

Toxicity of As, Pb, and Cr in Different Commercially Important Fishes Captured from the Bay of Bengal and Their Impacts on Human

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Abstract This study was conducted to discern levels of heavy metals and assess whether there are any significant toxic effects of the commonly exposed heavy metals, namely arsenic (As), chromium (Cr), and lead (Pb), on various organs of three commercially important marine fishes (Loitta: *Herpodon nehereus*, Rupchanda: *Pampus chinensis*, and Hilsa: *Tenualosa ilisha*) captured from Chattogram coast of the Bay of Bengal. The acquired results showed that, in contrast to the other two fish (*P. chinensis* and *T. ilisha*) under investigation, Loitta fish (*H. nehereus*) had the highest concentrations of As and Pb. However, there were substantial differences in the levels of Cr in all three species, with the greatest levels found in Rupchanda (*P. chinensis*), followed by Loitta and Hilsa (*T. ilisha*). The organ-wise accumulation for As and Pb were significantly higher in kidneys and gills, whereas the Cr concentration was the highest in gills with significant variation with the other three investigated organs (liver, kidneys, and muscles). Based on the calculated Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Carcinogenic Risk (CR), consuming the studied fish species poses no risk. In both adults and children, As had the highest EDI values, followed by Pb and Cr. The correlations among the three examined metals in each fish species were not statistically significant. However, hierarchical clustering showed that As and Pb may have come from more closely connected sources than Cr. To conclude, the heavy metal levels in the investigated fishes were within the permissible limit and found safe for human consumption.

Keywords: Bay of Bengal, heavy metals, Loitta, Rupchanda, Hilsa, Human consumption

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1. Introduction

Human activities in coastal areas and marine waters release numerous pollutants into marine ecosystems, making marine pollution a problem of global environmental concern [1]. Heavy metals (HMs), a group of metals and metalloids with a relatively higher atomic mass, are one of the factors influencing the pollution of the marine environment [2]. Due to lack of their biodegradability and capacity for bioaccumulation, they can stay in nature for an extended period and cause chronic toxicity [3,4]. The toxicity may pose a major threat to human health throughout the food chain, raising both carcinogenic and non-carcinogenic concerns [5,6]. Bioaccumulation and biomagnification of heavy metals

occur due to anthropogenic activities within an ecosystem. This could be deposited in the fish organ, and the accumulation is affected by various physiochemical and biological variables. While terrestrial species exhibit a strong pattern of biomagnification, marine and estuarine organisms show a less clear pattern. Excess accumulation of metals can be poisonous to humans leading to severe damage of living cells, and long-term exposure may even be fatal [7,8].

Fish can be a useful bioindicator for metal pollution since they are at a higher trophic level in the food chain [9,10,11]. For commercially important fishes, the hazards of this bio-toxicity are substantially larger [9,12], and the threats are mostly limited to various organs such as the gills, kidneys, muscles, and liver [13]. However, metal accumulation in these tissues varies considerably for several reasons [14]. Contamination of these HMs directly

contributes to environmental degradation by limiting the diversity of aquatic animals [15].

At very low concentrations, HMs such as arsenic (As), lead (Pb), and chromium (Cr) can be harmful to living organisms, resulting in several physiological system disruptions [16] with some carcinogenic effects on human health [17]. Additionally, the harmful effects of these metals extend to the marine biota [18], primarily on fishes and other beneficial invertebrates [19]. Due to different circumstances, the tolerances of marine organisms to HMs might vary [20], and there are substantial differences between species [21]. The differences may be attributed to various living and feeding habitats [5,22].

Hilsa (*Tenualosa ilisha*), designated as Bangladesh's Geographical Indicator (GI), is responsible for 12.23% of the nation's fish production. Along with this species, Loitta (*Harpadon nehereus*) and Rupchanda (*Pampus chinensis*) are two of the nation's most significant marine fishes [23]. However, accumulation of HMs from their estuarine habitat has been observed in these species, highlighting some non-carcinogenic arsenic (As) threats [24] despite their greater consumption rates and popularity. Thus, investigating the circumstances of metal accumulation for these species in the marine environment is crucial. Furthermore, continuous monitoring is suggested for highly consumed fish species, and checking the levels of metal accumulation at a regular interval is a prerequisite [24,25].

The sources of HMs include both anthropogenic [26] and natural [27,28]. However, anthropogenic sources of HMs are associated with the highest levels of HMs toxicity [28]. The management of industrial waste, traffic pollution, sewage discharge, building materials, tainted feeds, etc. are examples of anthropogenic sources of HMs [29,30]. On the other hand, this HMs pollution is more prevalent in metropolitan regions and more exposed to industrial locations [31]. Therefore, Chattogram, the largest port city in Bangladesh, is extremely prone to HMs toxicity due to a greater number of industries and agricultural mass production [32]. On top of that, residents of densely populated cities may experience worse effects from fish consumption and direct or indirect contact with contaminated water [33].

This study compares the accumulation of HMs in the gills, kidneys, livers, and muscles of three commercially important fish species in light of the relevance and potentially lethal effects of HMs. The goal of this study has been extended to include determining whether consuming these fishes poses harm to humans. To aid in developing future management strategies, more research is done on the mechanisms underlying the variety in HM source types and the variations in HM accumulation levels.

