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Health hazardous index based trace metals and essential acids analysis of size-dependent market available Hilsa fish, Bangladesh: Experimental and chemometric approaches

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ABSTRACT

With priority given to various-sized samples of market-available Hilsa (*Tenualosa ilisha*), human health consequences of trace metals along with total essential acids, including the fatty acid (FA) and amino acid (AA) profile were measured and compared to different size groups (G I, G II, and G III) using chemometric approaches. Essential amino acids were lower than nonessential amino acids. The G III contained the highest (97.55%) saturated and unsaturated fatty acids. The highest concentrated metal was found in G1 among the groups and the order of metal (mg/kg) was Zn (205.01) > Mn (37.37) > Fe (69.39) > Cu (1.47) > Cr (1.31) > Ni (0.42) > Pb (0.017) > Cd (0.005). Even though the adult group showed no health hazards for Hilsa consumption, non-carcinogenic risks have been identified for G1 fish consumption by children. Continued monitoring is recommended to overcome the health consequences caused by fish consumption.

1. Introduction

An increase in trace metal pollution in marine and riverine ecosystems has been highlighted as a significant environmental concern for fish as a result of frantically industrial and agricultural expansion (Yi and Zhang, 2012). Trace metals are high-density, nonbiodegradable metals and metalloids with long-term adverse impacts that accumulate in the aquatic environment and are transferred to the aquatic biota via multiple pathways (Hossain et al., 2024; Parvin et al., 2019). The natural metabolism of fish may absorb trace elements from nutrition, water, and/or sediment and transmit them to humans through the food web through bioaccumulation and biomagnification, endangering humans as fish comprise a substantial component of our daily diets (Gao et al., 2021). Transmission of trace metals follows the order: industry-topsoil-catchment-plankton-fish-human (Karmaker et al., 2024). Although some metals, such as Pb, Cd, and Hg, are extremely hazardous to human health even at low concentrations, others, such as Cu, Zn, Fe, Ni, and Mn, are beneficial to humans in small amounts (Islam et al., 2023).

Trace metal accumulation in fish tissue can be influenced by metal concentrations, exposure periods, and environmental conditions (Liu et al., 2011; Yi et al., 2011). Fish size also influences sensitivity to metal buildup (Yi and Zhang, 2012). As a consequence, recognizing the association between fish size and metal concentrations is crucial to determining metal content in their habitats and evaluating human-induced pollution risks (Muiruri et al., 2013; Raknuzzaman et al., 2016). Furthermore, whenever dealing with complicated datasets, to generate relevant results and interpretations, chemometric techniques are used. The utilization of multivariate chemometric techniques, specifically principal component analysis (PCA), and cluster analysis (CA), is increasingly being applied in environmental investigations, including trace metal assessment and monitoring (Chabukdhara and Nema, 2012).

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Hilsa, *Tenualosa ilisha*, a Clupeidae family member, is a significant fish in the Indo-Pacific zone, particularly bordering nations in the Bay of Bengal (De et al., 2019; Rahman et al., 2018). Bangladesh, featuring Hilsa as its national fish, accounts for 50 %–60 % of all *Tenualosa* sp. extracted throughout the world; then comes Myanmar (20–25 %), India (15–20 %), and the rest of the nations (5–10 %) (Parvin et al., 2023a). It is in high demand and has a significant economic value in West Bengal and Bangladesh because of its taste, flavors, and culinary characteristics (De et al., 2019). The lengthy chain of ω-3 (PUFAs) found in *Tenualosa* sp. has several positive effects on health (Hossain et al., 2014). Hilsa exhibits higher amounts of high-density lipoprotein (HDL) and lower amounts of low-density lipoprotein (LDL) (De et al., 2019). *Tenualosa* sp. is also a good source of minerals that are essential for growth, fertilization, and energy metabolism.

Hilsa fish available on the market come from almost all major riverine ecosystems in Bangladesh, as well as associated estuaries along the Bay of Bengal (Parvin et al., 2023a) Bangladesh, like many other countries, is dealing with a severe issue of marine and freshwater pollution as a result of the indiscriminate disposal of untreated wastewater from industrial operations, municipal waste discharge, and the use of phosphate fertilizers in agricultural fields adjacent to rivers (Hossain et al., 2024). Cement factories, textiles, batteries and plastic industries, oil refineries, phosphate loading activities, and other industries deposit their effluent into sea or river systems. On the one hand, commercially significant fish catches are steadily diminishing due to pollution and other anthropogenic activities, while on the other hand, the quality of fish is increasingly degraded by trace metal contamination (Chatta et al., 2016).

Majumdar and Basu (2010) and De et al. (2019) evaluated the mineral content of *Tenualosa* sp. tissues. The trace metal concentration in *T. ilisha* from the Gangetic Delta and coastal West Bengal (India), the Ganga basin, and the Indian Bay of Bengal was studied by certain researchers (Karmaker et al., 2024). Earlier studies on the nutritional makeup of Hilsa focused on larger groups and its health advantages. However, no systematic data is available on the essential acids, metal accumulation, and human health risks of market-available *Tenualosa* sp. in different-sized groups in Bangladesh. Importantly, specifically in Bangladesh and India, Hilsa is not only a commercially important species but also an inevitable component of the two neighboring countries' cultures (e.g., Eid, puja, Bengali New Year's Eve) (Jahan et al., 2017); a rigorous investigation of mineral content, essential acids, metal accumulation, and the influence of hazard index-based trace metals on human health must be urgently required. As a result, the present study was the first of its kind (concerning specific *Tenualosa* sp., essential acids, and trace metals) to satisfy the knowledge gap. As a consequence of this, the current investigation has been conducted to fulfil the following goals: (1) to assess the essential acid and micronutrients levels; (2) to estimate the concentration levels of eight trace metals (Cr, Mn, Fe, Ni, Cu, Zn, Pb and Cd) within various size groups (G I - G III); (3) to investigate how nutrient requirement sand metal accumulation can be affected by different body sizes; (4) to use chemometric approaches to evaluate the relationship between trace metals and their probable origins; and (5) to estimate the potential non-carcinogenic and carcinogenic threats to human health.

2. Materials and methods

2.1. Study area and sample collection

A total of 60 fish samples ranging from 400 g to >1000 g were purchased from three distinct wholesale markets in Dhaka city, namely: Newmarket, Karwan Bazar, and Mirpur-1 in August–December 2023. Based on their body weight, the fish samples were separated into three groups: 400–600 g (Group I), 700–900 g (Group II), and >1000 g (Group III) to evaluate the disparity in essential acid percentage and trace metal accumulation with the advancement of growth. After collection, the fish specimens were immediately placed in a hermetically sealed insulating container and brought to the laboratory.

2.2. Sample preparation

The collected fish specimens were thoroughly cleansed using both tap water and distilled water for the removal of any clinging pollutants. The total, standard, tail, head, and fork lengths as well as the body weight of the Hilsa were recorded and assembled group-wise, regardless of the collecting site. The inedible components (intestines, entrails, and skeleton) were extracted after the slicing of the fish specimens using a knife. The samples of muscle were homogenized into an extremely thin mesh using an electric food grinder and then preserved in a deep fridge (−18 °C) until analysis. Nutritional analysis was performed on representative samples in accordance with the various methodologies. All applicable rules and regulations were followed during specimen handling and analysis.

