International Journal of Public Health Practice and Promotion (IJPHPP)

VOLUME 1 ISSUE 1 (2026)



PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

International Journal of Public Health Practice and Promotion (IJPHPP)
Volume 1 Issue 1, Year 2026
https://journals.e-palli.com/home/index.php/ijphpp

Trace Metal Distribution in Five Fish Species from Selected Aquatic Environments in Bangladesh

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Article Information

Received: August 12, 2026
Accepted: October 31, 2026
Published: November 12, 2026

Keywords

Aquaculture Feed, Aquatic Contamination, Atomic Absorption Spectrophotometry, Bangladesh Fish Tissues, Trace Metals

ABSTRACT

The evaluation of trace metal in fish should be conducted not only on edible fish tissues, but also on internal tissues, since the concentration in the internal tissues may be significantly different from that in the edible tissues. The present exploratory study investigated the levels of lead, cadmium, chromium, nickel and copper in the five fish species of the Balu River and Meghna Ghat and a fish farm of the Gazipur district in Bangladesh. Fish was collected in January 2017 and laboratory was carried out in the period of February- July 2017 at the Bangladesh Council of Scientific and Industrial Research (CSIR). Before being analyzed by atomic absorption spectrophotometry, tissue and feed samples were digested. Supplementary environmental information was provided by water characteristics and six commercially available feeds. The level of copper was observed up to 142.79 ppm in liver of mrigal and 50.95 ppm in rohu liver. The concentration of nickel was found to be 131.15 ppm in chewa gut and 32.11 ppm in spotted snakehead muscle. Lead level was 1.36 ppm in mrigal muscle while lead in the rohu, mrigal and chewa muscle and other tissues was undetectable. These observations reveal that they are significantly enriched in a tissue-specific manner except for muscle. The interpretation of statistics and regulation is limited by unreported replication, detection limits and concentration weight basis. If there were more than one entry that conflicted, then those entries were not corrected; they remained unresolved. Results here support directed confirmatory monitoring of edible tissues, enhanced analytical record keeping, and simultaneous monitoring of fish, water, sediment and aquaculture inputs. They do not represent population exposure or nonconforming with the regulations.

INTRODUCTION

It is important for fish contamination assessment to differentiate between the presence of a metal and the consequences of eating contaminated tissue. Concentration measurements determine the presence of a chemical. Dietary risk is also influenced by the quantity consumed, frequency of exposure, body weight and the toxicological characteristics of the substance measured. Thus, a tissue survey can help with food safety by providing food items that require additional research. Its conclusions are less valid when organ measurements are compared as direct measures of consumer harm without taking into consideration the amount of the edible portion or the method used to calculate the concentration (Islam *et al.*, 2023).

The study in Bangladesh has shown that it is important to investigate metals in the commercially important aquatic organisms. The fish and shellfish of the Buriganga River were studied and the concentration data was linked with dietary exposure calculation by Ahmed *et al.* (2015). Their approach shows that chemical analysis and exposure assessment are complex, but related and separate investigations. The evidence for one river does not give either a direct estimate for another site or give any context. When comparing results between studies differences in species sampled, sample dates, environment and analysis methods are important.

The distribution of metals in fish is especially important as it relates to the design of monitoring. This analysis may only show the muscle levels and not the uptake, storage, or elimination levels. However, a high liver concentration does NOT necessarily reflect the concentration in the most often used part. In the present study, in addition to gill, liver, muscle and some other tissues have been taken for examination. This allows for examination of internal distribution but does not lose sight of an explicit focus on edible tissue. It also spares the best patterns from being obscured in the overall organ rank, such as muscle concentrations (Ahmed *et al.*, 2020).

Aquaculture and river fisheries raise other issues of possible exposure. Metals can be introduced to farmed fish via water, pond substrate, and purchased feeds. Water quality may change and the fish in rivers may come into contact with contaminated particles or food. These pathways provide plausible explanations for the tissue patterns and evidence establishing a pathway should show the connection between the environmental medium and the individual fish. The presence of a certain concentration in a market feed cannot be used as proof that the sampled fish fed on the feed. In the same way, the exposure history of a mobile organism cannot be reconstructed from a water measurement in the vicinity of a collection site (Shorna *et al.*, 2021).

Selected environments were two river environments and one aquaculture environment. Chewa was from Meghna Ghat. The sources of spotted snakehead and stinging catfish were the Balu River at Gazipur. Rohu and mrigal are of indigenous species and have been stocked from

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the local fish farm of Dhirasram, Gazipur. Thus there are fish included in the design in accordance with the various production systems. But there is some overlap among species and location. There is a simultaneous change in species and change in source environment when comparing chewa rohu. The results thus represent combinations of sampled species and setting, and not unique habitat effects (Bhattacharjee *et al.*, 2025).

Based on previous study of these tissues, both biological and environmental measurements should be taken into consideration. Canli and Atli (2003) investigated concentration in muscle, gill and liver of 6 species of Mediterranean fish and explored their relationships with fish size. Their investigation affords a methodological basis for length and weight to be noted in relation to the various tissue differences. These biological descriptors were not available in a form that allowed them to be adjusted in the present investigation. Comparisons of species are therefore given in a descriptive manner and differences observed are not solely related to feeding behaviour, age or contamination intensity.