2. Materials and Methods

2.1. Ethics Approval

The experimental procedures for this study were carried out as per the rules and regulations of the applied chemistry and chemical technology laboratory of Chattogram Veterinary and Animal Sciences University (CVASU). The investigation considered all guidelines and policies when dealing with hazardous compounds like HMs.

2.2. Sample Collection and Preservation

From January to April 2021, samples were collected from fish captured in fishermen's nets along the Chattogram coast of the Bay of Bengal at four distinct collection points (Patenga, Halishahar, Sadarghat, and Salimpur) per month (Figure 1). From each collection points, 15 individuals of Hilsa, Loitta and Rupchanda fishes were collected resulting in a total of 60 individuals for each species. The collected fishes were then transported to the laboratory. Prior to dissection, fish samples were kept in plastic bags at -20°C. The total length (in cm) and weight (in g) of each fish were measured. The mean total length and wet weight of Loitta, Rupchanda, and Hilsa were 23.9 ± 3.1 cm, 22.7 ± 2.4 cm, 40.8 ± 3.6 cm and 485.6 ± 37.9 g, 493 ± 62.7 g and 812.6 ± 88.1 g, respectively. The fishes were then dissected, and their target organs (gills, liver, kidneys, and muscles) were separated and cleaned with distilled water. After cleaning, organs were air-dried and stored at -20°C for further analysis. Three replications were kept for each organ of the fish.

In accordance with UNEP (United Nations Environment Programme) Reference Methods [64], all the prepared samples were digested. Total weight of the sample tissue was 5g/ sample. One (1) g of each organ sample was taken and put into the conical flask. Each conical flask was filled with 30 ml of nitric acid and placed on a hot water plate for boiling. An additional 30 ml of perchloric acid was added and thoroughly mixed after the proper boiling. The mixture was then heated at 60° C until 1 ml of the mixture was left. After being taken off the hot plate, the mixture was mixed with 100ml of purified water. This final mixture was then filtered using filter paper. After digestion, As, Pb, and Cr were analyzed using an auto analyzer in a graphite furnace (GBCGF 3000 with Zeeman background corrector). Each metal was examined three times in each digested sample. Standard solutions made from commercially available materials were used to calibrate the instrument. Analytical blanks were run in the same way as the samples and determined using standard solutions prepared in the same acid matrix. The samples were prepared while wearing sterile lab coats and clean powder-free latex gloves to prevent cross-contamination. The glassware was also cleaned using distilled water and chromic acid solutions.

2.3. Human Health Risk Assessment

The following formula was used to calculate each HM's estimated daily intake (EDI) [17,34,35].

$$EDI = \frac{Cn \times IGr}{BWt}$$

where Cn is the metal concentration in different organs (mg/kg dry-wt); IGr is the acceptable ingestion rate, which is 60 g/day for adults and 52.5 g/day for children [34,36]; BWt is the body weight: 70 kg for adults and 15 kg for children [34,35].

Target hazard quotient (THQ): THQ was calculated using the EDI and oral reference dose (RfD) ratio. As, Cr, and Pb have RfDs of 0.0003, 0.003, and 0.002, respectively [34,37]. A non-significant risk effect is

implied by a ratio value of less than 1 [38]. The THQ formula is written as follows [39]:

$$THQ = \frac{Ed \times Ep \times EDI}{At \times RfD} \times 10^{-3}$$

Where Ed is exposure duration (70 years) [36]; Ep is exposure frequency (365 days/year) [40]; At is the average time for the non-carcinogenic element ($Ed \times Ep$).

Hazard index (HI): According to the formula below, the hazards index (HI) for As, Cr, and Pb was determined [36]:

$$HI = \sum_{i=k}^n THQs$$

where, THQs is the estimated risk value of metals. Consumers are regarded to be at risk of a non-carcinogenic risk effect when the HI score is more than 10. [41].

Carcinogenic risk (CR): The following formula evaluates carcinogenic risk to determine the likelihood of developing cancer [42]

$$CR = \frac{Ed \times Ep \times EDI \times CSf}{At} \times 10^{-3}$$

Where CSf is oral slope factor of specific carcinogen (mg/kg-day). Available CSf values (mg/kg-day) are: As (1.5), Cr (0.5) and Pb (0.0085). The acceptable range of the risk limit is 10^{-6} to 10^{-4} [43]. CRs higher than 10^{-4} are likely to increase the probability of carcinogenic risk effect [34].

2.4. Data Processing and Statistical Analysis

Data were analyzed and visualized using R environment (version 26). The data obtained during the present study was summarized into mean and standard deviation (Mean $\pm$ SD). Kolmogorov-Smirnov and Shapiro-Wilk tests were employed to test the collected data normality and non-normal data were square rooted before the multivariate analysis. Kruskal-Wallis test with Tukey's post-hoc comparison (ANOVA, $P < 0.05$) was employed to determine the significant difference of HMs in investigated fishes. Cluster analysis (CA) was performed by Ward's linkage method to identify the possible source of HMs. Additionally, Principal Component Analysis (PCA) is used to reduce the data set and identify common patterns among the investigated heavy metals. Spearman rank correlation (r) was used to determine a significant correlation ($P < 0.05$) among the HMs in the studied fishes.