2.2.1. Extraction of lipid

The overall lipid content in the fish specimens was assessed using the Folch et al. (1957) technique, with certain modifications. After homogenizing the fish samples, approximately 10.0 g was added to a glass stopper conical flask. The flask was filled with a 50.0 mL mixture of 2:1 (v/v) chloroform and methanol, shaken vigorously, and left overnight. Following filtration through Whatman no. 1 filter paper, the residue was further immersed in a 50.0 mL solution of chloroform-methanol. The extracts were mixed after three repetitions of the process. A rotating evaporator set to 45 °C was used to concentrate the extract. After dissolving the concentrated extract in 50.0 mL of petroleum ether (40–60 °C), filter paper was used to filter the mixture. Then, it was dried by evaporation and put in the fridge so that it could be studied further.

2.3. Reagent

A 30 % H_2O_2 solution and a 68 % concentrated HNO_3 (guaranteed reagent) were provided by Sigma-Aldrich. Supleco was the source of Multi-Element Standard Solution XIII. For ensuring that the techniques used for analysis were accurate, certified reference materials (SRM2976, NIST) were applied. The blank quantity of metal contents was proven to be absent from the target metals, and the remaining reagents were of analytical quality.

2.4. Sample analysis

2.4.1. Proximate analysis

The proximate composition, including moisture (930.15), ash (942.05), protein (954.01), and fat (920.39C), was determined according to the procedures established by the Standard Association of Official Analytical Chemists (AOAC, 2000). Percentages (g/100 g) on a dry basis are used to express all the data.

2.4.2. Amino acid analysis

The muscles of the Hilsa shad were used to conduct amino acid assessments. After the grinding process, which involved using a pestle and mortar to obtain a fine powder, the materials were subjected to further crushing using 6 N HCl. Subsequently, the substance underwent filtration, and the resulting liquid was subjected to a heating process lasting 22 h at a temperature of 110 °C. The resultant solution was transferred to an evaporating vessel and exposed to evaporation in a water bath to remove the HCl. Afterwards, the solution was passed through filter paper (Whatman No. 41) and collected in a 25-ml volumetric flask. It was then mixed with 0.1 N hydrochloric acid to achieve dilution (Haque et al., 2017). The fluid was evaluated using an amino acid analyzer manufactured by Shimadzu in Japan. The analyzer exhibited two curves: one depicting the standard response and the other illustrating the known solution. The calculation of amino acids involved a comparison of the areas of the two curves.

$$\%\text{of Amino Acid} = \frac{\text{Area of Sample Solution}}{\text{Area of Standard Solution}} * \text{Concentration of Amino Acid}$$

2.4.3. *Fatty acid evaluation*

2.4.3.1. Fatty acid methyl ester (FAME) preparation. The corresponding methyl esters of fatty acids (FA) were used to calculate the relative amount of FA in fish oil samples. Fatty acid methyl ester (FAME) was produced using AOAC Official Procedure 991.39 with specific modifications. A glass tube containing about 25 mg (±1 mg) of fish oil was combined with 3.50 mL of methanolic NaOH. The tube was then covered with nitrogen and heated at 100 °C in a water bath for 5 min. After the tube was allowed to attain the temperature of the surrounding environment, a volume of 1.0 mL of boron trifluoride with a concentration of 14 % was added. Subsequently, the tube was blanketed with nitrogen and subjected to a temperature of 100 °C for a duration of 30 min. Once the tube was cooled to a temperature of 30 °C, 2.0 mL of isooctane was introduced and violently agitated for a duration of 30 s. A 5.0 mL solution of NaCl that was saturated was placed into the tube, and the top portion of the solution was passed through a column containing anhydrous Na_2SO_4. A vial was used to collect the filtrate, and 1.0 μL of the filtrate was inserted into a GC for FAME analysis (Ichihara and Fukubayashi, 2010).

2.4.3.2. FAME analysis in the gas chromatograph. The FA composition was examined using a gas chromatograph (Trace 1300 GC, Thermo Scientific, USA) equipped with an FID and a fused silica capillary column (TR-FAME, 0.25 m, 0.25 mm inner diameter, 30 m length, Thermo Scientific, PA, USA). The split injection approach, with a ratio of 20:1, involved setting a constant flow rate of 1.0 mL/min using nitrogen as the carrier gas. The oven was first set to a temperature of 150 °C and kept at that temperature for 5 min, while the injector was set to 250 °C. The temperature increased at a rate of 5 °C per min, reaching 200 °C and remaining at that level for 5 min. Subsequently, the temperature was increased to 240 °C at a rate of 10 °C per min and maintained for 5 min. The Supelco 37 Component FAME mix from the USA, which consists of methyl ester standards for fatty acids, was used to identify the fatty acids. The results were then shown as relative percentages by the automatic GC software (Chromeleon, version 7.00).

2.4.4. *Metal analysis*

From the three groups of fish samples, portions of muscle tissue weighing 5–10 g were randomly chosen for analysis, and the tissues were allowed to dry in a furnace heated to 70–73 °C until they reached a constant weight. The materials were then dried in desiccators to remove any remaining moisture before digestion. The samples were digested following the instructions of Mostafiz et al. (2020). A digital electrical balance was employed to accurately weigh 0.50 g of oven dried Hilsa shad samples, then 10.0 ml of 68 % concentrated HNO_3 acid and 5.0 ml of 70 % concentrated $HClO_4$ acid were mixed in that order. Digestion was continued over a hotplate (between 200 and 250 °C) until the solutions became transparent. After being diluted with distilled water, with a maximum volume of 50 ml, the solutions were filtered through 0.45-mm acid-resistant filter paper. The minerals Cr, Ni, Cu, Pb, and Cd were quantified with a NexION® 2000 ICP Mass Spectrometer manufactured by PerkinElmer in the United States. Shimadzu AAS-7000, Japan, a flame atomic absorption spectrophotometer, was utilized to assess minerals like Ca, Mg, Fe, Mn, and Zn. Phosphorus was quantified through a spectrophotometer with a UV–Vis wavelength, whereas potassium was determined with a flame photometer.

2.5. *Assurance/quality control (QA/QC)*

Reagent blanks, duplicate samples, and validated reference materials were utilized at random for the measurement of every digestion batch to ensure quality assurance and control (QA/QC) were detailed explained in supplementary information (Standard procedure for high quality data output, Tables S1–S3) The observed high sample recovery of 95 % to 103 % complied with the standard of sample recovery of 95 % to 104 % (Shorna et al., 2021).

2.6. *Chemometric investigation of measured results*

The data sets were subjected to univariate analysis such as standard deviation, mean, correlation and one-way ANOVA (with post hoc Tukey's test) analysis. Multivariate analysis performed on the data includes principal component analysis (PCA). The IBM SPSS (Version 25.0) was employed to analyze the data, and the threshold of significance was $p < 0.05$. Microsoft Excel 2013 was used to create the graphs. The study employed Pearson correlation analysis to establish the relationship between trace metal concentrations in the fish muscles.

2.7. *Human health risk assessment*

The risk of trace metal concentrations in Hilsa fish was evaluated applying the target hazard quotient (THQ) and target cancer risk (TR) methodologies. The muscle is taken into account in the risk assessment because those who live in the area exclusively consume the muscle of fish.