The study addresses three connected questions. The first was which measured metals were most prevalent in fish tissue that was available. The second deals with the question of muscle and whether or not it followed the same pattern as internal organs. The third relates to environmental information from water characteristics, water metals and commercial feed measurements. To answer these questions, measurements should be kept and distinct numerical patterns and irregularities reported should be separated from each other. This distinction is particularly important when an analytical record includes graphical values as well as summary tables which do not match entirely (Lipy *et al.*, 2021).

The aim of this study was to describe the distribution of Pb, Cd, Cr, Ni and Cu in 5 species of fish collected from selected aquatic bodies of Bangladesh. Some additional goals were to provide a summary of water and feed measurements, to evaluate the interpretive utility of tissue comparisons, and to classify the information needed to defensively assess food safety. The investigation takes the form of an exploratory environmental survey. The sampling was completed in 2017 and the results should be viewed as a historical snapshot and not a representation of the current contamination in Bangladesh.

LITERATURE REVIEW

Tissue Distribution and Biological Interpretation

An accumulated metal concentration can have different meanings depending on the biological location and chemical availability of the metal. Measured total may contain material involved in physiological activity and material in less-available forms. This distinction is created by Rainbow (2007) in a biodynamic context of aquatic invertebrates. While it is not possible to directly translate processes specific to an organism to the fish, the general rule is applicable: The toxicologically active fraction is not the total tissue concentration. In the present study, therefore, concentration patterns are used to establish priorities of analysis and not to diagnose tissue injury (Lipy *et al.*, 2021).

The study of fish from Tuzla Lagoon showed the importance of organ content and time of sampling in the study of fish contamination for different metals in the different seasons. Tissue concentration was found to be element dependent with large amounts in the liver for certain elements by Dural *et al.* (2007). These are comprised of findings that give qualitative context for the liver copper measurements reported here. It is still not appropriate to make direct numerical comparisons between numbers unless analytical recovery, units, sample preparation and weight are compatible. Identical organ patterns do not necessarily indicate that two sites have similar environmental contamination but they can substantiate a biological theory (Wahiduzzaman *et al.*, 2022).

The meaning of a monitoring result is also dependent on the species identity. If a species comes into regular contact with bottom material, it might have a different exposure mixture than another species using a different part of the water column. However, during the present investigation no direct measurements were taken of feeding ecology. The chemical measurements were not supplemented with stomach content analysis, movement assessment or ingested sediment estimate. Ecological explanations are thus regarded as hypotheses. This permits the separation of the characteristics typically found in a species and the exposure of the organism sampled (Mostafiz *et al.*, 2020).

Evidence from Bangladesh

Wahiduzzaman *et al.* (2022) studied the Dhaleswari River for seven different fish species such as spotted snakehead and stinging catfish and analyzed the presence of metals and their possible dietary implications. The results of their investigation indicate that species used in the present study are relevant to the larger scale contamination studies in Bangladesh. The measurements of their head and body, however, cannot be used interchangeably with those of the respective muscle, liver and gill parts. The comparison provides evidence of the need for monitoring and illustrates the importance of clear definitions of tissue when synthesizing studies (Maya *et al.*, 2025).

The literature also shows the importance of measuring of multiple environmental compartments in the case of Bangladesh. Water analysis represents conditions at collection, while uptake and retention are represented by tissue analysis, which is on a different timescale. Feed analysis can be used to determine chemicals which may be present in purchased products but for the attribution of exposure, documentation of feeding is required. The present record includes information from these compartments which lacks a fully paired sampling structure. Its relevance is in the discovery of opposing chemical signatures and certain measurements that should be confirmed. It is unable to set up a transfer sequence that is complete from industrial discharge to water, feed, fish and consumers.

Measurement Quality and Food Safety Inference

Careful handling of the values below detection is indicated because the nondetection in the analysis does not equal true nondetection. Nondetects can be replaced with arbitrary numbers and misleading descriptive statistics and associations can result, as Helsel (2006) showed. This is a direct consequence for the interpretation of datasets with a high number of entries reported as zero or less than detection limit. The detection designation below is maintained in the present analysis and also no new averages are calculated by substituting zero. The upper limits of these censored observations were not quantifiable as the numerical detection limits were not available (Ali *et al.*, 2020).

Food standards imply another requirement for measurement compatibility. Commission Regulation (EU) 2023/915 establishes maximum levels of contaminants in certain foodstuffs and information on the concentration basis, which for the applicable fish products is wet weight (European Commission, 2023). This is because an exceedance does not automatically occur when an organ is only reported as ppm. Here the regulation is used instead of imposing the European legal classification on the sampled fish of Bangladesh. Each needs to be separately verified for local applicability, product category and the measurement basis.

MATERIALS AND METHODS

Study Design and Sampling Scope

This was an exploratory observational study with fish, commercial feed and source water. In January 2017, live fishes were collected and brought in polythene bag with aerated water at Bangladesh Council of Scientific and Industrial Research, Dhaka. Laboratory activities were carried out from February to July 2017 at the Soil, Agronomy and Environment section. No experiments were reported involving the exposure to metals. Thus the results are based on field collected material that has been characterized in the laboratory. No record revealed repeated sampling of the season, nor a probability based selection of sampling locations.

Five fish species were taken as Rohu (Labeo rohita H. H.), Mrigal (Cirrhinus cirrhosus B. et S.), Spotted snakehead (Channa punctata M. H.), Stinging catfish (Heteropneustes fossilis T. et E.) and Chewa (Pseudapocryptes elongatus B. et S.). The occurrence of species, locations and tissues is summarised in table 1. Sample size (number of individual fish, pooling procedures and number of independent biological replicates) were not reported in a way that allows reconstruction of sample size. Therefore, the species level result is considered to be a reported analytical observation and not an estimate of a species population mean.