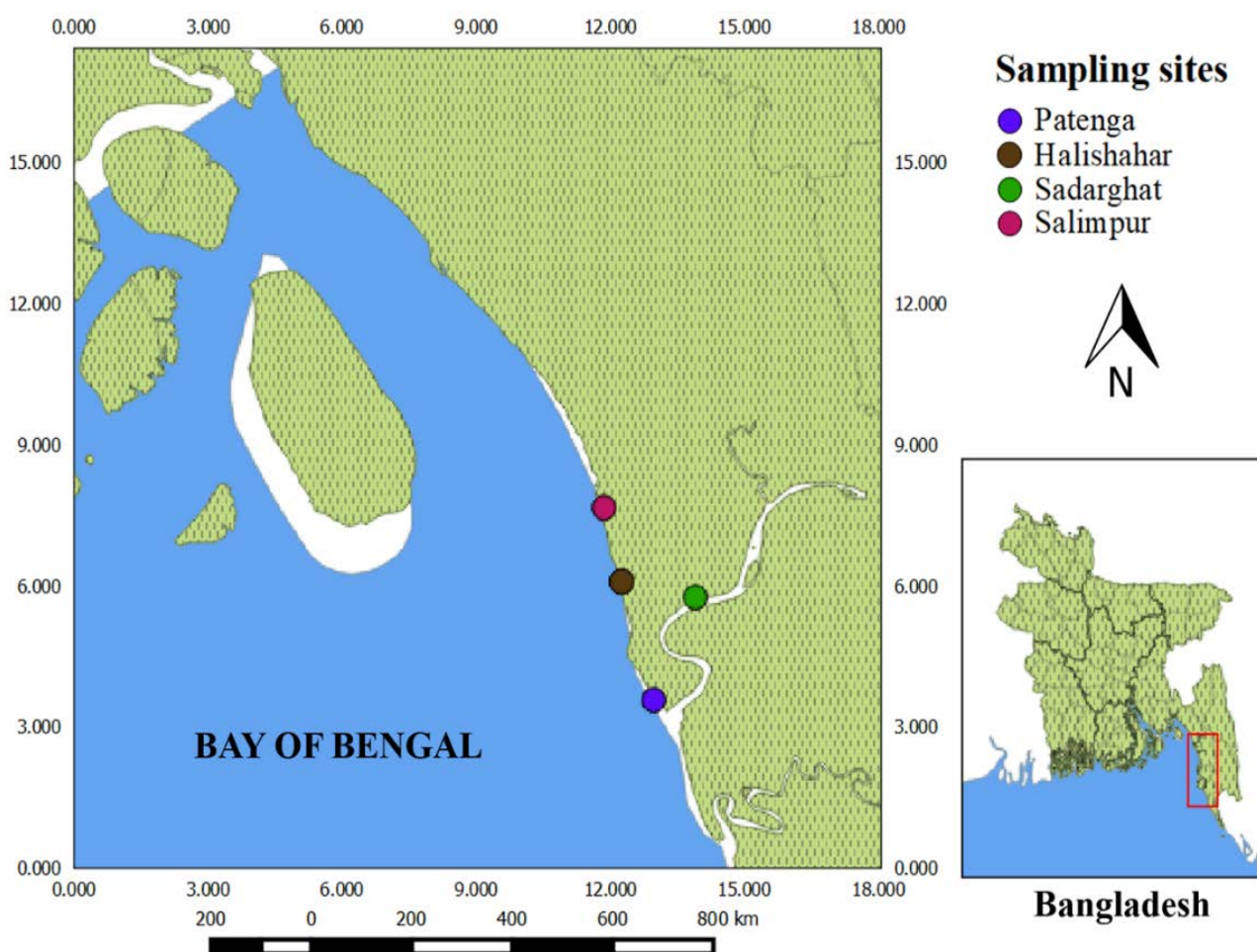


Figure 1. Location of sampling sites. Different colored circles depict different sampling location

3. Results

3.1. Arsenic (As) Concentration

The Loitta fishes had the greatest levels of arsenic (0.0493 ± 0.0137 ppm), which were significantly different from the other two fish species under study (Hilsa- 0.0321 ± 0.0112 ppm and Rupchanda- 0.0360 ± 0.0080 ppm)

($P < 0.05$). (Figure 2). The concentration of this HM is, however, most prevalent in the kidneys (0.0466 ± 0.0177 ppm) and gills (0.0391 ± 0.0218 ppm), whereas the liver (0.0168 ± 0.0081 ppm) and muscles (0.0223 ± 0.0104 ppm), respectively, have the lowest concentrations. Moreover, the concentration in kidneys and livers are significantly higher than the concentration in livers and muscles ($P < 0.05$, Figure 3).