2.7.1. *Estimation of non-carcinogenic and carcinogenic risk*

The THQ evaluated the health risks of non-carcinogenic associated with fish consumption for each metal, and the hazard index (HI) was calculated by combining the THQ values of every metal involved. The THQ assumes a threshold of exposure (known as RfD) at which even sensitive groups won't likely suffer detrimental health effects. On the contrary, HI represents the combined risk associated with all metals. Target risks (TR) for carcinogens were computed as the increased possibility of contracting cancer throughout an individual's lifetime due to exposure to that potential carcinogen (USEPA, 1989).

Eqs. (1), (2), and (3) were utilized to estimate the values of THQ, HI, and TR, respectively. Details of the parameters are presented in Table S4.

$$\text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{Cf} \times \text{CM}}{\text{WAB} \times \text{ATn} \times \text{RfD}} \times 0.001 \tag{1}$$

$$\begin{aligned} HI = {} & \text{THQ (for Pb)} + \text{THQ (for Cd)} + \text{THQ (for Cr)} + \text{THQ (for Ni)} \\ & + \text{THQ (for Fe)} + \text{THQ (for Mn)} + \text{THQ (for Zn)} + \text{THQ (for Cu)} \end{aligned} \tag{2}$$

$$\text{TR} = \frac{\text{EF} \times \text{ED} \times \text{FIR} \times \text{CF} \times \text{CM} \times \text{CPSo}}{\text{WAB} \times \text{ATc}} \times 0.001 \tag{3}$$

$\text{THQ} < 1$ indicates no noncarcinogenic risk; $\text{THQ} \geq 1$ signifies a health risk requiring precautionary measures (Salam et al., 2021; USEPA, 1989).

As per the USEPA guidelines, the acceptable value of TR varies from 10^{-6} to 10^{-4}.

3. Results and discussion

The feeding habits of fish influence their body composition (De et al., 2019). Hilsa, an omnivore, consumes phytoplankton as well as zooplankton. Fatty acid, protein, lipid, and amino acid content of fish muscle are the nutrients that are most commonly influenced by feeding habits, feed type, and feed abundance (Saito et al., 1999). Fish body composition, specifically moisture, fat, and crude protein content, can also be affected by feed quality and rate of feeding (De et al., 2019). The higher concentration of lipids and DHA in fish flesh is likely due to the higher concentration of these nutrients in their meals (De et al., 2011; Powell et al., 2010).

Humans predominantly consume muscle from larger fish species (Ahmed et al., 2016). Furthermore, Minerals, polyunsaturated fatty acids (PUFAs), and a balanced supply of animal protein are all found in fish. As a result, fish is a great source of these ambiguous nutrients for an appropriate diet (Galimberti et al., 2016; Parvin et al., 2023a, 2023b). Muscles from Hilsa are approved for nutritional analysis as Bangladeshi people prefer to consume the muscle.

3.1. Proximate composition

The proximate composition of Hilsa sizes (Fig. 1a and Table S5) demonstrated that the moisture content was greater in smaller-scale groups compared to larger-scale groups. Crude protein quantity (%) peaked in group I (59.45 ± 5.05) and gradually decreased as fish weight rose. Total fat (%) was substantially lesser ($P < 0.05$) in group I and increased with fish weight. Group I had the least amount of fat (13.29 ± 1.01), and Group III had the most (30.49 ± 2.04). In the current study, the crude protein content of small size Hilsa groups was higher, indicating that small fish have higher dietary requirements due to their higher metabolic activity. The Hilsa's fat percentage fluctuates throughout migration. The fat content of the fish increases as they go from the marine habitat to brackish water, and thereafter decreases progressively as they enter freshwater habitats. Hilsa acquires fat in marine and brackish water habitats before migrating to freshwater upstream and spending it on spawning (Rao et al., 2012). The present investigation demonstrated a progressive increase in fat content proportional to the fish's size as it grew. A previous study found that Hilsa (848.9 ± 86.8) from the Bay of Bengal had a crude protein content of 50.31 %, which is consistent with the current results (Hossain et al., 2014).

3.2. Assessment of minerals

Four minerals have been detected in three different sizes of Hilsa shad, and the concentrations of these minerals varied (Fig. 1b and Table S8). The order of average metal concentrations in fish samples is Ca > P > K > Mg, with Ca being the most abundant metal.

Calcium content in Hilsa fish samples ranged from 11.41 % to 9.34 % (Fig. 1b and Table S8). These findings are consistent with previous reports on fish and seafood (Parvin et al., 2023b). As anticipated, the species that regularly consume bones and incorporate them in their edible parts exhibited significantly enhanced calcium levels. The amount of P, Mn, Fe, and Zn in Hilsa are interfered by the high calcium content. Fig. 2a and Table S8 also show that the ranges for magnesium (0.04–0.14 %) and potassium (0.41–0.69 %) were quite similar to those reported for various seafood and fish in various geographical regions (Parvin et al., 2023b).

Among minerals, potassium and magnesium content were substantially greater ($P < 0.05$) in group I with respect to other groups (Table S8). Mineral concentration was greater in smaller groups than in larger groups, demonstrating that Hilsa fry requires more minerals in their diet. The study indicated greater amounts of calcium and smaller amounts of magnesium in every size group of Hilsa compared to the previous findings for fish from rivers and oceans (Rao et al., 2012.