Table 1: Sampling locations and examined tissues

Species	Collection setting	Tissues recorded
Rohu	Farm at Dhirasram, Gazipur	Muscle, gill, liver, air sac, fibre
Mrigal	Farm at Dhirasram, Gazipur	Muscle, gill, liver, air sac, fibre
Spotted snakehead	Balu River, Gazipur	Muscle, gill, liver, fibre
Stinging catfish	Balu River, Gazipur	Muscle, gill, liver
Chewa	Meghna Ghat	Muscle, gill, liver, gut, eggs

Water and Feed Collection

Water measurements were taken for Balu river, Meghna river and local farm. Six readings were taken (temperature, pH, electrical conductivity, total dissolved solids, dissolved oxygen, turbidity). The instruments listed in the experimental record were a thermometer, a pH meter, HACH multiparameter equipment, a HACH HQ30D dissolved oxygen meter and a HANNA turbidity meter. No calibration histories for instruments were provided or number of readings taken in the field. These measurements are therefore reported as the site observations. These are not meant to be used to interpret the seasonal averages or as complete descriptions of the water bodies (Aithal & Aithal, 2020).

Six types of commercial feed were analysed. The required fish feeds AIT Tilapia Finisher, AIT Fish feed and AIT Fish floating feed were collected from the Harinal Bazar area in Joydebpur. Nursery feed was collected from the fish project nearby Joydebpur Bazar. Krishibid group and Modern super standard feeds were purchased from the local market, Daxinkhan, Dhaka. The record didn't describe manufacturing batches, what was in the product or how long the product was stored or used at the sampled farm. Product names refer to the materials sampled and are not indicative of contamination throughout a manufacturer's product range (Wang *et al.*, 2024).

Tissue Preparation and Chemical Analysis

Fish were dissected into tissues according to the categories noted for each species. The preparation story relates to some 5g of wet tissue being dried for 48 hours then ground. 10 mL of nitric acid was added to a 0.5 g dried material, covered and allowed to predigest overnight. It was then heated in a fume chamber until it was clear. The digest was diluted to 25 mL in deionized water, filtered and placed in clean plastic containers. These steps outline the reported workflow and do not contain any presumed, unreported recovery performance.

The written procedure had a drying temperature of 60°C and preparation diagram had a drying temperature of 80°C. This discrepancy could not be clarified from the records available. Digestion was carried out at about 80 °C using about 1 g of material with 10 mL nitric acid, followed by heating for six hours and diluting to 25

mL. No information was provided on drying feed. The documentation of the water preservation and digestion was inadequate to duplicate a similar procedure for the aqueous samples.

Determination of metal was done by atomic absorption spectrophotometer Shimazdu AA7000. The analytical grade of reagent and deionized water were mentioned in the experimental description and commercial standards were used for calibration solutions. The results were found in parts per million (ppm). While one of the methods gave zinc as the metal passed, the numerical results gave lead, cadmium, chromium, nickel and copper as the metals passed. This paper therefore reports those five elements and makes no inference of zinc concentrations. No data on instrument wavelengths and calibration ranges, blank results, matrix recoveries, or numerical detection or quantification limits were provided (Braun *et al.*, 2021).

Concentration Basis and Record Reconciliation

A digest concentration, normally used to determine a concentration for solid material, will need the concentration measured in the solution, sample mass, the dilution factor, and the final volume of the solution. The corresponding dimensional relationship is Csolid = (Cdigest − Cblank) × Vfinal × D / mdry, using volume in litres and dry mass in kilograms. This is an expression for information required for traceability and not an affirmation that the information reported can be recalculated. Complete dilution records, individual instrument readings and blank measurements were not available. To this end no new conversion between dry and wet weight was made (Wu *et al.*, 2022).

Reported ppm values were retained without relabelling them as verified milligrams per kilogram wet weight. The tissue drying procedure indicates that it may have been intended to calculate dry matter; however, the denominator is not certain. ppm was kept as reported for water since the analytical calculation and matrix treatment were not adequately defined. The difference is significant for the interpretation of concentrations in water, feed and tissue. The correspondence of numbers does not necessarily mean equivalent exposures and vice versa, that absence of correspondence in numbers does not necessarily mean absence of accumulation factor, which should be based on validation.

Disagreements between figures and tables were noted but not adjusted. The figure show the Chewa muscle chromium as 0.086 ppm while the table show 0.66 ppm. The liver lead content of the Mrigal was 20.28 and 20.21 ppm. The figure and tabulated record assignments for chromium and nickel were different for stinging catfish gill and liver. The maxima were the same; establishment of the organ of the maxima was uncertain. These discrepancies were left in the results. The word fibre was retained as an anatomical label for the reported tissue due to the lack of an anatomical definition.

Analytical Strategy

Individual reported concentrations, locations and tissue patterns are summarized in an analysis. Comparisons highlight values that are preserved among the representations that are available. Independent replicate observations and their sampling structure were not available, so no new significance tests were calculated. Different organs did not provide estimates of biological variation as means and standard deviations were calculated across organs. Various tissues, such as muscle, liver, gills, etc., are different biological compartments (Holtom *et al.*, 2022). However, among them, there is only variation that answers the question of tissue distribution, but not in place of inter-fish (intra-location) variation within species. The original analysis involved analysis of variance, Tukey comparisons and Pearson correlations using the software Minitab 18 and Microsoft Excel 2010. But replicate data and complete test output were not available. There were some reported correlations that seemed to involve concentration rankings for several different elements without repeated measurements for any one of the elements in the different sampling units. These kinds of coefficients were not collected as evidence of environmental transfer. It would need to be confirmed; with paired observations, adequate replication, and a design separating species, tissue, site, and time.

