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Organ-specific accumulation of toxic elements in Hilsa shad (*Tenualosa ilisha*) from Bangladesh and human health risk assessment

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Abstract

Purpose: We aimed to determine the amount of some toxic elements in three organs of Hilsa shad, focusing on the possible exposure to human health through Hilsa consumption. This study was designed to determine the concentration of seven toxic trace elements (As, Cd, Cr, Cu, Ni, Pb, and Zn) in three distinct organs (n = 21) (muscle, liver, and gills) of Hilsa shad (*Tenualosa ilisha*) fish collected from the Bangladeshi coastal area. The samples were digested following a microwave digestion. Inductively coupled plasma mass spectrometer was used as analytical instrument. Estimated daily intakes (EDI) and target cancer risk (TR) were used to evaluate carcinogenic and non-carcinogenic risk.

Results: The mean concentrations (mg/kg-wet weight) of toxic elements in different organs of *T. ilisha* were determined as follows: in muscle, As (4.05), Cd (0.09), Cr (0.12), Cu (0.77), Ni (0.26), Pb (0.20), and Zn (10.64); in liver, As (2.83), Cd (0.84), Cr (0.18), Cu (6.17), Ni (0.55), Pb (0.23), and Zn (30.16) and in gills, As (3.45), Cd (0.05), Cr (0.08), Cu (1.06), Ni (0.51), Pb (0.78), and Zn (35.21). The liver showed higher concentrations of most elements than that of muscle except for As. Concentration of As, Cd, and Pb in the fish were found above the food safety guidelines, while other trace element concentrations were below the permissible range for human consumption. According to EDI and TR values, there were carcinogenic and non-carcinogenic risks from exposure to total As concentration from Hilsa fish consumption.

Conclusion: This study suggests that the toxic trace elements contamination levels in Hilsa fish from Bangladesh's coastal area need to be monitored on a systematic and regular basis to ensure the safety of this food item for human consumption.

Keywords: Trace elements, arsenic, hilsa shad, health risk, Bangladesh

INTRODUCTION

Trace elements are the most hazardous to aquatic ecosystems because they are widespread in the environment, widely dissolved in water, and rapidly absorbed by aquatic organisms[1-3]. Metals enter the environment naturally through air deposition, geological matrix attrition, and anthropogenic intrusions including sewage, industrial, and agricultural runoff[4]. Because of their toxicity, persistence, and bioaccumulation in food chains, trace elements in aquatic environments may have an impact on fish and other biological life[5,6]. The aquatic environment is one of the most regularly monitored areas where different organisms will be vulnerable depending on the type of contaminant, habitat, position in the food chain that the organism occupies, *etc.*[7]. Fish have been recommended as valuable biological indicators for the assessment of aquatic pollution because they are large in size, considered as resident species, adult, perennial, and easily identified in aquatic medium[8]. In addition, they have a longer life span and a higher position in the aquatic food chain[9], and thus have been suggested as useful biological markers in the assessment of aquatic environmental pollution[10,11].

The concentrations of trace elements accumulated in a fish's body greatly vary among its different organs[12-15]. Fish absorb trace elements by ingesting of particulate matter from water, taking of foods, grazing food from sediments through the gills, and adsorption on tissues. The distribution of metals in different tissues of fish depends on the mode of exposure, biokinetics of the elements, lipophilic properties, *etc.*[16-18]. Furthermore, the accumulation rates of trace elements in fish are affected by both absorption and removal processes[19-21].

However, very few studies in Bangladesh have concentrated on the increasing high levels of trace elements in different organs of the fish (e.g., muscle, gills, and liver). Gills and liver are considered as indicators for measuring metal accumulation in fish. Metal accumulation is also measured using the gills and liver as markers. Metal concentrations in gills represent the metal concentrations in a fish's living environment, while the liver mainly acts as metabolic organ where metals are also stored[22] and muscle is not an active tissue in accumulating trace elements but does act as storage organ[23-25].