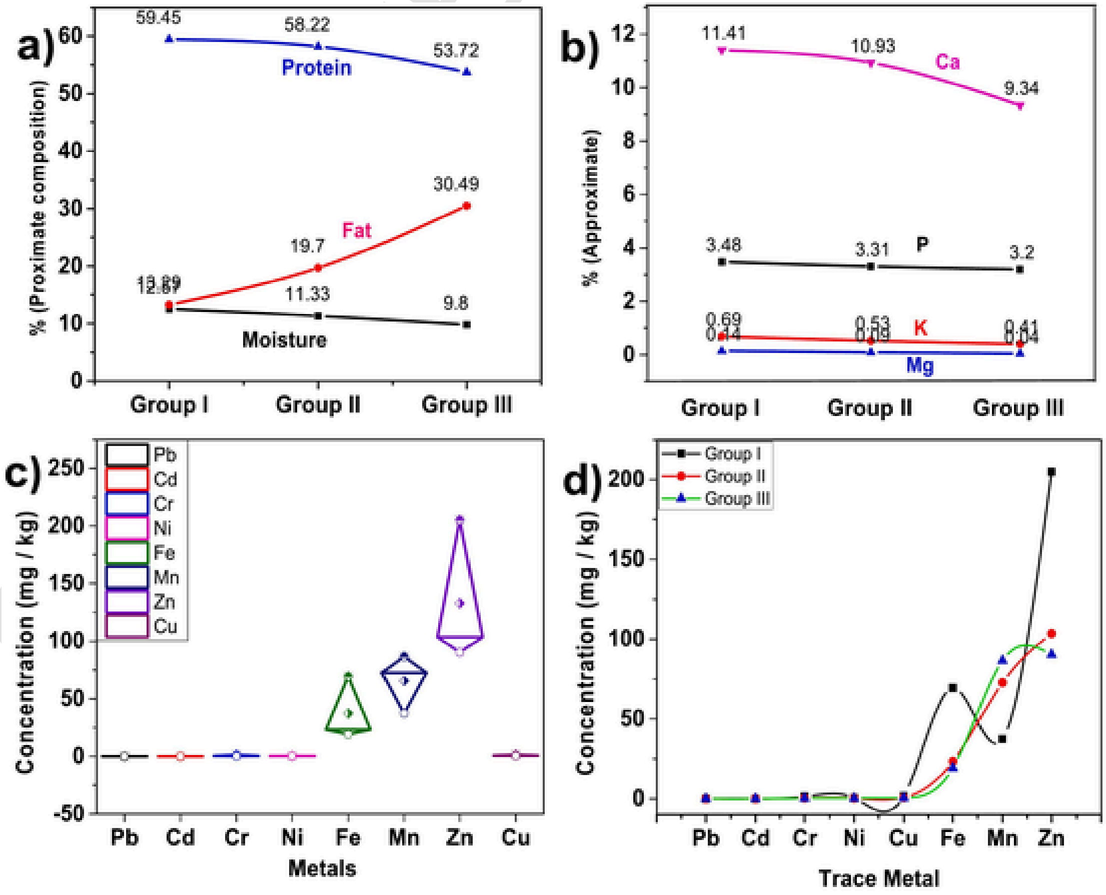


Fig. 1. a) Proximate composition, b) Essential minerals c) Trace metals content in average size group and d) Trace metals content in different size groups of Hilsa fish.

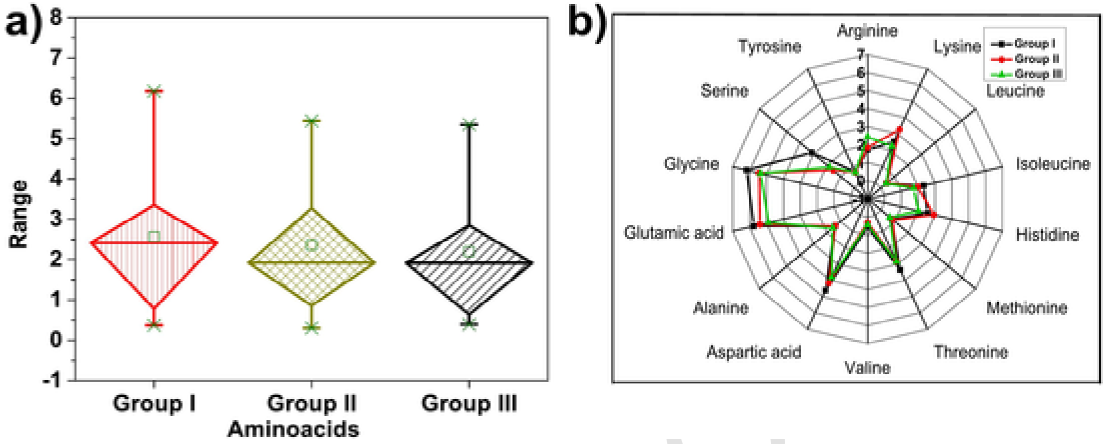


Fig. 2. a) Average amino acid in Group I, Group II, and Group-III of Hilsa fish, and b) Radar graph showing amino acid profile in different size groups of Hilsa fish.

3.3. Concentration of trace metals in Hilsa fish

Table 1 depicts the average concentrations and standard deviations (SD) of trace metals in Hilsa shad fish from three distinct markets in Dhaka, Bangladesh. Fig. 1c and d illustrates bifunctional and hazardous trace elements in average -sized and various market-available-sized Hilsa fish, respectively. The fish species showed average trace metal concentrations (measured in mg/kg) in the following order: Zn > Mn > Fe > Cu > Cr > Ni > Pb > Cd (Fig. 1c).

The Cr, Fe, and Zn contents were all noticeably ($P < 0.05$) greater in group I than in the other groups (Table 1). Mn concentration was, however, considerably ($P < 0.05$) greater in group III compared to the other groups.

The Zn concentration (205.01 mg/kg) was highest in group I, followed by group II of Hilsa shad. Group III had the lowest Zn concentration of 90.32 mg/kg (Table 2). In this study, however, Cd levels were reported to be 0.005 mg/kg in group I (the highest) and 0.003 mg/kg in group III (the lowest) of Hilsa shad (Table 1 and Fig. 1d). The Zn con-

Table 1
Concentration (Mean ± SD) of trace metals (mg/kg dry weight) in the muscle of the different size groups of *T. ilisha*. *$P < 0.05$; Here, SD indicates the standard deviation; Means followed by different superscripts, differ significantly from each other.

Size class				Trace Metal content (mg/kg) in Hilsa							
				Pb	Cd	Cr*	Ni	Fe*	Mn*	Zn*	Cu
Group I (400–600 g)	Mean	455 g	31 cm	0.017^a	0.005^a	1.31^a	0.42^a	69.39^a	37.37^c	205.01^a	1.47^a
	±SD	3.21	3.13	0.011	0.001	0.21	0.11	3.10	4.04	3.00	1.11
Group II (700–900 g)	Mean	744.0 g	40.5 cm	0.013^a	0.004^a	0.40^b	0.32^a	23.31^b	72.69^b	103.47^b	0.73^a
	±SD	35	3.84	0.002	0.002	0.05	0.06	2.05	3.20	3.30	0.20
Group III (>1000 g)	Mean	1103 g	47 cm	0.009^a	0.003^a	0.23^b	0.27^a	19.21^b	86.56^a	90.32^c	0.51^a
	±SD	56	3.21	0.001	0.0006	0.10	0.11	4.04	1.10	3.01	0.04
*F.S.G.				1.15 (0.3)§	0.20 (0.05)§	3.84 (1.00)‡	1.92 (0.50)†	n/a	n/a	192.31 (50.00)§	76.92 (20.00)§

F.S.G.: food safety guideline; § (EC, 1881/2006), ‡ (JECFA, 2003), † (WHO, 1989)* The guidelines presented in wet wt. were converted into dry wt., assuming an average of 74 % water present in tissues, and wet wt. were shown in parenthesis (Huss, 1995).

Table 2
Comparison of fish muscle in this study with other studies.

Aquatic species	Sites	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Rainbow trout	Iran	0.024	0.141	5.45	3.87	1.57	0.095	0.277	5.24	(Fallah et al., 2011)
Atlantic salmon	Chile	0.47	–	2.6	26.07	5.07		0.088	39.3	(Medeiros et al., 2014)
Red mullet	Black Sea, Turkey	0.25	–	1.14	29.4	7.51	0.85	0.37	19.81	(Durmus et al., 2018)
Red mullet	Mediterranean Sea, Spain	0.0011		0.35	–	–	–	0.005	3.65	(Martínez-Gomez et al., 2012)
Edible fish	Pearl River Delta, China	–	0.20–0.65	–	–	–	0.44–9.75	0.03–8.62	15.2–29.5	Leung et al., 2014
Marine fish	Hugly River, India	–	3.89	–	0.82–27.35	–	–	12.4–19.96	12.13–44.77	De et al., 2010
Tigris scraper	Keban Dam	0.00085	0.76	0.73	8.0	0.78	0.9	0.0386	4.3	(Varol and Sünbül, 2018)
Blue Carb	Mediterranean Lagoons	0.03–0.08	0.05–0.13	5.38–11.7	21.1–38.2	0.15–2.98	0.24–0.45	–	13.9–20.1	Mutlu et al., 2011
Six edible fish sp.	Tigris River, Turkey	0.002–0.05	0.78–1.68	0.05–2.81	16.55–175.88	0.10–10.29	0.16–2.76	0.11–0.33	10.08–22.34	Töre et al., 2021
Tilapia fish	Bangladesh	0.013	0.47	1.26	19.0.9	1.23	0.15	0.28	22.7	Parvin et al., 2023b
Group I	Bangladesh	0.005	1.31	1.47	69.39	37.37	0.42	0.017	205.01	Present study
Group II	Bangladesh	0.004	0.40	0.73	23.31	72.69	0.32	0.013	103.47	Present study
Group III	Bangladesh	0.003	0.23	0.51	19.21	86.56	0.27	0.009	90.32	Present study

tent in group I was above the FSG whereas its content in group II and group III was below the FSG. Mussel contains 190.89 mg/kg Zn near to the Zn content in Hilsa fish.

