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Heavy metal bioaccumulation in *Etroplus suratensis* residing in inland rivers and *Amblygaster sirm* in marine habitats in Sri Lanka: A comparative study

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Abstract

Inland and marine fish are primary protein sources of the Sri Lankan diet. Prolonged consumption of contaminated fish can cause critical health implications such as chronic kidney disease (CKDu). This study investigates the bioaccumulation of heavy metals in edible fish from inland rivers and coastal regions using *Etroplus suratensis* residing in inland rivers and *Amblygaster sirm* in marine habitats in Sri Lanka for the analysis. Inland fish samples were collected from Nikawewa and Mahakandarawa tanks in Kurunagala and Anuradhapura districts, respectively, while marine fish samples were collected from the coastal region near Trincomalee harbour. The analysis of heavy metal/ metalloid (i.e., Pb, Cr, As, Cd, and Ni) contents in fish samples revealed that muscle tissues of inland fish samples had significantly higher levels of Pb and Cr, exceeding the Maximum Permissible Limits (MPL). In contrast, marine fish exhibited negligible amounts of heavy metals across all tissues. Health risk assessments indicated that the Estimated Daily Intake (EDI), Total Hazard Quotient (THQ), and Carcinogenic Risk (CR) values for Pb and Cr in inland fish were significantly higher than those in marine fish, suggesting potential health risks. One-way ANOVA and Tukey's pairwise comparisons confirmed significant ($p < 0.05$) geographical and tissue-specific variations in heavy metal concentrations. Inland regions showed higher contamination levels than coastal regions. Gut tissues generally had the highest concentrations of heavy metals, followed by muscle, gill, and liver tissues. These findings underscore that the inland fish samples from the studied regions may pose adverse health effects, emphasizing the need for continuous monitoring and regulation to mitigate heavy metal contamination and safeguard human health.

Keywords: Aquatic pollution, bioaccumulation, CKDu, heavy metals, inland fish, marine fish

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Introduction

Heavy metal pollution in aquatic systems has emerged as a critical environmental issue globally and in the Sri Lankan context. Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are introduced into the environment through various anthropogenic activities such as industrial and agricultural activities (Rahman & Singh, 2019). For instance, studies have shown that industrial sewage, deep river sediments and agrochemical runoff are the most significant sources of heavy metal contamination in the Kalametiya lagoon in southern Sri Lanka (Kodikara et al., 2023). Another study observed high Pb and Cd concentrations in the Negombo lagoon and Hamilton canal in Sri Lanka (Chandrasekara et al., 2014). Similarly, pollution from landfill leachates has been noted in the Meda - Ela canal near the Karadiyana semi-controlled dumpsite in Sri Lanka, contributing to heavy metal accumulation in aquatic sediments (Koliyabandara et al., 2020). These metals tend to accumulate in the sediments due to their poor solubility in water which can disrupt the health of aquatic fauna, causing biochemical stress and affecting reproductive capabilities (Okerefor et al., 2020).

The bioaccumulation of heavy metals in fish and other aquatic organisms poses significant risks to human health, especially in communities that rely heavily on fish and shellfish collected from inland aquatic environments and oceans. Contaminated fish can transfer these metals up the food chain, affecting the immediate consumers and higher trophic levels, including humans. Chronic exposure to heavy metals through diet can lead to various health issues, including kidney damage (Lentini et al., 2017), neurological disorders (Jaishankar et al., 2014), and increased risk of chronic diseases such as CKDu (R. T. Perera et al., 2021). Prior studies indicate that exposure to heavy metals such as Cd, As, Pb might be associated with CKDu (Kulathunga et al., 2019; Wanigasuriya, 2014).