The presence of metals in fish tissues is of major concern for food safety as well as public health[26-28]. As a result, metal contamination in fish has become a major problem around the world, and many studies on metal accumulation in fish have already been conducted[28-32].

In Bangladesh, trace element pollution in fish has become a serious public health concern. Fish are the major source of protein for Bangladeshi people, and about five million coastal residentsd are directly or indirectly engaged in commercial fishing, particularly of Hilsa shad (*Tenualosa ilisha*) fishing. Hilsa shad is usually known as Indian shad, which is a highly migratory fish with a similar breeding behavior to Atlantic salmon. It migrates into the Padma and Meghna Rivers and their tributaries from the Bay of Bengal for breeding and nursing. Hilsa is the most dominant, commercially important, and widely consumed fish by the Bangladeshi people. As a single species, it contributes 12% of the total marine catch. Approximately, 2% of the country's population is directly or indirectly involved with Hilsa fishing[33]. Bangladesh contributes 75% of the global Hilda shad catch, and the rest comes from Myanmar (15%), India (5%), and 5% from Thailand and Iran[33].

The rapid unplanned industrialization, urbanization, and trans-boundary movement of water may contribute a considerable amount of industrial effluent and untreated domestic wastewater into the coastal water environment through rivers. As a result, trace elements contaminate coastal water, and contaminants can be deposited in aquatic organisms, including Hilsa fish, via bioconcentration, bioaccumulation, and food chain effects. Trace element contamination in Hilsa fish and other aquatic biota threatens human health via the food chain.

To the best of our knowledge, this was the first study carried out on the organ-specific trace element distribution in Hilsa shad in Bangladesh. The objective of this study was to determine the level of trace element contamination (As, Cd, Cr, Cu, Ni, Pb, and Zn) and its distribution in three organs (muscle, liver, and gills) of Hilsa shad, with a focus on the possible exposure to human health through the consumption of Hilsa shad.

EXPERIMENTAL

Collection of hilsa shad

The most consumed and commercially important fish, Hilsa shad (*Tenualosa ilisha*) (weight, 851.71 ± 10.57 g; length, 32.46 ± 3.27 cm) was collected from the central fish landing center, which is very close to the Bakkhali estuary of Cox's Bazar [Figure 1]. Cox's Bazar is a seaside tourist town with the world's longest sandy sea beach. Hundreds of factories and industries, such as ship breaking and building industries, paint and salt industries, fish and shrimp processing plants, textile manufacturing, fish and shrimp hatcheries, fish auction and landing center, unplanned urbanization, and several hotels, have an impact on the city. Every year, many local and international tourists visit the city. The sampling was conducted in winter (temperature range: 10-16 °C) from January to February 2016. The fish samples were transported to the Department of Fisheries, University of Dhaka, Bangladesh. They were washed with deionized water after transportation to remove surface adherence. With the use of a clean SS knife, the samples were chopped into small pieces according to target organs, namely gills, liver, and muscles, and separately freeze-dried (48 h) until a constant weight was achieved. Further chemical analysis of all processed samples was performed at the laboratory of the Yokohama National University, Japan.

Sample preparation

The samples were digested following a microwave digestion[34]. In a closed digestion vessel, 0.2 g of each organ of Hilsa shad was treated with 5 mL of 69% HNO_3 acid and 2 mL of 30% H_2O_2 and mixed for 20 min. The samples were then placed in the Berghof-MWS2 (Berghof speedwave®, Germany) digestion system. Acid solutions containing samples were put into a Teflon graduated cylinder after digestion, and the total volume was adjusted to 50 mL using Milli-Q water. The digested acid solutions were then filtered through a syringe filter (DISMIC®; 0.45 m) and stored in 50 mL PP tubes.

