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Biochemical and ecotoxicological characterization of *Carcinoscorpius rotundicauda*

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Abstract

Carcinoscorpius rotundicauda is an ecologically important horseshoe crab inhabiting estuarine and mangrove systems, yet its biochemical composition and contaminant status remain poorly documented in the northern Bay of Bengal. This study established a preliminary baseline nutritional and ecotoxicological profile of *C. rotundicauda* collected from Cox's Bazar, Bangladesh. Proximate composition, major minerals, and trace metals were quantified using standard analytical procedures, and descriptive visual analyses were applied to evaluate internal metal distribution and nutrient-to-metal association patterns. The specimen exhibited a moisture-rich (87.40%) biochemical profile, with protein as the dominant organic fraction (7.60%), followed by ash, amino acids, lipids, and carbohydrates. Its mineral composition was characterized by relatively high sodium (0.60%), with low calcium (0.20%) and magnesium (0.08%) concentrations. Among trace metals, copper (149.00 mg/kg) and zinc (52.30 mg/kg) dominated the internal metal burden, producing the hierarchy: copper > zinc > lead > cadmium > mercury. Lead (2.40 mg/kg) and cadmium (2.20 mg/kg) exceeded commonly cited food safety limits, whereas mercury remained extremely low (3.4×10^{-2} mg/kg). Nutrient-to-metal association analysis further showed that copper had the strongest associations with protein, amino acids, ash, and lipid, indicating a physiologically structured metal partitioning rather than random accumulation. Although based on a single specimen, this study provides one of the first integrated biochemical and ecotoxicological baselines for *C. rotundicauda* from coastal Bangladesh. The findings support its potential use in seafood risk screening, coastal pollution surveillance, and future bioindicator-based monitoring of estuarine habitats.

Keywords: *Carcinoscorpius rotundicauda*; Proximate composition; Heavy metals; Nutrient–metal interactions; Bioaccumulation; Bangladesh coastline

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1. Introduction

Horseshoe crabs (HSCs) are regarded as the oldest marine living fossils, as their ancestry dates back about 450 million years.[1,2] *Carcinoscorpius rotundicauda* is an HSC species and is found mainly across Asia, including the nutrient-rich but increasingly stressed coastal environments of Bangladesh.[3,4] Across Asia, adult HSCs (e.g., *Tachypleus tridentatus*, *T. gigas*, and *C. rotundicauda*) are harvested for consumption and are also retained as bycatch. They are traded in fish shops and seafood restaurants and are consumed directly or used in traditional Chinese medicine.[5-7] In Hong Kong, market surveys during 2020 to 2021 reported HSC consumption in 80 of 98 seafood shops, while gravid females and eggs remain especially valued in parts of Indonesia, Malaysia, Thailand, China, and Hong Kong.[5,7-9]

In addition to their genetic importance, HSCs serve crucial ecological roles as benthic bioturbators and as prey for migrant birds and shoreline predators.[6,10-12] They are also highly important in medicine due to their copper (Cu)-rich hemocyanin, and lysates derived from *Tachypleus* and *Limulus amebocytes* are already widely used for endotoxin detection.[13] Even though they play important roles in ecology and medicine, relatively little is known about their nutritional value or the levels of contaminants they carry in many regions, particularly in the northern Bay of Bengal.[14] The Bangladesh coast is very productive, but it is also becoming more polluted with metals from factories, aquaculture, and city runoff.[15,16] Sediment-bound pollutants such as lead (Pb), cadmium (Cd), zinc (Zn), Cu, mercury (Hg), and arsenic (As) can build up in benthic habitats where *C. rotundicauda* feeds, leading to bioaccumulation and trophic transfer.[17,18] Seafood and other marine invertebrates are still vital sources of nutrition in many coastal populations. Consequently, assessing both the biochemical content and contamination status of *C. rotundicauda* is crucial for dietary security, food safety, and ecosystem evaluation.[19-21]