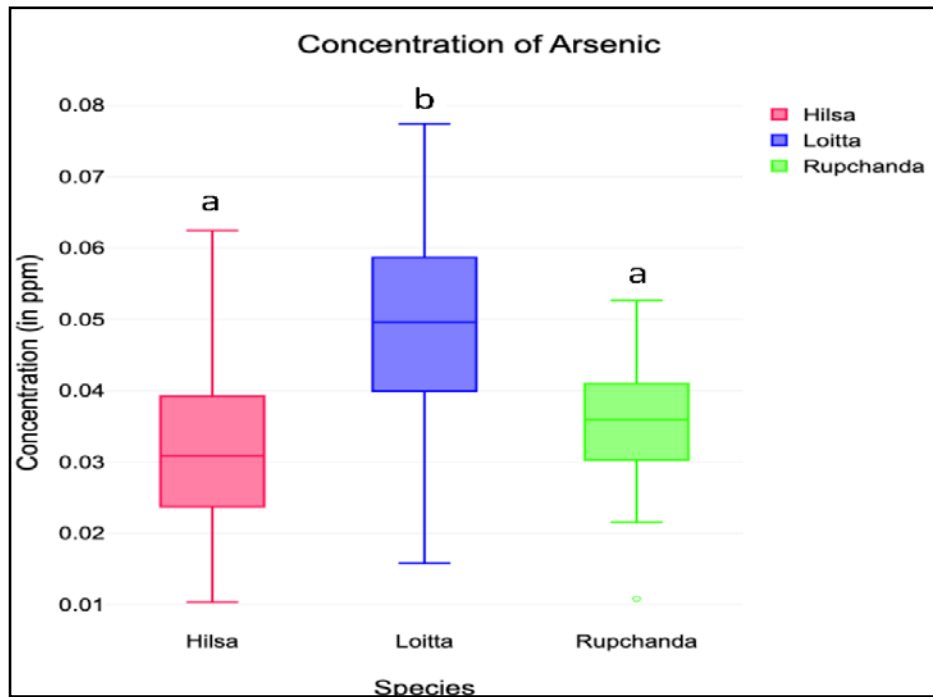


Figure 2. Species-wise Arsenic (As) concentration. Values are represented in ppm (mg/L). Values indicated by different letters are significantly different ($P < 0.05$)

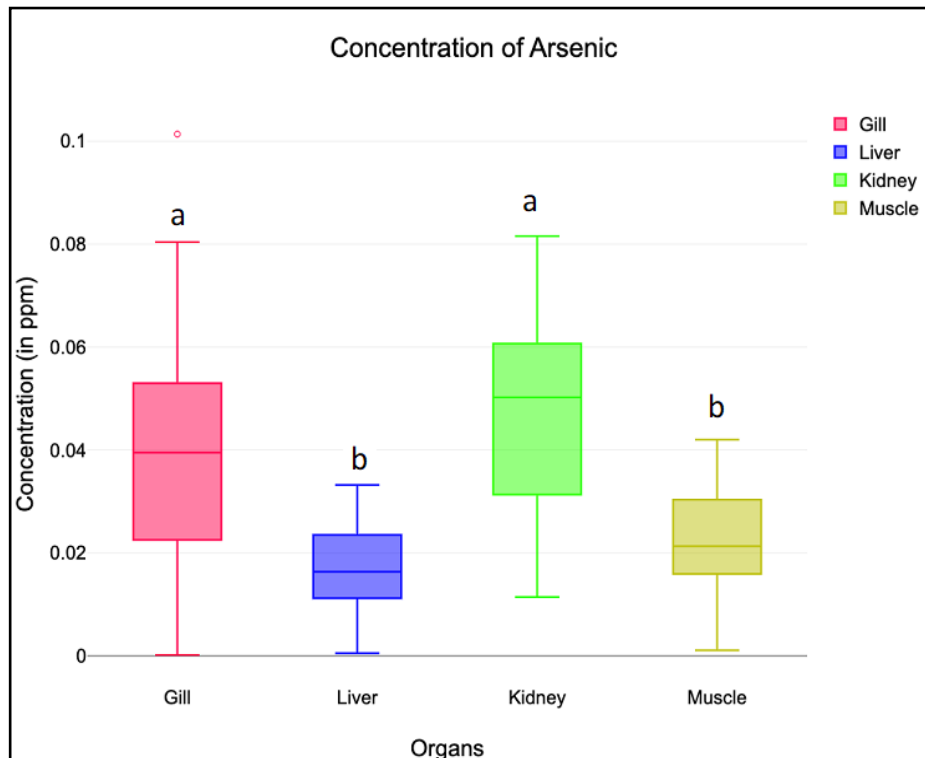


Figure 3. Organ-wise Arsenic (As) concentration. Values are represented in ppm (mg/L). Values indicated by different letters are significantly different ($P < 0.05$)

3.2. Lead (Pb) Concentration

Likewise the arsenic, lead concentration was found the highest in Loitta (0.0369 ± 0.0087 ppm) followed by Hilsa (0.0257 ± 0.0010 ppm) and Rupchanda (0.0255 ± 0.0082 ppm) (Figure 4). Here, the concentration of this metal is significantly greater ($P < 0.05$) in kidneys (0.0418 ± 0.0073 ppm) and gills (0.0383 ± 0.0090 ppm) compared to the liver (0.0135 ± 0.0059 ppm) and muscle (0.0205 ± 0.0022 ppm). The concentration among species also varied significantly ($P < 0.05$, Figure 5).

3.3. Chromium (Cr) Concentration

The recorded chromium concentration varied substantially ($P < 0.05$) among the fish species under study, with Rupchanda recording the highest value (0.0048 ± 0.0016 ppm), followed by Loitta (0.0042 ± 0.0014 ppm), and Hilsa (0.0012 ± 0.0006 ppm). (Figure 6) However, this concentration is largely found in the gills (0.0137 ± 0.0082 ppm), with much lower values observed in the other three organs under investigation ($P < 0.05$, Figure 7).