Excessive Zn levels, approximately 50 mg/day for weeks, might impair Cu availability in the body (Powers et al., 2003). Extremely high doses of Zn hinder immunity function and high-density lipoprotein (HDL). The zinc content of Hilsa can be influenced by food habits, protein source, and other components including calcium, phosphorus, and phytic acid (FAO/WHO, 2009). The lower mean Zn levels in rainbow trout's muscle (5.24 mg/kg) from Iran has been reported (Fallah et al., 2011) (Table 2).

Lead, cadmium, chromium, nickel, iron, manganese, zinc, and copper content (mg/kg) ranged between 0.009–0.017, 0.003–0.005, 0.23–1.31, 0.27–0.42, 19.21–69.39, 37.37–86.56, 90.32–205.01, and 0.51–1.47, respectively in different size groups which was below the food safety guideline (Table 1). In addition, all selected trace metals in the present study were far below than blue crab (Table 1). Ahmed et al. (2009) suggested that the Ni, Pb, Cd, and Cr content in pearl mussels were 9.19, 24.47, 2.64, and 164.58 mg/kg, which were higher than the present study, respectively.

Lead concentration in this study ranged from 0.009 (G III) to 0.017 (G I) mg/kg dry wt. which is within the safe limit, i.e., 1.15 mg/kg dry wt., as recommended by EC (1881/2006). The Pb content in Hilsa fish is quite lower than the other sources, such as fish species in the coastal area of Bangladesh (0.28–2.52 mg/kg dry wt.; Raknuzzaman et al., 2016) and fish species from the Tigris River, Türkiye (0.44–1.32 mg/kg dry wt.; Töre et al., 2021) (Table 2).

Cadmium is a hazardous element that occurs naturally in soil but is also distributed in the environment as a result of human activity (Raknuzzaman et al., 2016). In this study, Cd levels were reported to be 0.005 mg/kg in group I (the highest) and 0.003 mg/kg in group III (the lowest) of Hilsa shad (Table 1) and did not exceed the safe limit (0.20 mg/kg dry weight) as recommended by EC, 1881/2006. Although other studies showed lower levels of Cd (Sarker et al., 2021; Chakraborty et al., 2016), *Tenualosa ilisha* from the Shatt Al-Arab River, Iraq, was enormously contaminated with Cd (6.894 mg/kg) (Al-Najare et al., 2016). Chronic exposure to Cd can affect pulmonary function and gastrointestinal irritability, reduce mineral density in bones, and induce osteoporosis, while acute exposure can cause stomach illness, nausea, muscle cramping, and other symptoms (Alipour et al., 2015).

Chromium within an acceptable range in fish species can play an important role in lipid and glucose metabolism. However, in extreme circumstances, especially higher concentrations of Cr in fish species, it can cause respiratory difficulties and damage vital organs such as the liver, lungs, and kidneys (Ustaoğlu et al., 2024). The Cr concentration in this study ranged from 0.23 (G III) to 1.31 (G I) mg/kg dry weight. The Cr level was within the safe limit (3.84 mg/kg), it was quite lower than other marine and riverine ecosystems such as the coastal area of Bangladesh (0.6–8.8 mg/kg) (Raknuzzaman et al., 2016) and the Tigris River, Türkiye (3.12–6.72 mg/kg) (Töre et al., 2021). At the Padma-Meghna river confluence, Bangladesh, *T. ilisha* had the highest amount of Cr (11.14 mg/kg) (Sarker et al., 2021).

The iron concentration of market-available *Tenualosa ilisha* from Bangladesh was found to range between 19.21 (G III) mg/kg dry weight and 69.39 (G I) mg/kg dry weight, indicating that small fish contain a higher level of iron. Thus, the studied *Tenualosa* sp. could be a great source of Fe and have several health benefits. The majority of physiological actions in the body require Fe (Acharya et al., 2022). Fe content (172.4 mg/kg) from the east coast of India (Acharya et al., 2022) was almost triple that of the present study. Mutlu et al. (2011) also showed a higher level of Fe content (84.4–152.8 mg/kg) in blue crab, *Callinectes sapidus*, from Mediterranean Lagoons. However, Parvin et al. (2023a) showed a lower level of Fe content (3.32 mg/kg) in *Tenualosa ilisha* from coastal areas of Bangladesh.

The highest nickel level in Hilsa fish muscle was detected in group I with 0.42 mg/kg dry weight, and the lowest in group III with 0.27 mg/kg dry weight. The Ni level did not exceed the safe limit (1.92 mg/kg dry weight) as recommended by WHO (1989). The Ni concentration in *Tenualosa ilisha* in this study was compared with other fish species and crustaceans and found to be considerably higher than Parvin et al., 2023a and lower than that of the other countries in the world (Töre et al., 2021; Al-Najare et al., 2016; Mutlu et al., 2011).

Mn is a structural component of various enzymes and an active ingredient in their functions. It is an indispensable component for both animals and plants, and a shortage may induce skeletal and reproductive problems in mammals (Parvin et al., 2023a). Mn concentrations in G I, G II, and G III were 37.37 ± 4.04 mg/kg, 72.69 ± 3.20 mg/kg, and 86.56 ± 1.10 mg/kg, respectively. However, the highest Mn concentration (86.56 mg/kg dry weight) was found in G III, and the lowest was observed (37.37 mg/kg dry weight) in G I. Mn levels in fish species were higher than in several other studies in Bangladesh and other countries (Table 2). Some earlier research revealed lower manganese concentrations in *Tenualosa* sp., such as Parvin et al. (2023a), who reported 3.21 mg/kg in the coastal area of Bangladesh. According to Acharya et al. (2022), *Tenualosa* sp. from the east coast of India contains 18.2 mg/kg. In other countries, *Tenualosa* sp., other fish species, and crustaceans have remarkably similar Mn concentrations (Al-Najare et al., 2016; Töre et al., 2021; Mutlu et al., 2011).

Cu exists naturally and is required for growth and metabolism in all living species, but can be hazardous at greater doses (Ustaoğlu et al., 2024). Despite being a required trace metal, excessive intake of Cu can cause severe toxicological effects (Parvin et al., 2023a). Considering all sizes, the highest quantities of Cu were measured 1.47 mg/kg in G I, followed by G II (0.73) and G III (0.51). The Cu concentration in Hilsa fish of this study was also compared to other studies and found to be considerably lower than the studies in Bangladesh and other countries in the world (Sarker et al., 2021; Raknuzzaman et al., 2016; Acharya et al., 2022; Chakraborty et al., 2016; Mutlu et al., 2011). The concentrations of Cu in all of the studied fish and crustaceans' samples were below the recommended food safety guideline of 76.92 mg/kg (EC, 1881/2006).