RESULTS AND DISCUSSION

Water Characteristics

There were differences in the water observations between the three settings (Table 2). The least amount of dissolved oxygen was recorded in Balu river (2.30 mg/L) than the amount in Meghna River (3.55 mg/L) and farm (8.01 mg/L). The water of Meghna was found less conductive, less TDS and less turbid than the water of Balu. The farm reported the highest turbidity 186 NTU. These differences pertain to what was sampled and not sources

Table 2: Physicochemical observations in source water

Parameter	Balu River	Meghna River	Farm
Temperature (°C)	25.3	23.8	25.4
pH	6.71	7.70	7.76
Conductivity (µS/cm)	1262	202	25.8
Total dissolved solids (mg/L)	631	101	129.1
Dissolved oxygen (mg/L)	2.30	3.55	8.01
Turbidity (NTU)	119	36	186

of metals or exposure times.

Farm conductivity was reported as 25.8 µS/cm and its total dissolved solids was reported as 129.1 mg/L. This pairing is quite distinct from the numerical relationship of the two river samples and needs to be confirmed based on instrument records. It was kept without the introduction of an assumed decimal adjustment. Water temperature was 23.8-25.4°C and pH was 6.71-7.76. The repeated readings and calibration records were not available so an uncertainty interval or statistical comparison was not assigned to these site observations.

Metal Concentrations in Muscle

Results of the muscle measurement indicated that the predominant metal varied from species to species (Table 3). The highest muscle Ni concentration among the five species was found in spotted snakehead (32.11 ppm). The muscle of the stinging catfish, chewa, rohu and mrigal contained 8.09, 2.56, 1.98 and 0.12 ppm of Ni, respectively. This means that nickel could be detected in muscle of all five species. The high inter-species differences between the two Balu River species also attest that similar collecting environment does not result in a similar muscle profile.

Table 3: Reported muscle metal concentrations in the five fish species

Species	Pb	Cd	Cr	Ni	Cu
Rohu	BDL	BDL	BDL	1.98	0.88
Mrigal	1.36	BDL	BDL	0.12	0.55
Spotted snakehead	BDL	0.0091	BDL	32.11	0.81
Stinging catfish	BDL	0.018	0.35	8.09	0.11
Chewa	0.07	BDL	0.086 / 0.66	2.56	13.45

Lead concentration in mrigal muscle was found to be 1.36 ppm while in chewa muscle the lead concentration was found to be 0.07 ppm. The concentrations of lead in muscle of rohu, spotted snakehead and stinging catfish were below detection. The results reported 0.0091 ppm of Cd in spotted snakehead muscle and 0.018 ppm in stinging catfish muscle. It could not be detected in the other three species. The copper content in the muscle was highest in Chewa (13.45 ppm). Chromium was reported at 0.35 ppm in the muscle of stinging catfish; and there were conflicting chewa chromium entries, so a single defensible entry could not be selected (Alam *et al.*, 2025).

Rohu and Mrigal Tissue Profiles

Rohu exhibited a significant copper uptake in the liver. The liver concentration reported was 50.95 ppm while the concentration in muscle was 0.88 ppm, gill 0.97 ppm, air sac 5.67 ppm and fibre 0.42 ppm. The lead concentration reported in gill was 5.75 ppm, in air sac was 2.87 ppm and in fibre was 4.49 ppm. Not detected in liver, muscle. 5.11 ppm of chromium was found in air sac and none was detected in other tissues examined. Cadmium was not detected at all points on the rohu record.

The highest Cu concentration in the fish dataset was found in liver of the MRIGAL (142.79 ppm). Its liver chromium concentration was 24.52 ppm while the air sac was chromium 42.72 ppm. The elements found were, lead in muscle, gill, liver and fibre, liver was either reported as 20.21 or 20.28 ppm. No cadmium was detected in any of the mrigal tissues. These results distinguish mrigal from other varieties of fishes which are also reared in the ponds namely, rohu. They also demonstrate why it would be misleading to make a general statement that each metal was always found in the greatest amount in liver (Hossain *et al.*, 2024).

Balu River Species

The spotted snakehead appeared to be a nickel pattern in contrast to the liver copper enrichment of the farmed cyprinids. In the muscle, gill, liver and fibre of fish, nickel level was found to be 32.11 ppm, 11.64 ppm, 9.46 ppm and 10.53 ppm, respectively. Thus, the nickel level was highest in muscle for this species. The copper content in muscle was 0.81 ppm, but that in gill, liver and fibre was 3.07 ppm, 3.73 ppm and 6.32 ppm, respectively. Lead levels were not detected at any time and chromium was only present in fibre at 0.99 ppm (Proshad *et al.*, 2021).