Instrumental analysis

The samples were analyzed using inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700 series, USA) following the previous method[34]. Briefly, the calibration curve was prepared using a multi-element standard (SPEX CertiPrep®, USA) solution. For concentration calculations, calibration curves with $R^2 > 0.999$ were accepted. The relative standard deviation (RSD < 5%) was checked using a tuning solution before starting the analysis (1 μg/L each of Li, Y, Ce, Tl, Mg, and Co in 2% HNO_3). Dilution of the multi-

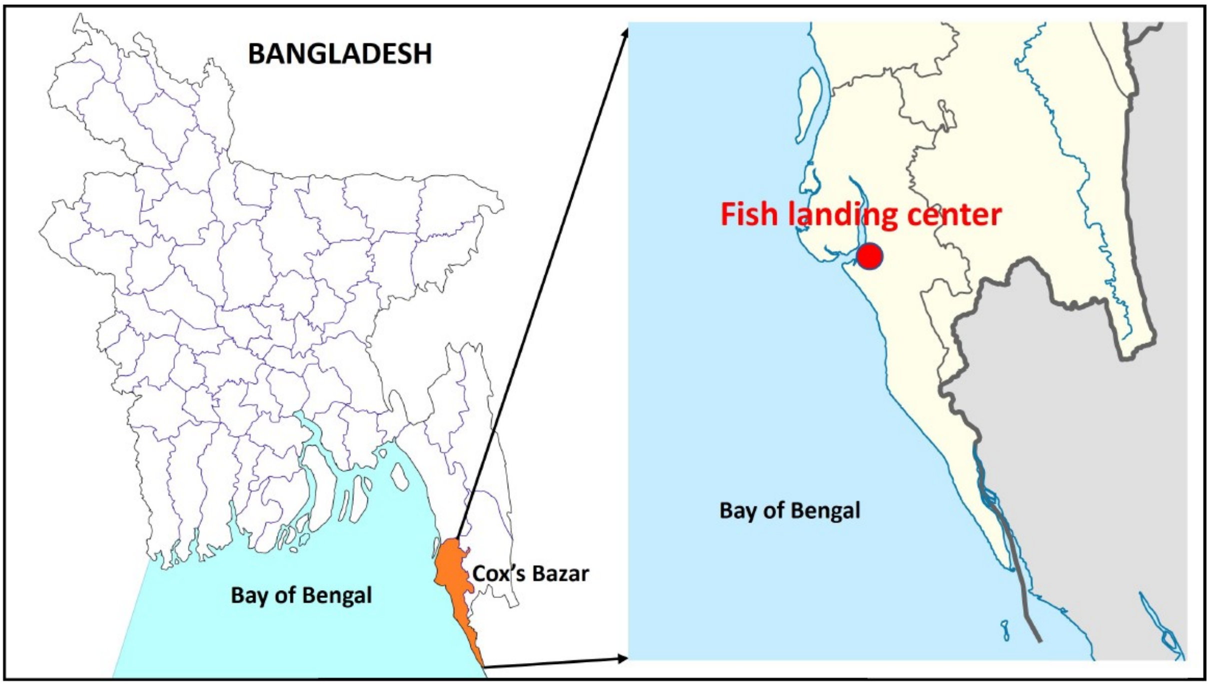

Figure 1. Map showing sampling site (main fish landing center at Cox's Bazar, Bangladesh).

element stock solution yielded working standards (0, 10, 20, 50, and 100 g/L). An internal standard method was used to determine trace element concentrations. In the current investigation, the trace element concentrations in Hilsa shad (*T. ilisha*) were assessed on a wet weight (ww) basis.

Quality control and quality assurance

To maintain strict quality control and assurance, Internal Quality Controls were maintained for all test batches and validated. The experiment included a blank, a certified reference material (CRM), and some samples that were examined in triplicate to remove the batch-specific error. NMIJ CRM 7402-a, cod fish tissue, as a certified reference material, was used to check the quality. The CRM recoveries ranged from 91% to 106%, with the triplicate reproducibility ranging from 93% to 97%, confirming the acceptable accuracy and precision of the method.