3.4. Analysis of amino acid content in fish

Amino acids are fundamental constituents of proteins and have a vital function in the process of protein synthesis, which is necessary for the growth and development of infants, children, and adults. While humans are incapable of synthesizing critical amino acids, they can obtain them through dietary means. The amino acid composition of fish protein is advantageous (Aubourg and Medina, 1999; Parvin et al., 2023b). Due to the similarity in amino acid composition between fish and humans, consuming fish can assist persons in obtaining the appropriate and sufficient quantities of amino acids. Isoleucine is essential for the synthesis of hemoglobin, as well as for the maintenance and control of blood sugar and energy levels. Leucine is crucial for the process of muscle protein synthesis, as well as for the body's ability to respond to stress. Glycine, an integral constituent of collagen in human skin, together with other indispensable amino acids such as alanine, synergistically form a polypeptide that facilitates the process of regeneration and repair of tissues (Witte et al., 2002). The current research reveals that Hilsa provides the required amino acids.

Fig. 2a, b and Table S6 depict the amino acid distributions of protein segments in fish fleshes. Group I muscle had significantly higher levels of isoleucine, threonine, and valine among essential amino acids (EAA) compared to other groups (Fig. 2a and Table S6), with a statistical significance of $P < 0.05$. The level of lysine, leucine, histidine, and methionine was considerably greater in group II contrasted with the other size groups ($P < 0.05$). Group III exhibited a higher arginine level (2.44 %) compared to the other size groups.

The muscles of group I included high levels of aspartic acid (4.61 %), glutamic acid (5.75 %), glycine (6.18 %), serine (3.13 %), and tyrosine (0.67 %), which are non-essential amino acids (NEAA). On the other hand, the muscles of group III had a higher abundance of alanine (1.58 %) compared to the other groups (Fig. 2b and Table S6). The levels of indispensable amino acids in Hilsa flesh were lower than the levels of total dispensable amino acids. The Hilsa muscle contains a greater amount of leucine and glycine compared to both salmon (Bekhit et al., 2009) and tiny indigenous fishes (Mohanty et al., 2014). Fifteen Glycine was the predominant non-essential amino acid in all size groups of Hilsa, with glutamic acid and aspartic acid following closely behind. In group I of Hilsa, the threonine level was the highest among all essential amino acids, followed by histidine, lysine, and isoleucine. In group II, the amino acids were arranged in the following sequence: lysine > histidine > threonine > isoleucine for Hilsa. In group III, the arrangement was threonine > arginine > lysine > histidine, with the amino acids arranged in descending order.

3.5. *Analysis of fatty acid profile in Hilsa fish*

Fatty acids, the very intricate macromolecules found in fish, suggest that they are nutritionally more accountable. In general, unsaturated fatty acids like docosapentaenoic acid are good for people's health and development, helping with things like brain activity and avoiding off acute illnesses like heart disease and inflammation (Calder, 2014; Parvin et al., 2023b). The fatty acid composition of Hilsa is affected by various internal and environmental factors, such as diets, shape, temperature, saltiness, age, and geographic position (FAO/WHO, 2009).

The FA composition of Hilsa shad is shown in Fig. 3 (a, b, c, d) and Table S7. Group II had lower levels of polyunsaturated fatty acid (PUFA) and greater levels of total saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) as compared to the other groups, according to the fatty acid analysis. In group III, the total amount of polyunsaturated fatty acids (PUFA) represented a larger percentage (21.44 %). C16 palmitic acid ($P < 0.05$) than the other SFAs was slightly higher in group II. De et al. (2019) found similar results. C12 lauric acid (0.28 %), and C20 arachidic acid (0.25 %) were higher in group III, but C22 behenic acid, C14 myristic acid, C17 heptadecanoic acid, C15 pentadecanoic acid, and C16 palmitic acid were slightly lower in group III among SFAs. In group I, the levels of C16:1 palmitoleic acid, C15:1 cis-10-pentadecenoic acid, and C17:1 heptadecenoic acid, which are all MUFAs, were considerably higher ($P < 0.05$). Conversely, the amounts of C20 cis-11-eicosenoic acid, C18 cis-9-oleic acid, and C24 nervonic acid were lower in group I fish than those in bigger size categories. C18 alpha-linolenic acid and DPA (0.78 %) were lower in group III, whereas EPA (11.18 %) and DHA (5.25 %) were considerably ($P < 0.05$) greater in group III among the PUFAs. The present study's Hilsa contained 9.15 to 21.44 % PUFA, which is almost twice as much as the previous report on coastal Hilsa (11.41 %) (Tacon, 1987). DHA levels were lower in juvenile Hilsa and increased considerably with age. However, as body weight grew, so did the overall SFA concentration. The findings revealed that early age groups have a larger DPA demand, conversely saturated and monounsaturated FA requirements increase with age. In Hilsa weighing 400–600 g, the most prevalent PUFA was EPA, followed by DHA and DPA, and this is comparable to that described before in *Sardinella lemuru*, a clupeid fish (Khoddami et al., 2009).