The following amounts of chromium, nickel, cadmium and copper were detected in stinging catfish muscle: 0.35 ppm, 8.09 ppm, 0.018 ppm and 0.11 ppm. Lead was found to be not detectable in the various tissues studied. The record always showed a maximum of 37.65 ppm nickel, a maximum of 2.64 ppm chromium, but in the figure the maximum for gill and liver and in the table the maximum for liver and gill. Thus, these maxima do not distinguish what was the organ of the maximum measurement but merely show organs of importance. Gill copper was always 0.54 ppm and liver copper was always 0.25 ppm.

Chewa Tissue Profile

Chewa gut contained 131.15 ppm nickel, 25.29 ppm copper, 10.83 ppm chromium, and 4.43 ppm lead. Liver contained 47.25 ppm nickel, 17.07 ppm copper, 14.50 ppm chromium, and 6.02 ppm lead. The concentration of gill nickel was 0.99 ppm with the other measured metals below the detection limit in that tissue. Nickel, copper and lead levels measured in eggs were 3.18 ppm, 0.66 ppm and 0.04 ppm respectively. No cadmium could be detected in any of the chewa tissues examined. This muscle chromium discrepancy did not affect the finding that the highest chromium values were found in internal organs

Table 4: Selected prominent concentrations in internal tissues

Species	Tissue	Metal	Reported ppm
Rohu	Liver	Cu	50.95
Rohu	Gill	Pb	5.75
Rohu	Air sac	Cr	5.11
Mrigal	Liver	Cu	142.79
Mrigal	Liver	Pb	20.21 / 20.28
Mrigal	Air sac	Cr	42.72
Stinging catfish	Gill or liver	Ni	37.65
Stinging catfish	Gill or liver	Cr	2.64
Chewa	Gut	Ni	131.15
Chewa	Liver	Ni	47.25
Chewa	Liver	Cr	14.50
Chewa	Gut	Cu	25.29

Note: Selected values summarize prominent observations and are not independent replicate means. Stinging catfish gill and liver assignments conflict between the figure and table. Mrigal liver Pb also differs between those representations. Weight basis remains unverified.

Feed and Water Metals

The highest cadmium concentration of the fish tissues analyzed was 0.093 ppm for the spotted snakehead fibre tissue. This finding compares to the low muscle value for this species, and liver being nondetectable in the same species. The level of lead in mrigal fibre was found to be 8.60 ppm and rohu fibre was 4.49 ppm. From these observations it was concluded that this category, fibre contained measurable amounts of several elements. It is associated with an unconfined anatomical territory and so is not possible to directly compare with skin, connective tissue or another named compartment in published investigations.

Copper had the highest concentration (in ppm) in all six commercial feed profiles (Table 5) and this concentration varied from 5.57 to 9.37 ppm. Nickel ranged from 1.18 to 1.96 ppm, and chromium ranged from 0.26 to 2.37 ppm. Only the sampled feed of Krishibid group contained lead which was 0.38 ppm. Cadmium was not detected in the one feed tested and was found in the remainder of the products in the range 0.069 to 0.199 ppm. The measurements do not establish presence in the sampled materials but only show that the material is present.

Table 6 shows that the water metal profile was sparse. The level of the chromium in Balu water was found to be 0.0097 ppm and in the farm water was found to be 0.0087 ppm. In Meghna water, the presence of nickel and copper were reported at 0.0132 ppm and 0.0059 ppm respectively. The cadmium content of the farm water was 0.0011 ppm. Lead was below detection at all three locations. Some tissues of fish may contain lead, and yet the water may not have been detected – this is not a contradiction. The measurements are for different matrices and exposure times, but the numerical detection thresholds were not available.

Table 5: Reported metal concentrations in commercial feed samples

Feed category	Pb	Cd	Cr	Ni	Cu
AIT Tilapia Finisher	BDL	0.069	0.36	1.95	7.31
Modern Super Standard	BDL	0.16	0.85	1.53	5.60
Krishibid Group	0.38	BDL	2.37	1.19	9.37
AIT floating feed	BDL	0.11	1.18	1.96	8.24
Nursery feed	BDL	0.12	0.82	1.86	6.16
AIT fish feed	BDL	0.199	0.26	1.18	5.57

Table 6: Reported metal concentrations in source water

Location	Pb	Cd	Cr	Ni	Cu
Balu River	BDL	BDL	0.0097	BDL	BDL
Meghna River	BDL	BDL	BDL	0.0132	0.0059
Farm	BDL	0.0011	0.0087	BDL	BDL

Principal Patterns of Tissue Distribution

For the most part, the conclusion was that the distribution of metals was element and tissue specific. Liver copper and nickel were especially high in both the farmed

species, and spotted snakehead muscle and chewa gut. The highest report value of chromium in the species was observed in mrigal air sac. These patterns do not allow a universal ranking to be made liver > gill and muscle. The general tendency to enrich in the internal organs is very useful when the exception is specifically mentioned. If not, then the extent of muscle contamination can be underestimated and the biological specificity of the observations becomes lost (Haq *et al.*, 2022).

Within setting comparison can be seen between the rohu and mrigal copper profiles. In both, there was a marked liver maximum, although the magnitude of this maximum was very different. However, a similar pattern does not imply a common source of exposure or a similar exposure. The fishes might have been different in size, feeding history, residence time or in physiological condition (Rakib *et al.*, 2024). There is no record available that quantifies any of these factors adequately. This comparison thus suggests the need for additional investigation of the copper accumulation in the liver at the farm level, without yet clarifying the relative roles of external exposure and biological regulation.