Fish are widely used in biological monitoring of heavy metals in aquatic ecosystems. They are particularly vulnerable to heavy metal exposure due to their ecological roles and consumption patterns such as diversity of their food sources, frequency and quantity of their feeding in the aquatic environment making them sensitive indicators of pollution levels (Authman, 2015; Hasimuna et al., 2022; Mehana et al., 2020). Heavy metal bioaccumulation in fish occurs primarily through bioconcentration from water and food chain transfer (Rajeshkumar & Li, 2018). Fish can accumulate heavy metals in various tissues such as muscle, gill, gut, and kidney. This information is crucial for monitoring the health of aquatic ecosystems and assessing bioaccumulation pathways (Keke et al., 2020; Mehana et al., 2020). This study evaluates the health risks linked to fish consumption by examining the levels of heavy metals and metalloids in fish from freshwater and marine environments in Sri Lanka.

Methodology

All chemicals used were analytical grade as received from Sigma-Aldrich (USA), BDH (UK) or Fluka (Switzerland). Ultrapure water was used during the experiments.

Sample collection

Fish samples were collected from two distinct inland regions and one coastal region in Sri Lanka. The inland fish samples were collected from Mahakanadarawa tank (GPS: 8.38, 80.55) in the Mihintale divisional secretariat of the Anuradhapura district and from Nikawewa tank (GPS: 7.87, 80.41) in Polpithigama divisional secretariat of the Kurunegala district in Sri Lanka. Marine fish samples were collected from the coastal region near Trincomalee harbor (Figure 1).