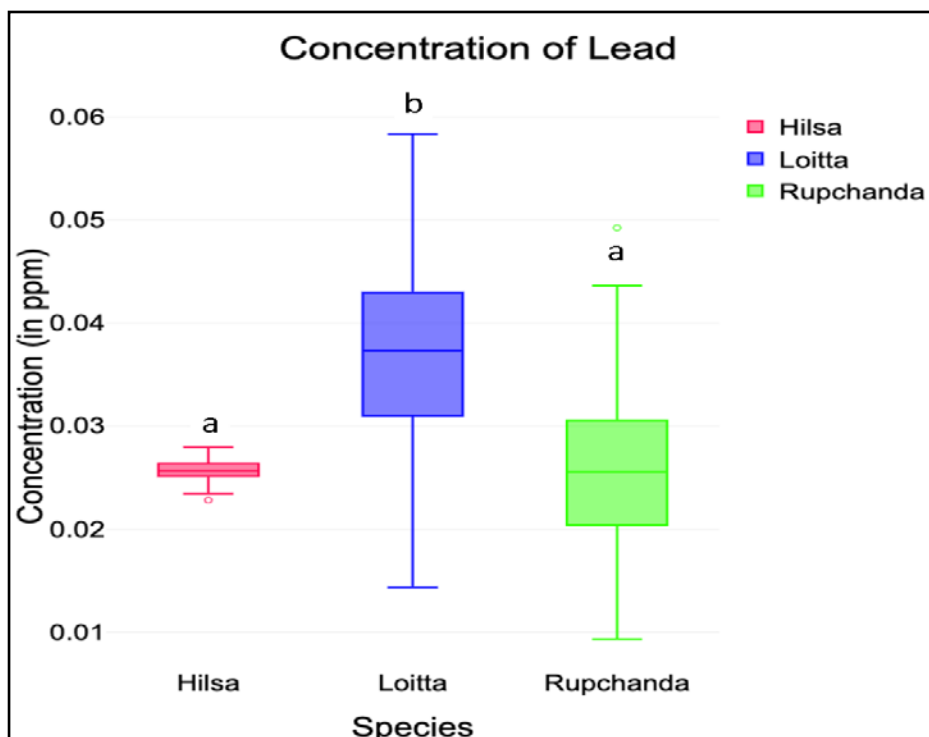


Figure 4. Species-wise Lead (Pb) concentration. Values are represented in ppm (mg/L). Values indicated by different letters are significantly different ($P < 0.05$)

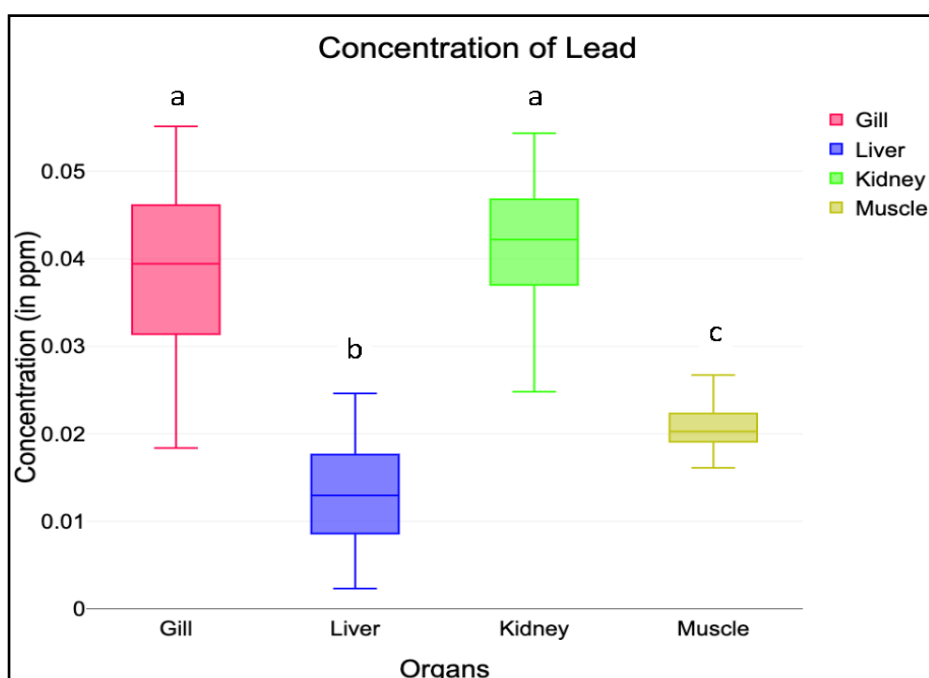


Figure 5. Organ-wise Lead (Pb) concentration. Values are represented in ppm (mg/L). Values indicated by different letters are significantly different ($P < 0.05$)