Health risk assessment

Estimated daily intakes (EDI) and target cancer risk (TR) were used to quantify the risk of trace element concentrations in the fish. Because the human population in the vicinity consumes fish tissues such as gills, muscles, and liver, all of these tissues were considered in the risk evaluation.

EDI

EDI (μg/kg-bw/day) were calculated for the detected trace elements by using Equation (1):

$$EDI = \frac{FIR \times C}{WAB} \qquad (1)$$

where FIR is the food ingestion rate (77 g/day for adults)[34], C is the metal concentration in samples (μg/g-ww), and WAB is the average body weight (60 kg for adults).

Carcinogenic health risk assessment

TR over a lifetime was used to assess the carcinogenic health concerns associated with fish consumption in this study. TR was calculated using Equation (2)[35]:

$$TR = \frac{EF \times ED \times FIR \times C \times CSF_0}{WAB \times TA} \times 0.001 \tag{2}$$

where EF is the exposure frequency (365 days per year), ED is the exposure duration (60 years for Bangladesh), FIR is the fish ingestion rate, C is the metal concentration in fish, WAB is the average body weight, TA is the exposure time (365 days per year × ED), and CSFo is the oral carcinogenic slope factor for As and Pb, cosidered as 1.5 $(mg/kg\text{-}d)^{-1}$ and 0.0085 $(mg/kg\text{-}d)^{-1}$, respectively[36].

Statistical analysis

Concentrations of trace elements, expressed as mg/kg-ww, are presented in the form of mean, standard deviation and range (minimum-maximum). Overall mean concentrations of trace elements were calculated as the average of the three organs. The dataset was normally distributed (evaluated by P-P plot). Variations of trace element concentrations among the three fish organs (muscle, liver, and gill) were tested by a one-way ANOVA analysis followed by Tukey's HSD post hoc test. Statistical analyses were carried out using SPSS (Version 25.0, IBM Corp., NY, USA) and the level of significance was $P < 0.05$.

RESULTS AND DISCUSSION

Trace elements concentration in muscle, liver and gills of Hilsa shad

The concentrations of seven trace elements detected in muscle, liver, and gills of Hilsa shad are presented in Table 1. The trace element concentrations in three organs of the Hilsa shad were detected in a wide range. The trace element concentrations were found in three organs of Hilsa shad in the following ranges: Cr (0.06-0.17 mg/kg), Ni (0.08-0.72 mg/kg), Cu (0.52-1.1 mg/kg), Zn (7.99-17.49 mg/kg), As (2.25-5.59 mg/kg), Cd (0.05-0.17 mg/kg), and Pb (0.10-0.31 mg/kg) in muscle; Cr (0.08-0.32 mg/kg), Ni (0.17-2.01 mg/kg), Cu (2.15-17.16 mg/kg), Zn (23.12-43.40 mg/kg), As (1.72-4.44 mg/kg), Cd (0.35-1.43 mg/kg), and Pb (0.16-0.34 mg/kg) in liver; and Cr (0.05-0.10 mg/kg), Ni (0.19-1.71 mg/kg), Cu (0.84-1.30 mg/kg), Zn (27.66-47.90 mg/kg), As (2.32-4.07 mg/kg), Cd (0.02-0.08 mg/kg), and Pb (0.58-1.03 mg/kg) in gills. The mean concentration of the metals examined in Hilsa decreased in the order of: Zn > As > Cu > Ni > Pb > Cr > Cd in muscle; Zn > Cu > As > Cd > Ni > Pb > Cr in liver; and Zn > As > Cu > Ni > Pb > Cd > Cr in gills. When total metal concentration was taken into account, the investigated metals in Hilsa followed a decreasing order of: Zn > As > Cu > Ni > Pb > Cd > Cr. The highest mean concentration of most metals, except As and Zn, was found in the liver, while muscle showed the lowest concentration except for As [Table 1]. Our results corroborate the previous findings[41,42]. The toxic elements accumulated in the liver at high concentrations, resulting from the liver's higher accumulating ability[41,43,44]. Many scientists believe that fish liver is the most accurate environmental indicator for measuring both water pollution and chronic trace element intake[45,46]. Fish muscle tissue, on the other hand, is not as active in binding metals. As a result, compared to other tissues and organs, the accumulation of metals in muscle is minimal[3,47].