For this reason, muscles have been treated separately and interpreted in terms of the consumption of flesh. Of particular interest is that the nickel concentration measured in spotted snakehead was greatest in the muscle of that species, with a mean of 32.11 ppm. If every fish were given an organ hierarchy of the same type, this observation would be hidden. It warrants confirmatory tests on separate muscle sample(s) collected, with analytical duplicates, and verified concentration units (Riad *et al.*, 2025). However, it does not measure on its own, the frequency of such concentrations in Balu River fish or the dose consumers may receive.

Interpretation of Individual Metals

The frequency of lead occurrence was different between species and organs. The muscle lead concentration in Mrigal was detected and that in the liver of Mrigal was reported to be much higher compared to the values reported for rohu in which no muscle or liver lead was detected but gill, air sac and fibre lead are detected. Lead was also found in the muscle and internal tissues of Chewa. These observations indicate species-tissue combinations for retesting. They cannot prove that lead was not present in the rest of the tissues, because it wouldn't be detected if it is below the analytical sensitivity. Because there are no numerical limits, it is not possible to compare the maximum concentrations that the sample might contain and remain undetected (Hasan *et al.*, 2021).

Cadmium was detected at low levels in the fishes, mainly in the two species from the Balu river. Cadmium was still detected in 5 products on the feed record and a cadmium value was detected on the farm water record. This distribution can neither confirm nor disprove the transfer of feed cadmium to the cultured fish or the lack of such transfer. No tissue measurements were taken with detection quantiles; and feeding records were not associated with the products (Priyanka Das Pinkey *et al.*, 2024). A defensible interpretation from these data is only based on the distribution of the detections in the matrices and does not involve the calculation of a transfer efficiency.

Care is especially needed in interpreting chromium, where the analytical record shows the concentration of all the elements, without chemical speciation. The total chromium result gives no indication of the amount of chromium in a specific oxidation state. A toxicity estimate assuming that all chromium measured was in one toxicological form would therefore need to be justified, which is not provided in this instance. The present data gives support to validate the presence of chromium in selected organs like mrigal air sac and chewa liver. They do not allow for a quantitative cancer risk assessment for an unmeasured chromium species.

One element that came up repeatedly in the tissue profiles was nickel, which should be followed up more closely. Dietary nickel assessment by EFSA Panel on Contaminants in the Food Chain (2020) shows that interpretation is variable with the conditions of exposure and the population assessed. Neither consumption distributions nor consumer characteristics were measured in the present data-set. The nickel observations should therefore be used for selecting the appropriate fish and tissue for assessing the exposure, but not be interpreted as a statement of clinical injury. This is especially pertinent to the elevated spotted snakehead muscle presence.

All muscle profiles measured contained copper, notably in liver of the two farmed species. It is its numerical preponderance in feed that gives reason for investigation of aquaculture inputs, but the presence of a chemical in a feed does not provide a basis for an explanation of tissue concentration without other evidence. No attempt was made to quantify feed intake, assimilation, growth dilution and elimination (Lipy *et al.*, 2021). Furthermore, if there is a high concentration in the liver, there is not necessarily an equivalent concentration in the muscle. The outcome helps to document and monitor real feed consumption on tissue, as well as to verify the abnormal dimensions of the liver.

Environmental Conditions and Exposure Pathways

The low dissolved oxygen observation made in Balu water sets the environmental background to the fish collections. It cannot, however, prove that low oxygen levels was responsible for the nickel patterns, or for the uptake of the metals in a specific sense. Such an interpretation would need a series of measurements and the experimental or observational design to isolate the effects of oxygen from other effects. The water observations are those relating to the water sampling setting. Their greatest contribution is to suggest parameters that should be measured in conjunction with future contaminant measurements, particularly where there is a significant change in water conditions with time.

There is also a temporal mismatch between the river water

and fish profiles. A water sample shows what was in the water at the time of the sampling, while a fish may have picked up material at an earlier time/place. The fraction measured in water may not be representative of some particles and/or food that may be associated with the measured metal. These possibilities account for the fact that one should not rule out prior exposure based on a low water concentration. They are all at best hypotheses, as a paired analysis for sediment, suspended matter, prey, and repeated water samples was not sufficiently documented. All of these potentially pathways cannot be distinguished in the present survey (Hossain *et al.*, 2024).

The results must be evaluated at the sampled product level. Krishibid Group sampled material had the highest feed copper and chromium values and AIT fish feed had the highest cadmium value. Thus, without batch replication, such observations can't be used to determine manufacturer rankings or consistent differences between brands. There are different types of feed and they can be used at different stages of production and/or may include different ingredients. The follow up investigation should involve comparisons of the same type of feed from different batches and document the feed origin prior to determining whether the variation is of practical importance with regard to aquaculture management.

Statistical Interpretation and Analytical Independence

Original correlations do not provide evidence of transfer from water to fish due to uncertain observational basis of these original correlations. The coefficient from combining multiple metals in water and multiple metals in a tissue is a measure of similarity between two chemical profiles. It is not a measure of the variation of one contaminant over and over again across different exposure units. This difference is significant because the various elements have varying behaviour in the environment, detection limits, and sensitivity in the lab. Even if there is no environmental transfer relationship, one relatively high coefficient could dominate a large coefficient.

A related restriction is the correlation between organs. Thus there may be a good correlation between copper levels in rohu and mrigal as both these species are known to have a liver maximum. Such an association refers to tissue structure (Mortuza, 2024). Two species do NOT show equivalent exposure levels, nor do they show that one species predicted another at independent sampling dates. Organ identity should not be regarded as an interchangeable replicate, but is part of the explanation for the pattern. The future analyses should use fish identifiers and measure organs in a design-based analysis that considers the dependence.