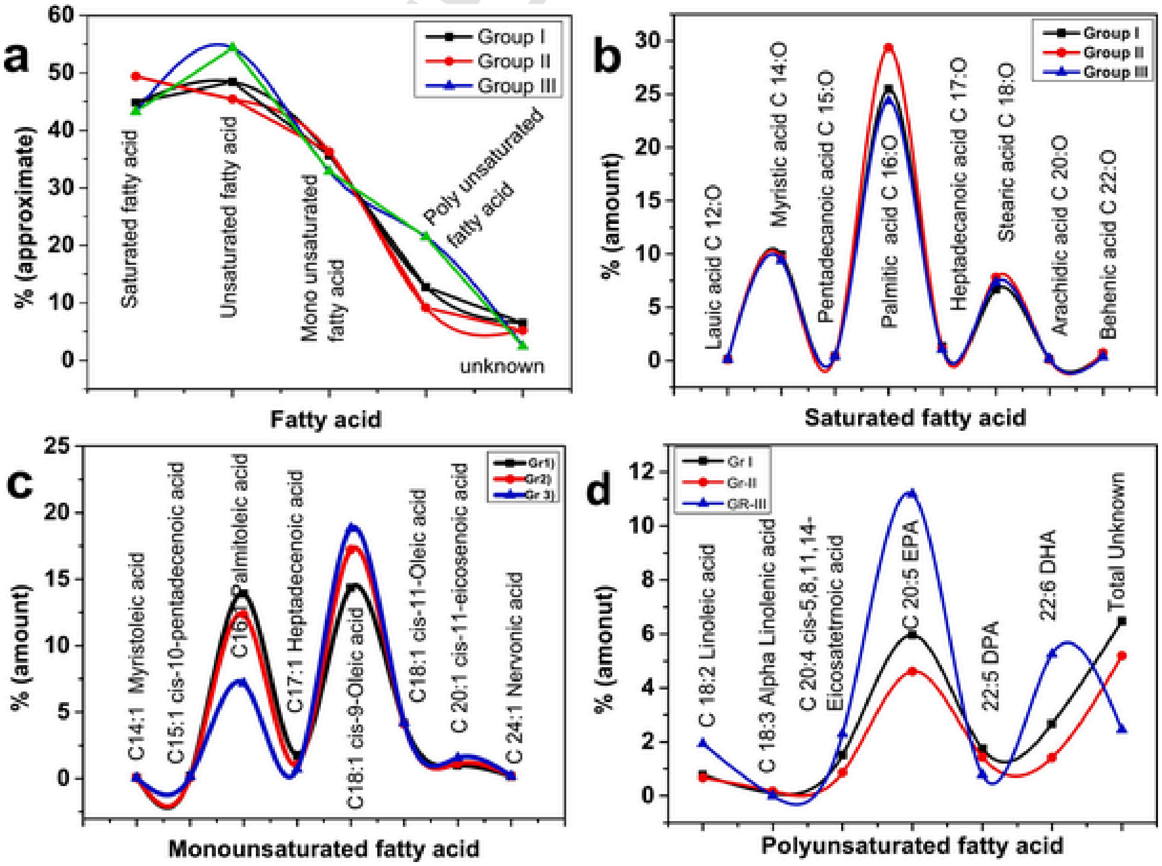


Fig. 3. Group I, Group II, and Group-III of Hilsa fish a) Fatty acid, b) saturated fatty acid, c) monounsaturated fatty acid, and d) polyunsaturated fatty acid of different size groups.

3.6. *Chemometric analysis*

3.6.1. *Pearson's correlation*

Table 3 shows Pearson's correlation of several metals in fish muscles at the 0.05 and 0.01 levels of significance.

The correlation table demonstrates how heavy metals are statistically related in correlation coefficients (r). The results of a two-tailed *t*-test are shown by an asterisk (*), and the significance level is represented by the symbol r. There is a profound and positive linear association between Zn & Fe (0.997), Fe & Cr (0.987), Cu & Pb (0.982), Zn & Cr (0.981), Ni & Cd (0.897), Cd & Pb (0.843), Ni & Pb (0.839), Cu & Ni (0.837), and Cu & Cd (0.814), which is significant at the 1 % level of significance. Some notable positive relationships were noticed between Ni & Cr (0.768), Cu & Cr (0.759), Fe & Ni (0.690), and Cr & Pb (0.666), and they are statistically significant at $p < 0.05$. However, a statistically significant negative correlation is observed between Zn and Mn (0.972) as well as Mn and Fe (0.955) at $p < 0.01$.

3.6.2. *Principal component analysis (PCA)*

The variable variance explained by the extracted components is displayed in Table S9 of communalities. For subsequent analysis, only communality values >0.5 were taken into consideration. Cr, Fe, Mn, and Zn accounted for >95 % of the deviations in comparison to other trace elements.

The total number of factors extracted is represented by the eigenvalue, which ideally matches the number of items that were subjected to factor analysis. The extracted factors are shown next, along with their corresponding eigenvalues. In order to figure out how many different factors the variables represent, eigenvalues greater than one are required. The eigenvalues of the first component (6.17) and the second component (1.42) surpass a value of 1, however, the eigenvalues of the remaining components are <1, as indicated by the results displayed in Table S10. Consequently, the specified set of eight variables represented two components. Furthermore, the first two components account for 77.13 % and 94.86 % of the variance characteristics, respectively, according to the cumulative variance percentage that is obtained from the sum of squared loadings that was extracted. Therefore, two components accurately reflected the characteristics or components that the previously described trace metals highlighted.

The scree plot is generated by displaying the eigenvalues against each component, and it is employed to ascertain the number of factors to retain. The region of interest is where the curve starts to flatten. The slope starts to flatten between components 2 and 3, as seen in Fig. 4. notably, factors with values of 3 or below has an eigenvalue that is <1. As a result, just two factors were kept.

The variable loadings on the two derived components were displayed in the rotated component matrix table (Fig. 4). Fig. 4 colored Table represent Rotated component matrix^a of trace elements present in Hilsa. Extraction method: Principal Component Analysis. Two components were extracted; Rotation Method: Varimax with Kaiser Normalization. ^{a}Rotation converged in 3 iterations.

The factor's influence on the variable is exactly proportionate to the loading value. After extracting two variables, the eight items were split into two groups according to whether or not the most important items in components 1 and 2 had comparable responses. Fig. 4 shows the relative placements of the trace elements in the first two factors.

3.7. *Human health risk assessment through Hilsa fish consumption*

Identifying, collecting, and exposing hazardous compounds, along with the connection between exposure, dosage, and detrimental impacts on the human body, are all part of the assessment of human health risks (Sobhanardakani, 2017a, 2017b). Hilsa contains excessive amounts of essential and toxic elements, which might be harmful to human health (Korashy et al., 2017; Morcillo et al., 2016). Fig. 5 represents human health risk assessment through Hilsa fish consumption. To assess the possible health risks to humans of target metals when ingesting Hilsa from various Bangladeshi markets, the THQ, HI, and TR indexes are used.

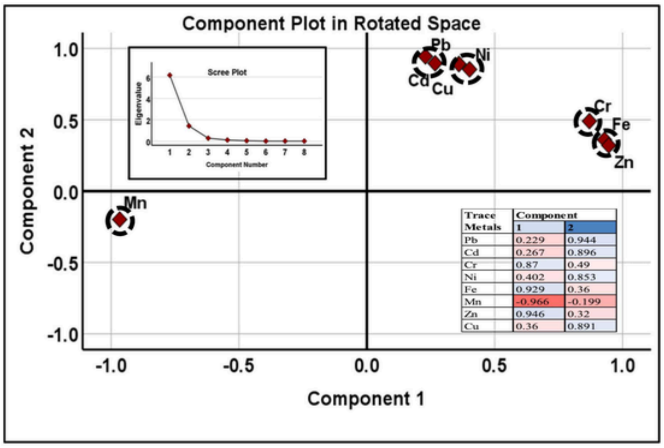
3.7.1. *Noncarcinogenic risk*

Fig. 5a, Tables S11, and S12 exhibit the THQ for each size group of *T. ilisha*. The average trace metal concentration in Hilsa was used to evaluate THQ for Bangladesh's adult and child groups in three different size groups (groups I, II, and III). The THQ values for the targeted trace metal in the group I followed the descending order: Zn > Fe > Mn > Cu > Cr > Ni > Pb > Cd in the adult group and the child group. For both adult and child groups, the THQ values for the specified trace elements in group II maintained the diminishing sequence: Zn > Mn > Fe > Cu > Cr > Ni > Pb > Cd. Finally, the THQ values in group III were in the descending order of Zn > Mn > Fe > Cu > Ni > Cr > Pb > Cd for both adult and child groups. *T. ilisha* muscles had the lowest noncarcinogenic risks for all metals in group III; nevertheless, group I has the lowest Mn. Compared to adults, children faced more than twice as many noncarcinogenic health risks. Overall, HI values for both adult and child groups followed a declining order: group I > group II > group III. Table S11 showed that the THQ value of each metal and HI of all metals were <1 in all sizes of Hilsa for the adult group, indicating that Hilsa shad consumption would not pose any substantial health hazards. However, HI value was >1 in group I (1.079) Hilsa fish for the child group, which displayed acute non-carcinogenic health threats to humans. Although group II (0.688) and group III (0.675) did not exceed the limit value (HI = 1), they also showed greater value compared to the adult group. Table S12 represents the THQ and HI of all metals for the child group. Sarker et al. (2021) estimated the THQ for all size groups of *T. ilisha* and organs. None of the metals exceeded the accepted limit (<1), except for Cr (VI), which was higher in the smallest-sized gills of *T. ilisha*. The highest THQ value of Cr in *T. ilisha* muscle was 7.268 and 1.646 for children and adults, respectively. Ustaoğlu et al. (2024) stated that the THQ of trace metals in the two fish species in the Terme River, Türkiye, did not surpass 1. Moreover, the combined metals' hazard index (HI) values in *C. gibelio*

Table 3
Pearson correlation among heavy metals present in Hilsa fish available in different local markets.