Among the three organs, gills had the highest mean content of Zn (35.21 mg/kg) and the lowest concentration of Cd (0.05 mg/kg). Moreover, a significant concentration of As (3.5 mg/kg) was also observed in gills, which was higher than that of the liver (2.8 mg/kg) [Table 1]. Metal contamination in the surrounding water environment is often reflected in the gills of fish[48]. Gills come into close contact with suspended particulates in water, and, as a result, different metals from the aqueous environment may be absorbed. The elevated metal concentrations in gills could be linked to trace element pollution in the living

Table 1. The concentrations (mg/kg; ww) of trace elements in the three organs (muscle, liver, and gills) of Hilsa shad (n = 21) and different national and international FSG values

Fish organs		**Concentrations of trace elements (mg/kg; ww)**						
		As	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Muscle	Mean	4.05^{a}	0.09^{a}	$0.12^{a,b}$	0.77^{a}	0.26^{a}	0.2^{a}	10.64^{a}
	SD	1.18	0.04	0.03	0.21	0.22	0.08	3.2
	Range	2.25-5.59	0.05-0.17	0.06-0.17	0.52-1.1	0.08-0.72	0.10-0.31	7.99-17.49
Liver	Mean	2.83^{b}	0.84^{b}	0.18^{a}	6.17^{b}	0.55^{a}	0.23^{a}	30.16^{b}
	SD	0.97	0.44	0.09	5.11	0.65	0.06	6.75
	Range	1.72-4.44	0.35-1.43	0.08-0.32	2.15-17.16	0.17-2.01	0.16-0.34	23.12-43.40
Gills	Mean	3.45^{a}	0.05^{a}	0.08^{b}	1.06^{a}	0.51^{a}	0.78^{b}	35.21^{b}
	SD	0.6	0.02	0.02	0.15	0.53	0.21	7.27
	Range	2.32-4.07	0.02-0.08	0.05-0.10	0.84-1.30	0.19-1.71	0.58-1.03	27.66-47.90
Overall mean		3.4	0.3	0.1	2.7	0.4	0.4	25.3
FSG		2[37]	0.05[38]	1[39]	20[38]	0.5[40]	0.3[38]	50[38]

Horizontally, ^{a,b}denote significant difference at $P < 0.05$ among the different organs of Hilsa shad for each metal. SD: Standard deviation; ww: wet weight; FSG: food safety guideline.

environment of fish. Furthermore, metal absorption onto the gill surface can affect the overall metal levels in gills[29,45]. In addition, relatively higher concentrations of metals in the liver and gills of fish have also been reported previously[49,50].

The maximum concentration (5.59 mg/kg-ww) of As was detected in muscle, while the lowest concentration (1.72 mg/kg-ww) was found in the liver [Table 1]. The mean concentration of As in muscle, liver, and gills exceeded the food safety guideline values [Table 1]. Delgado-Andrade *et al.*[51] (2003) reported the total As in fish muscle ranged from 0.39 to 12.58 mg/kg from southeast Spain. Arsenic concentrations in Bangladeshi freshwater fish species have been recorded in the range of 1.01-15.2 mg/kg-dw in earlier studies[52]. The maximum arsenic concentration level permitted for fish muscle is 2.0 mg/kg-ww according to the New Zealand food safety standard[37]. Chronic exposure to arsenic can cause dermatitis, mild skin pigmentation keratosis, reduced nerve movement velocity, and lung cancer. Arsenic can be found in two forms in fish, inorganic and organic. Organic As, while present in large concentrations in fish, is far less hazardous than inorganic arsenic and can be eliminated in urine efficiently and quickly without transformation[53]. Inorganic As, on the other hand, is thought to be carcinogenic in fish consumed by humans[54].