Analysis of variance is also limited by the lack of independent observations. Variability within tissue categories should be replicated to estimate the residual variability within each tissue category in comparison among tissue categories. If the users don't have that information, there can't be any significant difference verified from the single concentrations reported. Likewise, uncertainties intervals should be backed up by a biological or analytical replication. The descriptive technique of the present study thus provides continuing numerical data without the unsupported inferential precision. This decision also blocks the mis-representation of standard deviation between anatomically different tissues as uncertainty with a species population average.

Food Safety Implications

The muscle lead observation in mrigal and muscle nickel observation in spotted snakehead are priority observations to be confirmed. But it is not possible to classify the reported ppm until a concentration denominator is known. When the sample is dried, the amount of water removed causes the concentration, expressed as a percentage of sample mass, to increase. Thus, a sample specific conversion between dry matter and wet weight is needed to determine dry matter measurement and limit weight. The assumption of a universal moisture percentage would lead to an input of an unsupported value. Verification should start from the original weighing records or from new measurements performed for the purpose of determining the wet and dry masses (Hasan *et al.*, 2021).

Realistic consumption information would be needed for dietary exposure assessment as well. The mixture that someone who only eats the muscle experiences is different from the mixture that someone who eats the liver, gut or eggs of the fish experiences. Cumulative intake is dependent on three factors: portion size, preparation and frequency (Ali *et al.*, 2020). No data was gathered with regard to these variables during this investigation. Thus, there are no indications from these observations that could be used to support the population hazard quotients, hazard indices or cancer risk estimates. They form a basis on which to choose species and tissue classes for a follow-on exposure study to assess concentrations and consumption patterns that are defined in a compatible fashion.

The difference between contamination evidence and exposure assessment is similar to the approach adopted by Ahmed *et al.* (2015), who also estimated the risk indicators along with the aquatic organism metal concentration in the study area of Bangladesh. This present data set does not contain the parameters required to re-compute that extra step. So it would be inappropriate to take any of their consumption assumption or risk findings as if they were measured at the current sites. A study on another river provides evidence of the relevance of the research question, site-specific exposure has to be established independently.

The results do not necessarily mean that all fish from all the sampled environments should be avoided. They also don't set a benchmark for determining the safety of all muscle samples. Both claims would be beyond the sampling and analytical evidence. The proportionate implication is the targeted confirmation, specifically focusing on the muscle measurements that were

prominent, or involved detectable lead. For internal organs, results are relevant to the extent that the tissues are eaten or used for indications. It should be made clear in public communications that the species is identified, the tissue sampled, the time the sample was collected and the uncertainty, and that an exploratory survey is not a consumption advisory (Shorna *et al.*, 2021).

Implications for Monitoring and Management

Separation of edible tissue surveillance from environmental pathway investigation should occur as part of an improved monitoring programme. The surveillance for edible tissue concentrations would involve fish of an independent origin with known size and weight and be reported on a confirmed wet weight basis from known fish locations. These fish would be correlated with water, sediment, suspended material and feed inputs documented as pathway investigations. Sampling events may be the same for the two components, with different analytical objectives. This would enable an elevated muscle result to serve as confirmation and exposure assessment and not automatically presume that an elevated water result was the cause.

The entire preparation process should be complemented with quality assurance. Examples of useful records are procedural blanks, duplicate digestion results, certified reference material recoveries, calibration checks and element specific detection limits. These records allow to assess contamination added during preparation as well as incomplete recovery from a complex matrix. The current study does not provide evidence of the influence of these factors on the measurements reported (Bhattacharjee *et al.*, 2025). In a follow-on study, the quality information should be provided with the results and non-detects and unusually high results should be considered in the same transparent manner.

The value of the survey for management also depends on the time at which it is conducted. Samples taken in 2017 can not be used to determine current conditions as the industrial activity, river flow, aquaculture, or environmental management may have changed. These values are to record previous observations and to provide questions for further sampling. A contemporary survey should have sufficient methodological similarity to enable it to be compared with the previous survey, with enhanced replication and traceability. Evidence of change would then have to come not from a direct comparison between measurements made under incompatible conditions, but from locations, tissues, concentration bases and analytical performances that are comparable.

Limitations and Research Priorities

Analytical confirmation should start with those measurements that are most likely to impact interpretation. If tested one more time, low concentration samples only would be repeated, leaving the central findings unconfirmed. The results of high copper and nickel should be confirmed with a separate digestion, appropriate dilution and differencing against calibration performance. Blank correction and carryover records would be useful to differentiate a reproducible tissue signal from preparation or instrumental artefacts. The priority of each observation in the order of confirmation should be in accordance with the necessity of its significance in reaching the scientific conclusion and possible relevance for edible tissue surveillance.

There should also be a differentiation between the uncertainty on a measurement and the environmental significance of the measurement. A concentration can be reproducible from sample to sample and not representative of a population. However, multiple field sampling cannot bridge a gap that is in the calculations or a data entry error. Both uncertainties need to be properly documented, through laboratory and field activities, in a coordinated way. This differentiation gives a practical foundation to fortify the study without discarding observations that can be verified by means of the repeat analysis under a well-defined sampling procedure independently repeatable by traceable observations.