	Pb	Cd	Cr	Ni	Fe	Mn	Zn	Cu
Pb	1							
Cd	0.843**	1						
Cr	0.666*	0.657	1					
Ni	0.839**	0.897**	0.768*	1				
Fe	0.543	0.569	0.987**	0.690*	1			
Mn	−0.423	−0.447	−0.933**	−0.541	−0.955**	1		
Zn	0.517	0.542	0.981**	0.647	0.997**	−0.972**	1	
Cu	0.982**	0.814**	0.759*	0.837**	0.655	−0.521	0.629	1

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



Trace Metals	Component	
	1	2
Pb	0.229	0.944
Cd	0.267	0.896
Cr	0.87	0.49
Ni	0.402	0.853
Fe	0.929	0.36
Mn	-0.966	-0.199
Zn	0.946	0.32
Cu	0.36	0.891

Fig. 4. Principal Component Plot in Rotated Space analyzed by scree plot of the characteristic roots (eigenvalues) and component plot in rotated space of trace metals in Hilsa fish

(3.29E−02) and *S. cephalus* (3.17E−02) were below 1. The results of THQ and HI indicate that consumers should not be concerned about non-carcinogenic health hazards from consuming individual or combined elements in fish species.

3.7.2. Total cancer risk (TR)

Fig. 5b, Tables S11, and S12 reveal the target risks (TR) of Pb due to exposure from Hilsa shad consumption. As shown in Table S11, the TR of Pb for the adult group was G I (9.20×10^{-9}) > G II (7.01×10^{-9}) > G III (4.87×10^{-9}). Also, for the child group (Table S12), the TR values of Pb were G I (4.3×10^{-8}) > G II (3.27×10^{-8}) > G III (2.27×10^{-8}). It is notable that for children, all hilsa group values were higher than those for adults. Cancer risks $>10^{-4}$ are regarded as undesirable; cancer risks below 10^{-6} are regarded as negligible; and the risks between 10^{-6} and 10^{-4} are generally accepted as legitimate (Raknuzzaman et al., 2022. Pb showed negligible cancer risk for both adult and child groups. The present study found that the estimated TR values were in the tolerable risk range (10^{-6} to 10^{-4}). At the end of the day, this study suggests that there may not be a cancer risk associated with hilsa consumption from the market. Karmaker et al. (2024) supported this finding, stating that no carcinogenic risk was determined for consumers, with the exception of children's larger gills. However, Raknuzzaman et al. (2022) studied Hilsa shad and discovered that the TR value for Pb was 4.42×10^{-6}, near the permissible limit. High lead levels can cause significant damage to the brain, liver, and kidneys, eventually leading to death. In men, high-level exposure can damage the organs that produce sperm (Raknuzzaman et al., 2016). In pregnant women, lead may cause miscarriage. The THQ and CR data observed in *T. ilisha* and other fish species did not indicate any potential risks to human health in the upper Meghna River (Sarker et al., 2020), the Meghna estuary (Ahmed et al., 2019), or several wholesale markets in Dhaka city (Atique Ullah et al., 2019). Furthermore, Pb showed health risks when consumed by crustaceans (Raknuzzaman et al., 2016). According to Ustaoğlu et al. (2024), two edible fish species in Türkiye's Terme River pose a minimal carcinogenic risk to humans. Although the fish species were proven to be safe for human consumption, there is a risk of cancer if consumed continuously for 70 years. Therefore, the potential health risk to people from metal exposure through fish and crustacean consumption should not be underestimated.

4. Conclusion

The focus of the investigation aimed to analyze the nutrients and trace metal content of economically valuable Hilsa shad fish, as well as determine any possible health risks related to consuming them. The findings of this study demonstrated substantial differences in accumulated nutrients and trace elements among the three marketable size groups of Hilsa fish. The variation in trace metal concentrations and sizes may be species-specific, depending on habitats, food and feeding patterns, metabolic processes, and water qualities. The results also revealed that adult consumers in the investigated areas could consume *T. ilisha*, as the trace metals posed no health risks. However, due to the potential non-carcinogenic risk to children from consuming market-available Hilsa fish in Bangladesh, excessive fish consumption should be avoided to reduce the detrimental impacts of metal biomagnification. As Hilsa fish on the market come from almost all major riverine ecosystems in Bangladesh and associated estuaries along the Bay of Bengal, routine monitoring of Hilsa is required before their introduction into the market, and these results should be confirmed occasionally by conducting more detailed studies in the bay to monitor better and understand the current situation. Therefore, examining the metal bioaccumulation under laboratory conditions, extensive source-specific repeated sampling across different seasons, and preventive measures are recommended as future perspectives.

CRediT authorship contribution statement

Md Kamal Hossain: Writing – original draft, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Afroza Parvin:** Formal analysis. **Fahima Islam:** Writing – original draft, Formal analysis. **Badhan Saha:** Data curation. **Md Alamgir Kabir:** Software, Resources, Formal analysis. **Umme Fatema Shahjadee:** Resources. **Amin Hossain:** Writing – original draft, Formal analysis. **Mohammad Moniruzzaman:** Resources. **Priyanka Dey Suchi:** Software.

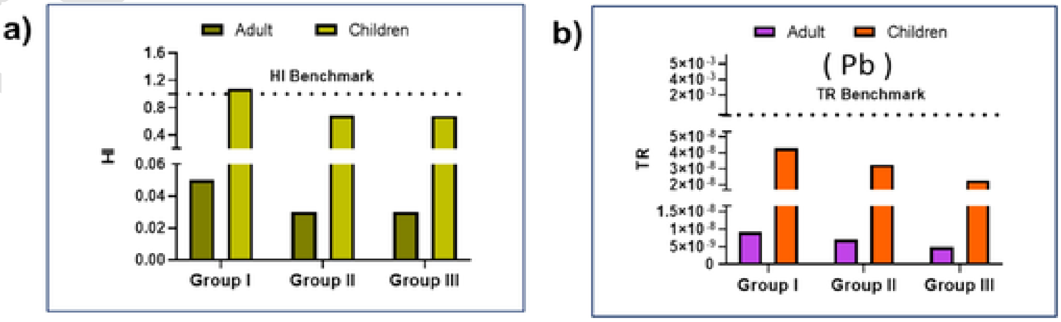


Fig. 5. Health risk (HR) for adult and child groups a) Hazard Index (HI) and b) Target cancer risk (TR) of trace metals in Hilsa fish.

Uncited references

Chemosphere, n.d
De et al., 2010

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116975.

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