There has been extensive research on HSCs regarding conservation, biomedical applications, and general biological properties, but less on their mineral content, heavy metal (HM) burden, and nutrient–metal interactions. Most prior research has focused on morphology, reproductive biology, or physiological responses,[22] resulting in a significant deficiency in dietary and ecotoxicological assessment, especially in South Asia. This limitation creates challenges in studying the species' trophic ecology, adaptive physiological processes, and bioindicator capacity.[19,23]

Traditional nutritional and toxicological evaluations frequently use summary tables or basic bar charts, which may not adequately represent the complex interrelationships among nutrients, minerals, and contaminants.[24,25] Combined visual techniques, such as treemaps, dual-axis comparison plots, and nutrient–metal association matrices, can help clarify complex biochemical datasets.[26,27] These tools are especially helpful for organisms like HSCs, where factors such as physiological condition, molt stage, sediment interactions, and feeding habits can alter the biochemical composition and the accumulation of contaminants.[28,29] Visualization can help interpret limited datasets and establish relationships between biodiversity and contaminant trade-offs.[30-32]

In this regard, the present study provides descriptive biochemical and ecotoxicological information on the *C. rotundicauda* species collected from Cox's Bazar, Bangladesh. This study does not aim to compare populations; instead, it establishes baselines for proximate composition, mineral profile, and metal levels. To define the nutritional profile, proximate nutrients (protein, total lipid, carbohydrate, moisture, ash, and total amino acids) and major minerals (calcium [Ca], sodium [Na], and magnesium [Mg]) were measured. To assess food safety, toxic and essential metals, including Pb, Cd, Hg, Cu, and Zn, were analyzed and compared with the Codex Alimentarius Commission (2015) standards established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).[33] Due to the lack of inferential statistics, descriptive statistics were used to describe the metal distribution and the relative contribution of each metal to the total measured metal burden. This study also serves as a baseline for future studies on quality assessment, bioindicator, and sustainable management of this important species.

2. Materials and methods

2.1. Study area

C. rotundicauda was collected from the Moheskhali Estuary in Cox's Bazar, Bangladesh (Figure 1). This tidal estuary is dominated by mangroves, a clay-like, muddy bottom, and salt marsh plants in the intertidal zone. These characteristics indicate an evolving coastal habitat influenced by persistent tidal flooding and physicochemical fluctuations. The mean temperature and salinity of the water were recorded as 27.47 °C and 23.1 PSU, respectively. Dissolved oxygen concentrations fluctuated from 4.15 to 5.05 mg/L, whereas total dissolved solids varied from 5.05 to 24.87 g/L. Mean nutrient concentrations for nitrate, nitrite, phosphate, and silicate were 0.45, 0.09, 0.08, and 0.07 mg/L, respectively. Water transparency averaged 0.69 m, and electrical conductivity was 36.11 mS/cm across the sampling site.

2.2. Sample collection

During low tide, *C. rotundicauda* were collected by hand from muddy intertidal habitats. To minimize