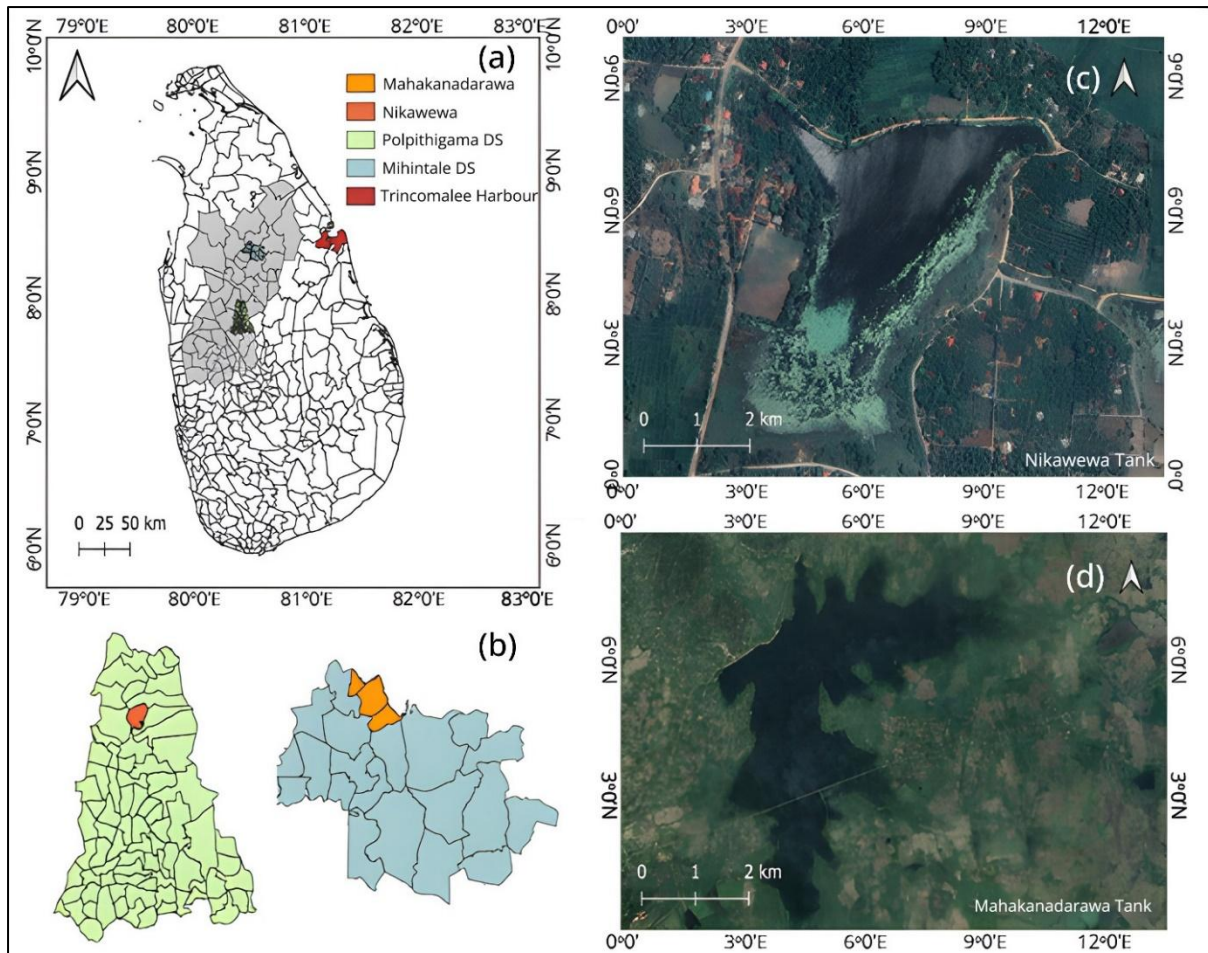


Figure 1: Fish sampling locations of (a) Sri Lanka (b) Polpithigama divisional secretariat and Mihintale divisional secretariat (c) Nikawewa Grama Niladhari division (d) Mahakanadarawa Grama Niladhari division

Employing a random sampling technique, twenty-two *Etroplus suratensis* (koraluya) samples were collected from Mahakanadarawa tank, and fifteen samples were collected from Nikawewa tank. Additionally, twenty-eight samples of *Amblygaster sirm* (hurulla) were collected from the coastal region near Trincomalee harbor. All fish samples were selected to be approximately uniform with an average length and weight of $(20.0 \pm 1.0 \text{ cm})$ and $(30.0 \pm 1.0 \text{ g})$ respectively. The samples were then placed in sterile polyethylene bags and preserved at -20°C during transport.

Sample preparation

All the fish samples were washed with distilled water and carefully dissected to extract muscle tissue, gut tissue, gill tissue and liver. Duplicate samples of each tissue type were prepared. Each sample was then labeled and stored at -20 °C until further processing.

Detection of the heavy metals/metalloids

The fish tissue samples were subjected to microwave-assisted digestion utilizing a microwave digester (ETHOS EASY, Italy). Each sample weighing 0.200 g was digested with 8.00 mL of concentrated nitric acid (HNO₃) and 2.00 mL of ultrapure water. The resulting solutions were diluted to a final volume of 25.00 mL by adding ultrapure water and filtered through 0.45 µm nylon syringe filters.

The concentrations of Pb, Cr, Cd, As and Ni in digested samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS; 7800-Agilent, Germany). Calibration was performed by using multi-element ICP-MS standards (Multi-element calibration standard 2A). Quality control measures included uncertainty measurement based on an internal standard procedure (ICP-MS internal standard mix) and the analytical method was validated in accordance with ISO/IEC 17025 standards. Before analyzing the samples, an HNO₃ acid blank was run.

Health risk assessment

Health risk assessment was carried out to evaluate the potential health risks associated with consuming contaminated fish. For the calculations and formulas, the guidelines from the United States Environmental Protection Agency (US-EPA) Regional Screening Level (RSL) were utilized (USEPA, 2001; Ai et al., 2022).

Estimated daily intake (EDI)

Estimated daily intake (EDI) values were calculated based on the concentrations of heavy metals in fish tissues.

$$EDI = \frac{IR \times C_i \times Ef_r \times ED_t}{BW_a \times At_n} \quad \text{Eq. 1}$$

i – Individual heavy metal/metalloid

IR – Daily ingestion rate (0.2 kg/day)

C_i – Concentration of heavy metal/metalloid (mg/kg)

Ef_r – Exposure frequency (365 d /y)

ED_t – Total exposure duration (70y)

BW_a – Body weight for adults (60 kg)

At_n – Average time on non-carcinogenic

Target hazard quotient (THQ)