Cadmium is an extremely hazardous element that occurs naturally in soil but is also distributed throughout the environment as a result of human activity. Even at low quantities, it could be one of the most poisonous trace elements[55] and could be particularly harmful to fish genetic material[7]. Cadmium accumulates in various organs and has a significant potential for bioconcentration in fish. The highest Cd content was found in the liver 1.43 mg/kg-ww and the lowest concentration was found in gills 0.02 mg/kg-ww. Other fish species had a similar Cd distribution pattern (highest levels in the liver, lowest in muscle)[45,56]. Cadmium levels in fish muscle were found to be in the range of 0.51-0.73 mg/kg-dw in samples from the Dhaleshwari River in Bangladesh[57]. A maximum Cd level of 0.05 mg/kg in fish muscle is set by European Community regulation[38].

The liver had the greatest concentration of Cr (0.32 mg/kg-ww), while gills had the lowest concentration (0.05 mg/kg-ww). Cr levels in edible fish muscles from Bangladesh ranged from 0.47 to 2.07 mg/kg-dw[58], and Cr (4.64 mg/kg) was found in *Mystus bleekeri* of Dhaleshwari River[32]. Matasin *et al.*[59] (2011) reported similar Cr concentrations in edible *Silurius glanis* tissue (0.23 mg/kg).

Copper is necessary for good health, but excessive amounts can cause issues such as liver and kidney damage[60]. Cu concentrations in the liver were the highest (17.16 mg/kg-ww), while the lowest (0.52 mg/kg-ww) were found in muscle [Table 1]. Copper concentrations in fish muscle from the Dhaleshwari River, Bangladesh, have previously been found to be in the range of 5.17-9.45 mg/kg[57]. Copper levels in fish muscle from the Northeast Atlantic have been recorded in the range of 0.11-0.97 mg/kg and 0.04-5.43 mg/kg-ww in the Iskenderun Bay, Turkey[61,62]. The concentration of Cu in this investigation was below acceptable levels when compared to international guidelines [Table 1].

Nickel is found in the environment at extremely low quantities; however, it can cause a number of respiratory health problems, including lung inflammation, fibrosis, emphysema, and malignancies[63]. Among the metals, the highest Ni concentration was recorded in the liver (2.01 mg/kg-ww), which exceeded the WHO recommended food safety guideline (0.5 mg/kg-ww), while the lowest concentration was observed in muscle (0.08 mg/kg-ww)[40] [Table 1]. The highest concentration of Ni in the liver of the present study differed from previous findings[64,65]. Nickel concentrations in fish have been recorded in the range of 0.11-12.88 mg/kg-dw for Iskenderun Bay fish species[62].

Lead is a non-essential and toxic element which may induce neurotoxicity, toxicity in the kidney, and a variety of other health problems[66]. Gills had the highest Pb content (1.03 mg/kg-ww), exceeding the European Community limit of 0.3 mg/kg, while muscle had the lowest (0.2 mg/kg-ww). Pb levels in fish species have been previously reported as 0.09-6.95 mg/kg-dw in Iskenderun Bay, Turkey[62]. Our findings are consistent with the findings of Storelli *et al.*[64] (2006), who found that gills had the greatest amounts of Pb. Furthermore, the previous study found that Pb concentration in the range of 1.76-10.27 mg/kg in various edible Bangladeshi fish[58]. Gills were found to contain levels of lead above detection thresholds, which is consistent with earlier results that gills were the primary site of metal accumulation[29,65]. Gills serve as a direct metal absorption site from water[64].