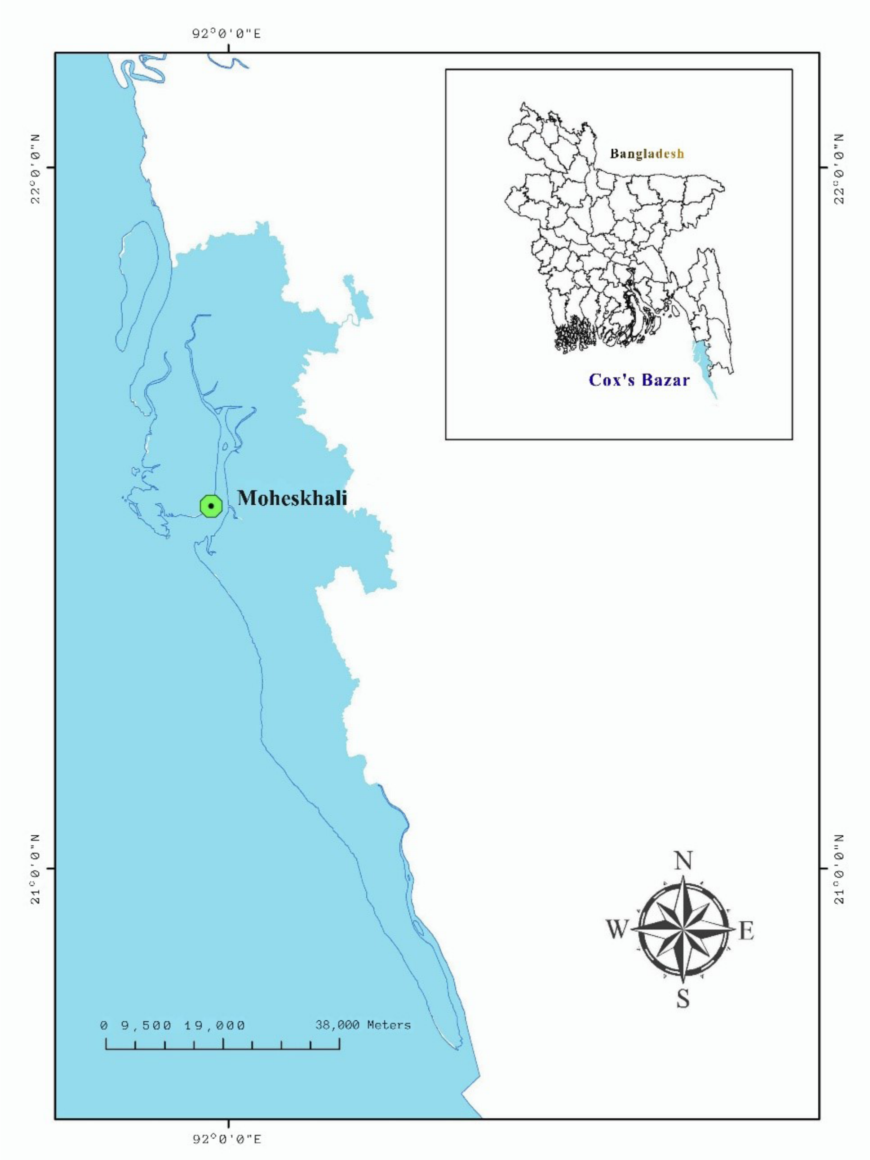

Figure 1. Map of the horseshoe crab collection site in Cox's Bazar, Bangladesh

contamination and ensure safe handling, all specimens were collected using nitrile gloves. The collection sites were characterized by soft muddy sediment, a typical feature of estuarine mangrove habitats. A total of 10 samples were collected, and only one sample was analyzed.

2.3. Determination of proximate composition

2.3.1. Determination of ash content

The ash content of *C. rotundicauda* was determined according to the Association of Official Analytical Chemists (AOAC) method 930.22. A homogenized sample (2–5 g) was incinerated in a muffle furnace at 550 °C for six hours until complete combustion of organic matter. The crucible was cooled in a desiccator and weighed. *Ash* content was calculated using **Equation 1**:

$$A = \frac{Wa}{Ws} \times 100 \quad (1)$$

where A is the ash content (%), Wa is the weight of the ash (g), and Ws is the weight of the sample (g).

2.3.2. Determination of moisture content

Moisture content was determined according to the AOAC method 945.15 by oven drying at 105 °C until constant weight. The moisture content was calculated using **Equation 2**:

$$M = \frac{Wi - Wd}{Wi} \times 100 \tag{2}$$

where M is the *moisture* content (%), Wi is the initial weight of the sample (g), and Wd is the dry weight of the sample after drying (g).

2.3.3. Determination of fat (lipid) content

Fat content was determined using the AOAC method 922.06 through acid digestion, followed by solvent extraction and evaporation of the lipid fraction. Fat content was calculated using **Equation 3**:

$$F = \frac{Wl}{Ws} \times 100 \tag{3}$$

where F is the fat content (%), Wl is the weight of the extracted lipid (g), and Ws is the weight of the sample (g).