Non-carcinogenic risks were assessed using the target hazard quotient (THQ). Equation 2 indicates the THQ_i calculation of individual heavy metals. RfD_i values of As, Cd, Pb, Cr, and Ni are considered 3×10^{-4} , 1×10^{-3} , 1.2×10^{-3} , 3×10^{-3} , 1×10^{-3} mg/kg/ day (Choquenaira-Quispe et al., 2022; Román-Ochoa et al., 2021).

$$THQ_i = \frac{EDI_i}{RfD_i} \quad \text{Eq. 2}$$

Target Cancer Risk (CR)

Carcinogenic risks were evaluated using the Target Cancer Risk (CR) metrics. The total target cancer risk is calculated using the following equation, (Equation 3)

$$CR_T = \sum_{i=1}^n CR_i \quad \text{Eq. 3}$$

The cancer risk (CR) are defined as; a safe limit with $CR < 10^{-6}$; an acceptable limit with $10^{-4} > CR > 10^{-6}$; a threshold risk limit at $CR > 10^{-4}$; and a risk limit set at $CR > 10^{-3}$ (Mao et al., 2019).

Data analysis

The heavy metal data obtained through ICP-MS was subjected to preprocessing using Python statistics (Pandas v. 2.2.2) and Minitab (2023). Descriptive statistics, including mean and standard deviation were calculated to summarize the concentrations of heavy metals in different fish tissues. Comparative analyses were performed using one-way ANOVA and Tukey's pairwise comparison tests to identify significant differences in heavy metal concentrations among the various fish tissues and collection regions. The data set met the assumptions for one-way ANOVA, including normal distribution and homogeneity of variance.

Regression analysis was conducted to explore the relationships between heavy metal concentrations to predict heavy metal levels in fish tissues in three selected locations separately. Maps were generated using QGIS (v.3.28.2) and Google Maps, while GPS data was obtained using the Garmin eTrex 20x device.

Results and discussion

Bioaccumulation of toxic metals in fish

Table 1: The average heavy metal concentrations (mg/kg)

| Fish Type | Locati on | Tissue | Heavy metal concentration (mg/kg) | | | | |
|--------------------------------|-------------------------------|------------------|-----------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | | | Pb | As | Cr | Cd | Ni |
| <i>Etroplus suratensis</i> | Nikawewa tank (n = 15) | Muscle | 1.57±0.05 ^a | 0.06±0.01 ^a | 1.76±0.09 ^a | 0.04±0.01 ^b | ND |
| | | Gut | 0.31±0.03 ^b | 0.03±0.00 ^b | 0.80±0.05 ^b | 0.03±0.02 ^b | ND |
| | | Gill | ND | 0.01±0.00 ^b | 2.03±0.03 ^a | 0.06±0.04 ^a | ND |
| | | Liver | ND | 0.03±0.00 ^b | 1.44±0.01 ^a | 0.02±0.01 ^b | ND |
| | Mahakanadarawa T. (n = 12) | Muscle | 0.51±0.03 ^b | 0.01±0.00 ^b | 0.22±0.03 ^c | 0.02±0.00 ^b | 0.06±0.03 ^c |
| | | Gill | 0.55±0.02 ^b | 0.02±0.01 ^b | 0.25±0.04 ^c | 0.01±0.01 ^b | 0.34±0.00 ^b |
| | | Gut | 0.71±0.13 ^b | 0.08±0.01 ^a | 0.96±0.03 ^b | 0.09±0.02 ^a | 0.67±0.02 ^a |
| <i>Amblygaster sirm</i> | Trincomalee harbor | Muscle (n=19) | 0.001±0.000 ^c | 0.001±0.000 ^c | 0.003±0.002 ^d | 0.001±0.000 ^c | 0.001±0.000 ^c |
| | | Gill (n=19) | ND | 0.001±0.000 ^c | 0.027±0.002 ^c | 0.003±0.001 ^c | 0.015±0.006 ^c |
| | | Liver (n=15) | ND | 0.002±0.001 ^c | 0.003±0.000 ^d | 0.010±0.001 ^b | 0.002±0.000 ^c |
| MPL | | | 0.5 | 2 | 1 | 0.3 | |