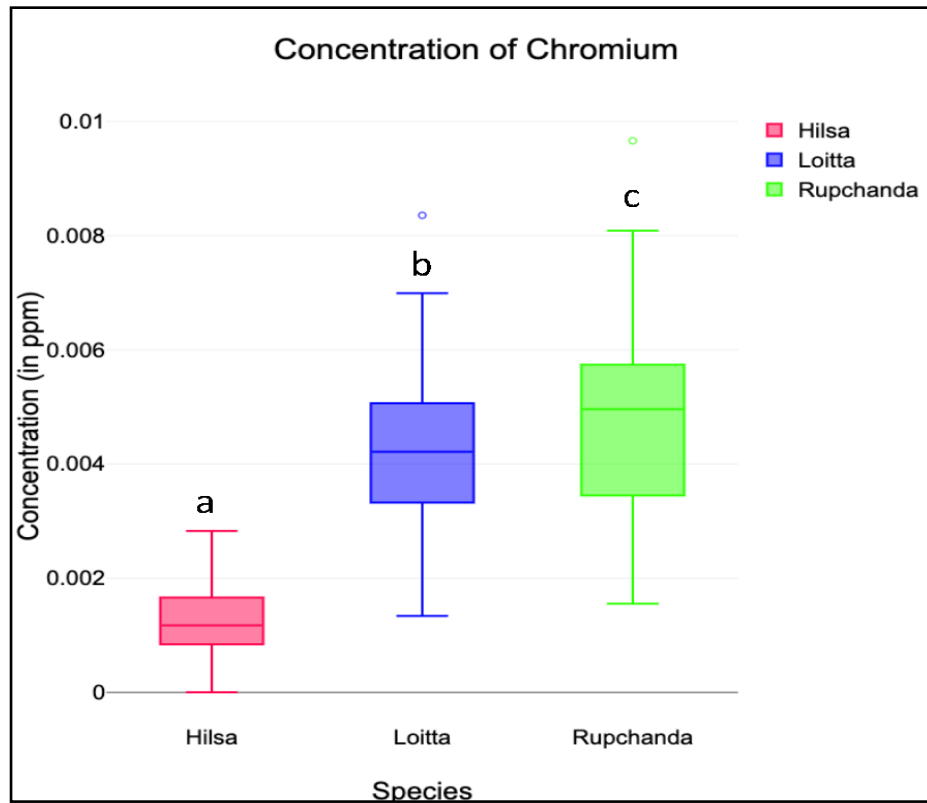


Figure 6. Species-wise Chromium (Cr) concentration. Values are represented in ppm (mg/L). Values indicated by different letters are significantly different ($P < 0.05$)

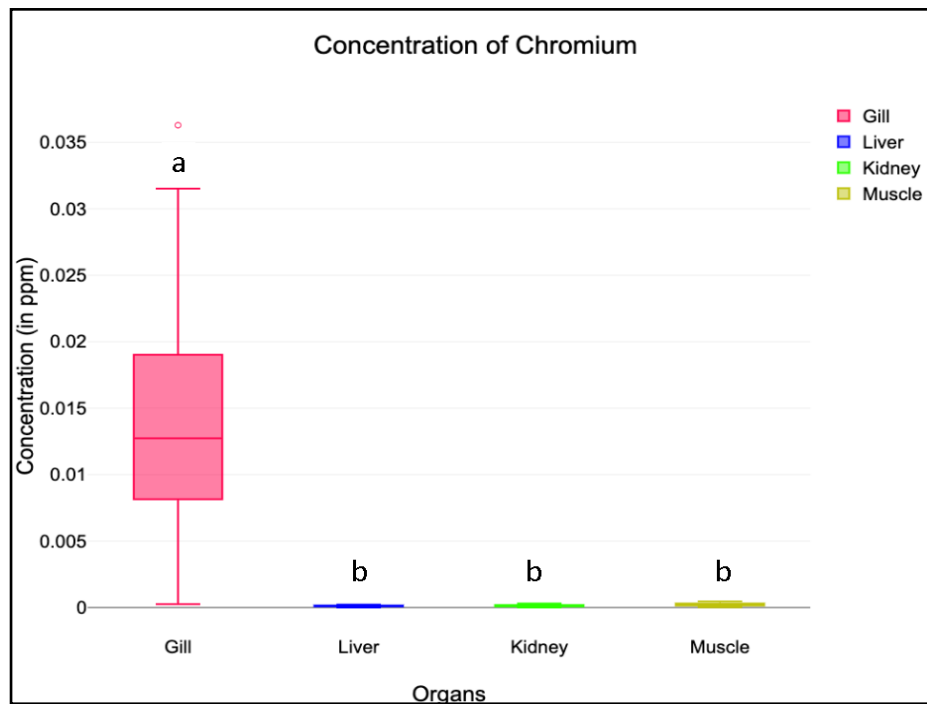


Figure 7. Organ-wise Chromium (Cr) concentration. Values are represented in ppm (mg/L). Values indicated by different letters are significantly different ($P < 0.05$)

3.4. Source Identification

The relationships between the three heavy metals under investigation are shown in Table 1. However, the correlation between them was not significant in each instance. This association shows that these metals accumulate differently in each species and are highly species-specific. However, the hierarchical clustering of

heavy metals from various collection locations reveals the formation of two separate clusters (Figure 8). In this case, As and Pb are more closely connected, which means they might originate from the same sources, while chromium might come from different sources. The principal component analysis (PCA) of investigated heavy metals likewise revealed the same result (Figure 9).

