic non-carcinogenic effects.

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