MPL - Maximum Permissible Limits (mg/kg) - CODEX STAN 193-1995 (Levels that exceeds MPL given in bold)

ND: Not Detected

Detection Limits: Pb- 0, As- 0, Cr- 0.4959 ppb, Cd- 0, Ni- 17.81 ppb

Data reported as mean±SD. Statistically significantly different values denoted by a, b, c, d down the columns based on Tukey's pairwise comparisons post-hoc one-way ANOVA at a p<0.05

All the fish sample data were subjected to statistical evaluation. The analysis of variance (ANOVA), post-hoc Tukey's pairwise comparisons, and regression analysis for the concentration of Pb, As, Cd, Cr and Ni were performed. The significance cutoff for all tests was set at 0.05.

Muscle tissues from inland fish in Nikawewa area showed high levels of Pb (1.57 ± 0.05 mg/kg) and Cr (1.76 ± 0.09 mg/kg), both of which are substantially higher than the MPL levels. In the Mahakanadarawa tank, muscle tissues had Pb levels (0.51 ± 0.03 mg/kg) above the MPL (Table 1). Cr concentrations were highest in the gill tissue of *Etroplus suratensis* from Nikawewa (2.03 ± 0.03 mg/kg) and gut tissue (0.96 ± 0.03 mg/kg) from Mahakanadarawa. These levels significantly exceed those observed in *Amblygaster sirm*, where Cr concentrations were as low as in muscle tissues (0.003 ± 0.002 mg/kg) and liver tissues (0.003 ± 0.000 mg/kg). Pb and Cr have a high affinity for proteins and bind strongly to muscle tissues, rich in proteins. These metals can be distributed via the bloodstream to various tissues, due to their large mass and metabolic activity, muscle tissues often act as reservoirs for heavy metals (Sheikhzadeh & Hamidian, 2021). In contrast, the study by Rajeshkumar and Li (2018) indicated that Pb concentration was the highest in the liver of inland fish species, Cd levels were relatively uniform across all organs, and Cr was mainly enriched in the liver. This noted that the total metal bioaccumulation was greatest in the liver and gills, with the lowest accumulation in muscle tissues (Rajeshkumar & Li, 2018). Marine fish exhibited negligible amounts of heavy metals across all tissues, well below the MPL (Table 01). Marine fish muscle had very low concentrations of Pb (0.001 ± 0.000 mg/kg), As (0.001 ± 0.000 mg/kg), Cr (0.003 ± 0.002 mg/kg), Cd (0.001 ± 0.000 mg/kg), and Ni (0.001 ± 0.000 mg/kg), highlighting the lower contamination levels in marine environments. These findings underscore that the regular consumption of inland fish may be a health risk due to the significant toxic metal contamination in both Mahakanadarawa tank and Nikawewa tank, with potential sources including surface water from municipal and industrial discharges, runoff from lawns, streets, and farmlands, and agricultural and residential waste products.

Tukey's pairwise comparisons, based on sampling locations, confirmed that Pb levels in inland fish in Nikawewa and Mahakanadarawa areas were significantly higher than those in the coastal region ($p < 0.05$). As and Cd concentrations followed a similar pattern, with Mahakanadarawa showing the highest levels, Nikawewa having intermediate levels, and the coastal region having the lowest levels ($p < 0.05$). Chromium (Cr) levels were highest in Nikawewa, followed by Mahakanadarawa, and the coastal region had the lowest concentrations ($p < 0.05$). Furthermore, Ni levels were highest in Mahakanadarawa and significantly lower in the coastal region and Nikawewa ($p < 0.05$).

Regression analysis further confirmed that marine fish had significantly lower Pb, As, Cr, Cd and Ni concentrations ($r = -0.54$, $P = 0.00$, $r = -0.02$, $P = 0.00$, $r = -0.45$, $P = 0.00$, $r = -0.01$, $P = 0.01$, $r = -0.16$, $P = 0.00$) compared to inland fish.