These priorities for more detailed tissue sampling are also included in the numerical record. In rohu, nickel concentration was found to be about 3.07 ppm in air sac, 1.98 ppm in muscle, and 1.87 ppm in liver tissue. Nickel was much lower in muscle in mrigal compared to the largest values recorded for the other species. From these comparisons it is suggested that an investigation which only considers one metal or one organ may result in a different ranking of species than an investigation considering another combination. Before sampling, future comparisons should thus be specified in terms of the primary tissue and element sampled, but also include the presence of other organs for interpretation.

It is necessary to use different sampling definitions for egg and gut measurements. Nickel, copper and lead were detected in chewa eggs but data regarding the reproductive stage and handling of the egg sample were not recorded. The gut result is also unable to differentiate material in the gut from metal related to the gut wall unless definitions of cleaning and dissection procedures are given. The observation of Ni at 131.15 ppm is therefore of importance to be confirmed with a well-defined sample. Analysis of cleaned gut tissue and separated contents might be replicated to address the ambiguity material to ecological and dietary interpretation.

A method-specific sensitivity should also be used to evaluate nondetection patterns. Cadmium could not be detected in many of the fish tissues, but when it was detected it was small compared to the concentrations of the other metals. Together with this combination makes the numerical detection limit particularly important. Each result should be reported as being above the quantification limit in a future report and values detected which are not reliably quantified should be identified. This reporting would ensure that apparently exact decimal data would not be assumed to have the same analytical accuracy. It would also enable comparisons to be made

where necessary between matrices which have different preparation factors.

Observations are available but the industrial processes that cause contamination cannot be determined. The choice of sites was based on concerns of pollution; but discharge composition, upstream conditions and distance from identified outfalls were not investigated. Association of a tissue concentration with a particular industry would, therefore, require further evidence. A more extensive source investigation should establish possible discharges, perform multiple upstream and downstream sampling and analyze the metal trends within multiple environmental compartments. Such a design could be a starting point for the evaluation of sources, while minimizing the possibility of assigning responsibility based on the proximity of the source.

For confirmation to be of any practical use it is important to maintain the traceability from collection to reporting. Each fish should be assigned a unique identifier associated with the location of the fish, the date of collection, fish measurements, the mass of the different tissues, the digestion batch, and the instrument run. Feed and water identifiers should be co-locations for co-pairing purposes. A data record should be reproducible and preserve codes that are below detection, for analytical replicates and for independently collected specimens. These records would allow for re-evaluation of the relatively large measurements and examination of the transcription mistakes and calculation of the uncertainty without having to reconstruct the sampling design subsequent to the analysis.

The main drawback is that there is incomplete documentation of numbers of independent samples. No fish identification numbers, collection counts, nor pooling data are provided, so an assessment of the representativeness of the reported values is not possible. There is also no length, weight, sex or age information to limit the biological interpretation even more. The selected fisheries were not representative of all the fisheries in Bangladesh and the species were not captured from all the fisheries. These features decrease the possibility of generalization and the separation of species effect from location effect. They don't remove the measurements that are reported, but set the questions that can be answered from those measurements.

Detection limits, recoveries and final weight basis were not available and analytical uncertainty is therefore large. There are indications of drying temperature inconsistencies and a number of discrepancies between table and figure values that would require reconciliation with actual laboratory records. The entries were hence not changed to corrected values but instead the unresolved entries were disclosed in this paper. There is also a need to clarify tissue labels, for instance fibre and air sac. It may be advisable to keep this distinction in any future reuse between the original recorded category and the verified anatomical definition. This would make the superiority of the both in terms of the comparability with other tissue studies.

Future studies should focus on nickel in the muscle of spotted snakehead, lead in the muscle of mrigal, and the highest levels in the internal organs. The patterns observed would be the subject of repeated collections throughout the seasons to determine if the pattern continues. Where possible, more than one species should be employed at each site and there should be reference sites chosen by explicit criteria. Samples of feed should detect batches and actual farm use. Sediment and water must be collected simultaneously with fish and the manner in which nondetects are handled must be defined prior to statistical analysis. All of this would lend weight to making environmental and dietary interpretations.

CONCLUSION

The present study documents significant variations of the reported level of trace metals in various tissues of five fish species obtained from selected aquatic environment of Bangladesh in 2017. The liver of mrigal and rohu fish showed a significant amount of copper, nickel in chewa gut, and nickel in spotted snakehead muscle. Lead was also found in the muscle of mrigal. These results prove that there does not exist a universal accumulation sequence that can describe the accumulation of the muscle and the internal organs. The species, the tissues and the elements must be kept explicit both in reporting and interpretation. Measurements of water and commercial feed are useful to give additional background information but are not indicators of the sources of tissue metals. Due to incomplete replication records, unreported analytical limits, uncertain concentration basis and conflicting entries, it is not possible to reliably statistically generalize or regulate. There is evidence for targeted confirmatory monitoring, enhanced laboratory traceability and coordinated sampling of edible tissue and environmental compartments. The latter exposure assessment should be conducted based on the verified wet weight concentrations and consumption data measured locally. Until fulfilling those requirements, the current results should be used to inform investigation but not to make definitive estimates of consumer risk.

Data Availability and Ethical Reporting

Analytical observations are provided in the tables and texts. Fish records individually identified by the operators and individual instrument readings and complete quality assurance outputs were not available for further analysis. No human subject data was obtained. The available experimental documentation did not establish an animal ethics approval identifier or a formal exemption. No approval or exemption is asserted in this paper.

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