The highest mean concentration of Zn (35.21 mg/kg-ww) was detected in gills, while the lowest concentration (10.64 mg/kg-ww) was observed in muscle [Table 1]. The greatest Zn concentrations in fish gills have been reported in several earlier investigations[45,56]. Gills help with a variety of physiological functions, including as osmoregulation and gas exchange. The exchange of trace elements between fish and their aquatic environment influences fish gills[67]. Furthermore, the elevated Zn content might be linked to the outflow of various fish processing plants as well as from fish and shrimp hatcheries where zinc oxide (ZnO) is typically used to provide oxygen to fish and shrimp larvae. Certain edible fish in Bangladesh had Zn levels ranging from 42.8 to 418 mg/kg[58], and the concentration of Zn in different fish of the Black Sea in Turkey ranged from 38.8 to 93.4 mg/kg[68].

Health risk assessment

The EDI and the oral reference dose (RfD) of detected trace elements from Hilsa consumption among Bangladeshi coastal adults are shown in Table 2. Human exposure to trace elements can take various forms, including inhalation and skin contact. Food consumption is frequently considered as one of the most significant exposure routes. With the exception of As, the average EDI of other metals in fish were below their respective RfD, indicating that regular Hilsa fish intake would not pose a health concern. However, dietary As intakes (4.4 µg/kg-bw/day) from Hilsa fish consumption surpassed the RfD (0.3 µg/kg-bw/day), potentially resulting in As-induced adverse consequences in human health.

Table 2. TR of trace elements due to the consumption of Hilsa fish collected from the Bangladeshi coast

Metals	Overall mean concentration (μg/g-ww)	Estimated daily intake (EDI) (μg/kg-bw/day)	RfD (μg/kg-bw/day)	TR
As	3.4	4.4	0.3	6.63×10^{-4}
Cd	0.3	0.4	1	
Cr	0.1	0.2	3	
Cu	2.7	3.4	40	
Ni	0.4	0.6	20	
Pb	0.4	0.5	3.5	4.42×10^{-6}
Zn	25.3	32.5	300	

TR: Target cancer risk; RfD: reference dose.

Table 2 shows the TR of As and Pb resulting from Hilsa fish intake. The TR values for As (assuming 10% inorganic As in marine fish)[51] and Pb from sea food consumption were 6.63×10^{-4} and 4.42×10^{-6}, respectively. In general, cancer risks of less than 10^{-6} are regarded as negligible, cancer risks of more than 10^{-4} are unacceptable, and risks of 10^{-6} to 10^{-4} are within the acceptable range[35,36]. Pb showed a carcinogenic risk that was near the permissible limit, whereas As had a risk that was in the unacceptable range. As a result, the possible health risk posed by metal exposure from Hilsa fish intake should not be overlooked.

CONCLUSION

The purpose of this study was to investigate the distribution pattern of some trace elements in various organs of commercially significant Hilsa fish and to assess the potential health risk associated with the consumption of this fish. The results of this investigation indicated a significant degree of differential accumulation of the investigated trace elements in three distinct tissues of Hilsa shad. The higher concentration of detected trace elements was found in the liver, while muscle exhibited the lowest amounts of analyzed metals. Except for Zn and As, which surpassed international quality requirements, trace element quantities in the tissue were below permissible limits for human consumption. The EDI and TR values indicated a high likelihood of carcinogenic and non-carcinogenic health consequences from consuming Hilsa fish. To reduce the negative consequences induced from As bioaccumulation, excessive consumption of Hilsa fish should be avoided. Where urgent control and more thorough investigations are needed, this circumstance should not be overlooked. Our findings suggest that further research is urgently needed to draw a conclusion about the trace element contamination in Hilsa shad of Bangladesh.

DECLARATIONS

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Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Raknuzzaman M, Habibullah-Al-Mamun M, Hossain A

Performed data acquisition, as well as provided administrative, technical, and material support: Tokumura M, Masunaga S

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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