ANOVA analysis revealed statistically significant differences ($p < 0.05$) in heavy metal concentrations (Pb, As, Cr, Cd, and Ni) across different fish tissues from the Mahakanadarawa tank, Nikawewa tank, and Trincomalee harbor. Tukey's pairwise comparisons grouped gut tissues with the highest Pb concentrations ($p < 0.05$), and muscle tissues as second highest while gill and liver tissues showed intermediate levels. Furthermore, gut tissues had significantly higher As concentrations ($p < 0.05$) compared to other tissues. Tukey's test grouped gill and gut tissues

with the highest Cr concentrations ($p < 0.05$), while muscle and liver tissues had lower levels. Higher Cd concentrations ($p < 0.05$) showed in gut and gill tissues, while liver and muscle tissues had lower levels. Also, Tukey's test grouped gut tissues with the highest Ni concentrations, while gill, liver, and muscle tissues had lower levels.

The regression analysis confirmed that gut tissues had significantly higher Pb, As, Cd and Ni concentrations ($r = 0.11$; $P = 0.00$, $r = 0.06$; $P = 0.00$, $r = 0.06$; $P = 0.00$, $r = 0.33$; $P = 0.00$, respectively) while gill tissues showed higher Cr concentrations ($r = 1.64$; $P = 0.00$) compared to other tissues, highlighting the potential bioaccumulation pathways of metal accumulation through ingestion of metal rich sediment and foods (Rajeshkumar & Li, 2018).

Health risk assessment

Table 2: Health risk assessment of bioaccumulation of toxic metals in inland fish and marine fish tissues.

| Location | Fish species | Total EDI | THQ | | | | | | CR | | | | | |
|---------------------|----------------------------|-----------|-----|----|----|----|----|------|----|----|----|----|----|-----|
| | | | Pb | As | Cr | Cd | Ni | THQT | Pb | As | Cr | Cd | Ni | CRT |
| Nikawewa | <i>Etroplus suratensis</i> | | | | | | | | | | | | | |
| Mahakanadawawa | | | | | | | | | | | | | | |
| Trincomalee harbour | <i>Amblygaster sirm</i> | | | | | | | | | | | | | |

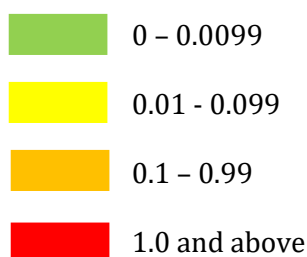
EDI: Estimated daily intake

THQ: Total hazard quotient

CR: Carcinogenic risk

THQT: Total risk assessment based on non-carcinogenic effects due to Pb, As, Cr, Cd, and Ni

CRT: Total risk assessment based on carcinogenic effects due to Pb, As, Cr, Cd, and Ni



The non-carcinogenic and carcinogenic health risk assessments were conducted for inland and marine fish. The highest toxic metal intake was observed in the Nikawewa region through inland fish consumption due to the higher total EDI value.

The non-carcinogenic risk assessment reveals that muscle tissues of inland fish in the Nikawewa region have a higher THQT value than the other areas (Table 2). The individual estimate health risk levels / Total Hazard Quotient (THQ) for Pb, Cr and THQT exceed 1 (1.50, 1.96 and 4.23, respectively). This indicates that these toxic metals pose a potential risk for non-carcinogenic adverse health effects. THQT values and THQ values for all toxic metals in the analyzed fish muscle samples from the Mahakanadarawa tank are lower, staying below the level of concern (THQ < 1). Additionally, THQT value of 0.02 for marine fish poses a considerably lower risk of non-carcinogenic adverse health effects compared to inland fish samples from Nikawewa and Mahakanadarawa with higher THQT values 4.23 and 0.90 respectively.