Table 1. Spearman rank correlation analysis among the metals

Tenulosa ilisha	As	Pb
Pb	0.0678 (0.6059)	
Cr	0.1260 (0.052)	0.1302 (0.3205)
Harpadon nehereus	As	Pb
Pb	0.0176 (0.8939)	
Cr	0.0155 (0.9063)	0.1175 (0.3702)
Pampus chinensis	As	Pb
Pb	0.1331 (0.3096)	
Cr	0.1797 (0.169)	0.0342 (0.7947)

** Correlation is significant at the 0.01 level (2-tailed). The value in the parenthesis indicates P value.

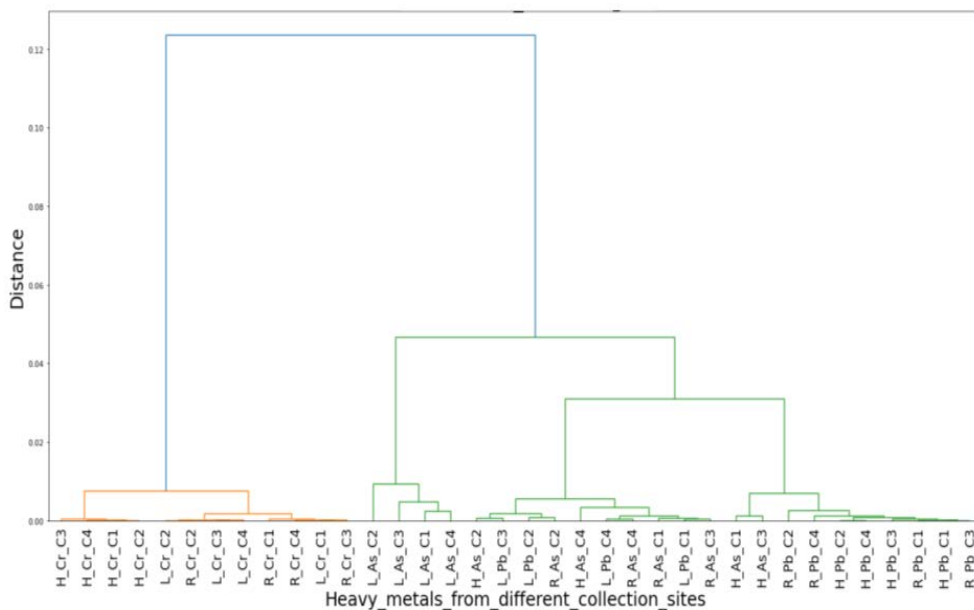
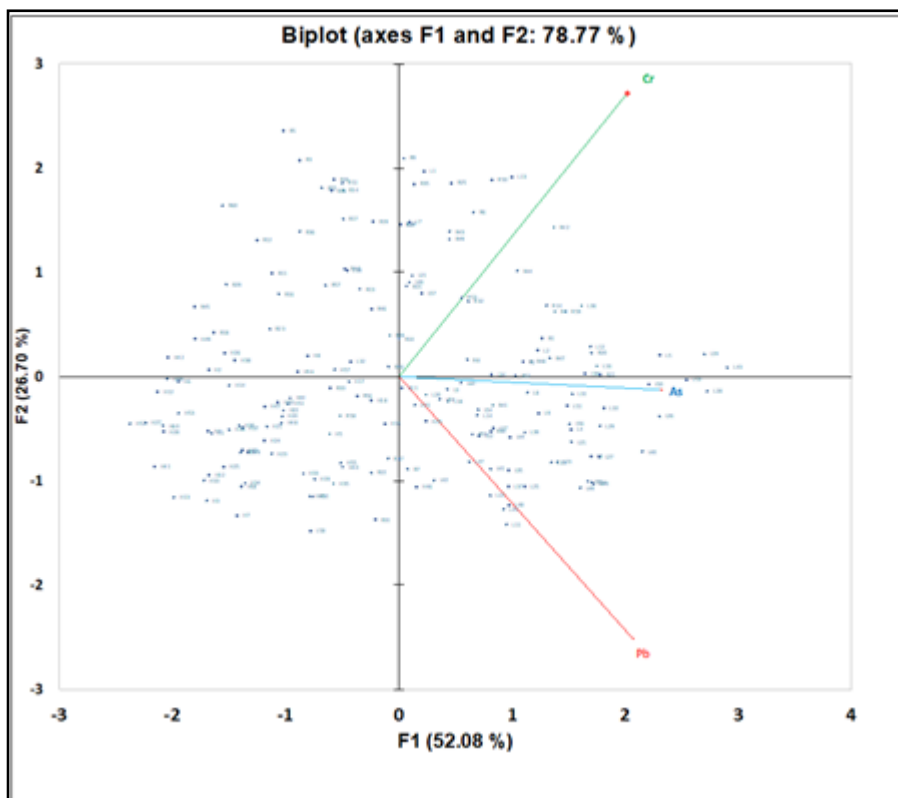
**Figure 8.** Hierarchical clustering analyses of studied heavy metals from different collection points**Figure 9.** Principal Component Analysis (PCA)-Biplot of heavy metals from different collection sites

Table 2. Estimated daily intake (EDI), non-carcinogenic (THQ), and carcinogenic (CR) risks of studied heavy metal

Fish Species	Heavy Metal	EDI (Adult)	EDI (Children)	THQ (Adult)	THQ (Children)	CR (Adult)	CR (Children)
Tenualosa ilisha	As	0.0275	0.1125	0.0918	0.3750	4.13×10^{-5}	1.69×10^{-4}
Tenualosa ilisha	Pb	0.0220	0.0899	0.0110	0.0449	1.87×10^{-7}	7.64×10^{-7}
Tenualosa ilisha	Cr	0.0010	0.0043	0.0003	0.0014	5.32×10^{-7}	2.17×10^{-6}
HI				0.1032	0.4214		
Harpadon nehereus	As	0.0422	0.1727	0.1409	0.5756	6.34×10^{-5}	2.59×10^{-4}
Harpadon nehereus	Pb	0.0316	0.1291	0.0158	0.0645	2.69×10^{-7}	1.10×10^{-6}
Harpadon nehereus	Cr	0.0036	0.0148	0.0012	0.0049	1.82×10^{-6}	7.43×10^{-6}
HI				0.1580	0.6452		
Pampus chinensis	As	0.0308	0.1259	0.1028	0.4199	4.63×10^{-5}	1.89×10^{-4}
Pampus chinensis	Pb	0.0218	0.0893	0.0109	0.0446	1.86×10^{-7}	7.60×10^{-7}
Pampus chinensis	Cr	0.0040	0.0166	0.0013	0.0055	2.04×10^{-6}	8.32×10^{-6}
HI				0.1151	0.4701		