2.3.4. Determination of protein content

Protein content was determined using the AOAC method 981.10 (Kjeldahl method). The nitrogen content was quantified through digestion, distillation, and titration. Protein content was calculated using **Equation 4**:

$$P = \frac{(Vs - Vb) \times N \times 14.007 \times Cf}{W \times 10} \tag{4}$$

where P is the protein content (%), Vs is the volume of titrant for the sample (mL), Vb is the volume of titrant for the blank (mL), N is the normality of acid, 14.007 is the atomic weight of nitrogen, Cf is the protein conversion factor (5.75), and W is the weight of the sample (g).

2.3.5. Determination of carbohydrate content

Carbohydrate content was estimated by difference. It was calculated using **Equation 5**:

$$C = 100 - (A + M + F + P) \tag{5}$$

where C is the carbohydrate content (%), A is the ash content (%), M is the moisture content (%), F is the fat content (%), and P is the protein content (%).

2.4. Determination of minerals and heavy metals

Inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS) were used to find the levels of minerals and HMs in *C. rotundicauda*. An Agilent (United States) 7900 instrument was used to measure HMs by ICP-MS, and a Shimadzu (Japan) 7000 instrument was used to measure Ca, Cu, Mg, Na, and Zn using AAS. Before use, all glassware was immersed in 10% (v/v) nitric acid for 24 hours, then rinsed thoroughly with ultrapure water to remove contaminants. All chemicals and reagents used in the analysis were of analytical grade.

2.4.1. Sample digestion

A microwave digestion approach (ETHOS EASY, Italy) was applied to digest the sample. In short, 0.5 g of each sample was placed in a polytetrafluoroethylene digestion vessel and mixed with 7 mL of nitric acid and 1 mL of hydrogen peroxide. Subsequently, the vessels underwent microwave digestion. After digestion, the mixtures were chilled to room temperature and transferred into acid-cleaned 25 mL volumetric flasks in the right amounts. A Whatman No. 44 filter paper was used to filter the digests, and distilled water was added to reach a final volume of 25 mL. The same steps were used to prepare a reagent blank.

2.4.2. Preparation of calibration standards

To prepare calibration standards for ICP-MS analysis, a multi-element stock solution (10 mg/L) was diluted in series to produce working standards at 5, 10, 25, 50, and 100 µg/L. A standard blank was prepared in the same way, but without the stock solution. Working standards were prepared for AAS analysis using certified stock solutions obtained from Fluka Analytical (United States) and Sigma-Aldrich (United States). These standards met the requirements of ISO/IEC 17025 and ISO Guide 34. The stock concentrations were 1000 ± 4 mg/L and were traceable to the National Institute of Standards and Technology. For AAS, the calibration ranges were: 0.2 to 4 mg/L for Ca, 0.2 to 2 mg/L for Cu, 0.02 to 2 mg/L for Mg, 0.03 to 1 mg/L for Na, and 0.05 to 2 mg/L for Zn. Samples with concentrations outside the calibration range were diluted to the appropriate level before measurement.

2.4.3. Instrumental analysis

An Agilent (United States) 7900 ICP-MS with an Octopole Reaction System, an autosampler, and a nebulizer was used to determine HM concentrations. Instrumental operating conditions were optimized as follows: a nebulizer gas flow of 0.91 L/minute, a radio frequency power of 1,200 W, a lens voltage of 1.6 V, a cool gas flow of 13.0 L/minute, and an auxiliary gas flow of 0.70 L/minute. A Shimadzu 7000 AAS was used to determine levels of major and trace minerals, including Ca, Cu, Mg, Na, and Zn. Table A1 shows the conditions for the AAS analysis.

For ICP-MS, calibration curves were constructed by plotting concentration against counts per second, whereas for AAS, calibration curves were generated by plotting concentration against absorbance. Analytical runs were performed one after the other, using acidified deionized water as the instrument blank, followed by calibration standards, reagent blanks, and sample solutions. During elemental assessment, blank readings were taken, and the measured values were adjusted accordingly.