The carcinogenic health risk assessment of muscle tissue samples of inland fish in the Nikawewa tank indicated the Carcinogenic risk (CR) values for Pb, Cr and CRT value (0.008, 0.009 and 0.017 respectively) exceeded the substantial risk limit ($CR > 10^{-3}$), indicating potential carcinogenic health risk. Pb does not directly cause carcinogenic issues but can induce genotoxic effects indirectly by interfering with DNA repair mechanisms and Pb exposure increases the production of reactive oxygen species. This oxidative stress can contribute to the initiation and progression of cancer (Soliman, 2018). Furthermore, CR values for Pb and CRT value (0.003 and 0.017 respectively) for samples of inland fish in the Mahakanadarawa tank also exceeded the substantial risk limit ($CR > 10^{-3}$). However, CR and CRT values of muscle tissue samples of marine fish stayed within a safe limit ($CR < 10^{-6}$). These non-carcinogenic and carcinogenic health risk assessments of fish sample suggest the long-term consumption of inland fish can cause severe adverse health effects than the consumption of marine fish.

Several studies have suggested that prolonged consumption of fish with even trace levels of toxic metals may pose potential health risks (Wang et al., 2022). Contamination of tank sediments with heavy metals can lead to the bioaccumulation of these metals in inland fish through various pathways (Emenike et al., 2022).

The statistical evaluation of heavy metal concentrations in fish from different regions and tissues provided several key insights. Heavy metals such as Pb, As, Cd, and Ni are known to accumulate in fish through various pathways, including ingestion of contaminated food and sediment, absorption through gills, and direct contact with contaminated water.

Conclusions

This study provides a comparative analysis of heavy metal/metalloid bioaccumulation in fish from inland rivers and coastal regions in Sri Lanka, offering valuable insights into bioaccumulation pathways and associated health risks. The findings highlight the significant disparity in heavy metal concentrations between inland fish and marine fish. Inland fish from the Nikawewa and Mahakanadarawa regions exhibited notably higher levels of Pb, As, Cr, and Cd compared to marine fish from the Trincomalee coastal region. The muscle tissues of inland fish exhibited the highest levels of Pb and Cr exceeding the MPL levels, highlighting the potential

health risk involved with consuming fish. In contrast, marine fish showed low levels of heavy metals across all tissues. Health risk assessments indicated that the EDI values for heavy metals in inland fish were significantly higher than those for marine fish. The THQ and THQT values for Pb and Cr in inland fish exceeded 1, indicating a potential risk for non-carcinogenic adverse health effects. Similarly, the CR values for Pb and Cr in inland fish also exceeded acceptable risk limits, indicating a potential carcinogenic health risk. In comparison, marine fish posed a significantly lower risk, with THQ, THQT, and CR values well below the levels of concern. The statistical analyses, including ANOVA and Tukey's pairwise comparisons further confirmed the significant geographical and tissue specific variation in heavy metal concentrations. Inland regions, particularly Nikawewa and Mahakanadarawa, showed higher contamination levels compared to the coastal region. Additionally, gut tissues generally had the highest concentrations of heavy metals, followed by muscle, gill, and liver tissues in inland fish. Although humans typically do not consume fish guts, heavy metals in the gut can be absorbed into the bloodstream of fish through the intestinal lining and accumulate in the other tissues. This process can eventually introduce heavy metals into the human body through consumption of fish. These results underscore the importance of continuous monitoring and regulation of heavy metal pollution in aquatic environments, especially in regions with significant agricultural activities. The study highlights the urgent need for mitigating measures to reduce heavy metal contamination and its impact on human health, particularly for communities relying on inland fish as a primary food source. The long-term consumption of inland fish from the studied regions poses a severe health risk due to significant toxic metal contamination. There is a critical need for comprehensive policies and effective management strategies to address and mitigate heavy metal pollution in Sri Lanka's aquatic ecosystems. Future research should focus on identifying specific sources of contamination and developing targeted interventions to protect both environmental and public health.

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Conflict of Interest

Authors have no competing interests to declare.

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