3.5. Health Risk Assessment

Table 2 displays the estimated THQ, EDI, and carcinogenic risk of heavy metals. In adults and children, the EDI was found in the order of As>Pb>Cr in all three investigated species. As a consequence, in Hilsa, Loitta, and Rupchanda fishes, the THQ values are higher for As followed by Pb and Cr. The Hazard Index (HI) for all three investigated fishes are less than 10 for both adults and children, suggesting these fishes are safe for consumption considering these three heavy metals. The results of the CR values in adults and children indicate that there is no probable carcinogenic risk for any of the three marine fish species under study.

4. Discussion

The current study investigates the different HMs (As, Pb and Cr) levels in the three most economically important marine fishes in Bangladesh, namely Hilsa (*Tenualosa ilisha*), Loitta (*Harpadon nehereus*) and Rupchanda (*Pampus chinensis*). The liver and muscles in the current investigation exhibited reduced HM accumulation, while the gills and kidneys recorded the greatest levels. Previous research from many parts of the world found that muscles only accumulate a small quantity of heavy metal [44,45,46,47]. Nevertheless, this can be closely related to the development and growth of the species [48]. Additionally, different fish organs respond to bioaccumulation in different ways [49], and muscle is likely the organ least susceptible to metal accumulation [14]. The higher metal concentration in gills can be related to its role in purifying toxic materials by rapid diffusion [50,51]. The liver mainly accumulates essential trace elements because of its role in metabolism [52] and the toxic materials are predominantly excreted through the functioning of the kidneys [40].

The variation in metal contents in fish is mainly due to the fish species and is usually altered by the feeding habits, body temperature, metabolism, and capacity for bioaccumulation of a species. However, chromium is the least recorded HM in the current studies in all three investigated fishes, which is consistent with other findings [36,53]. Chattogram coast is nowadays widely exposed to industrialization [54] which eventually led to higher contamination of As and Pb [55]. Our study also recorded a higher concentration of As and Pb than Cr.

The sources of heavy metal are both anthropogenic and natural [56]. However, most of the pollution is caused by human activity [57]. The results of this investigation indicate that while chromium is obtained from different sources, the sources of arsenic and lead may be the same. According to past studies [58], the probable sources of As and Pb can overlap. These sources mostly include industrial and domestic waste, oil spills, agricultural chemicals, etc. [58,59]. On the contrary, the distribution and source of Cr are unique and unrelated to other metals [60], which is similar to our findings. The sample collection zone was close to the territory of the Chattogram City Corporation (CCC) area. Apart from that, popular tourist destinations include Patenga, Haulishahar, and Sadarghat. Together with the Salimpur, there were some shipbreaking yards. The possible source of the HMs pollution in the sampling zone could be shipbreaking yards, ship transportation, and industrial effluents.

The health risk assessment of heavy metals is more focused on the muscle of fish that humans consume the most and is a significant indicator of metal contamination [61]. The results of the present investigation are consistent with those of [36] in that the levels of EDI for these three heavy metals in the investigated fishes are much below the suggested limit. In addition, both the individual and total THQ are below the recommended value of 1, indicating that consuming these fish does not pose a significant risk to humans. According to 2004 recommendations from the US FDA and EU regulations, each HM's estimated cancer risk (R) for both adults and children was also lower than the risk of potential risks (1×10^{-4} to 1×10^{-6}) [7]. The results, similar to other studies [62,63] suggested that the danger of Cr may be lower than that of As and Pb. Its increased RfD values might be the cause of this [25]. However, potential non-carcinogenic hazards for arsenic, fueling the potential risks of heavy metals for non-carcinogenic concerns [24]. As a result, the findings of this study should not be interpreted as necessarily coming to the same conclusion as many other works of literature [19,37].

5. Conclusion

The levels of heavy metals in various commercially significant fish species were compared. The findings show that fish species and organs differ significantly among themselves. The Chattogram coast may have the same

sources for arsenic (As) and lead (Pb), and the examined marine fishes exhibit a high prevalence of both contaminants. However, the concentrations of investigated heavy metals are within the bounds of national and international standards. Therefore, it suggests that these fish are secure enough to consume as a significant source of protein. To keep this safety level, heavy metal discharge and dumping are advised to be continuously monitored.

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Author's Contribution

Conceptualization: SIA, MFBQ; Methodology: ZNP, MAM, SIA, SR; Laboratory work: SIA, ZNP, MAM; Data Analysis: SIA, SR, MFBQ; Writing-Original Draft Preparation: SIA, ZNP; Writing-Review and Editing: SIA, SAAN, MFBQ; Visualization: SIA, SR; Supervision: SIA, MFBQ, SAAN.

Competing Interest

The authors declare no competing interest.

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