2.4.4. Quality control

To ensure analytical reliability, blank digests and standard blanks were used at every step of the process. Each sample was analyzed, and the average of three measurements was calculated to obtain the final value. The National Institute of Standards and Technology provided certified reference materials used to verify and standardize the analytical method. Calibration curves were then created based on these materials (Table A2). Recovery analysis was used to further assess method performance. The recovery values for the target HMs ranged from 85% to 110%, which shows that the analysis is accurate. The data are shown as mean ± standard deviation. Before analysis, all glassware and sample containers were immersed in 20% nitric acid, rinsed repeatedly with deionized ultrapure water, and dried in an oven.

2.5. Framework for computational analysis and visualization

To integrate the nutrients, minerals, and HMs into a united computational platform capable of generating high-resolution graphics suitable for publication, a customized computational approach was developed. All data analyses were performed in Python 3.10 using Google Colab, and the pandas, numpy, matplotlib, seaborn, and squarify libraries. Data were preprocessed by normalization, scaling, and transformation. These steps were meant to make sure that the nutrient and metal datasets could be compared.[34,35]

Treemap visualizations were created to show the mineral and proximate components of the sample.[26,36] This allowed visualization of the most important biochemical components in a hierarchical manner while preserving size differences. The most important part of the proximate profile, moisture, was removed from the treemap so as not to skew the scale. To ensure visual consistency, the treemaps were color-coded and given precise numbers for easier interpretation. The amounts of toxic metals and essential trace elements were presented in a dual-axis bar chart. This is because the toxic metals (Pb, Cd, and Hg) were vastly different from nutritional metals (Cu and Zn). Consumer safety was evaluated using the acceptable limits established by FAO and WHO. Values for metals that could not be detected were marked as below the detection limit. To improve the plot, gridlines, bold axis labels, and high-contrast color schemes were removed.[27]

This study used a normalized interaction matrix for the specimen to explore potential co-occurrence patterns between nutrients and metals. Min–max normalization was applied separately to nutrients and metals for each individual, and the resulting values were multiplied to determine the interaction scores. This descriptive approach provides a way to display the relationship between nutrients and metals in a biologically relevant manner, without using inferential statistical analysis, which is not appropriate for small sample sizes. Hatched cells indicate where metals are not found. There was only a single specimen; hence, the results were interpreted based on levels, the relative rank of the metal burden, and the relative proportions of the metals in the specimen. This descriptive, visual approach is a transparent way to present biochemical and metal profiles that cannot be statistically analyzed.

3. Results

3.1. Proximate and mineral composition

Moisture constituted the principal component (87.40% of the wet weight) (Figure 2), a value almost similar to the typical findings for marine arthropods and HSCs, where soft tissues commonly exhibit moisture levels ranging from 70% to 80%.[37-40] After moisture, protein was the major organic fraction at 7.60%, followed by ash (3.20%), lipid (1.30%), carbohydrate (0.52%), and total amino acids (2.20%) (Figure 2). This compositional pattern is consistent with those in *Tachypleus gigas,* where the roe and muscle tissues have also been found to be rich in proteins with relatively low lipid and carbohydrate contents and generally similar to the proximate profile of other marine crabs and shellfish.[37,39,41]

The macro-mineral profile was characterized by the relatively high Na content (0.60 %) compared to Ca (0.20 %) and Mg (0.08 %). Increased Na combined with moderate Ca concentrations is characteristic of marine invertebrates and has also been identified in HSC tissues and roe, where Na, potassium, and Ca are the main constituents of the mineral fraction.[38,39]

Trace metal concentrations showed relatively high concentrations of Cu (149.00 mg/kg) and Zn (52.30 mg/kg), while the levels of Pb (2.40 mg/kg) and Cd (2.20 mg/kg) were low, and the concentrations of Hg were extremely low (3.4×10^{-2} mg/kg). The elevated Cu and Zn concentrations reflect their essential physiological roles in crustaceans, where Cu is a key component of hemocyanin, and Zn is involved in enzymatic functions and exoskeletal development. These metals are actively accumulated via gill uptake, dietary intake, and binding to proteins

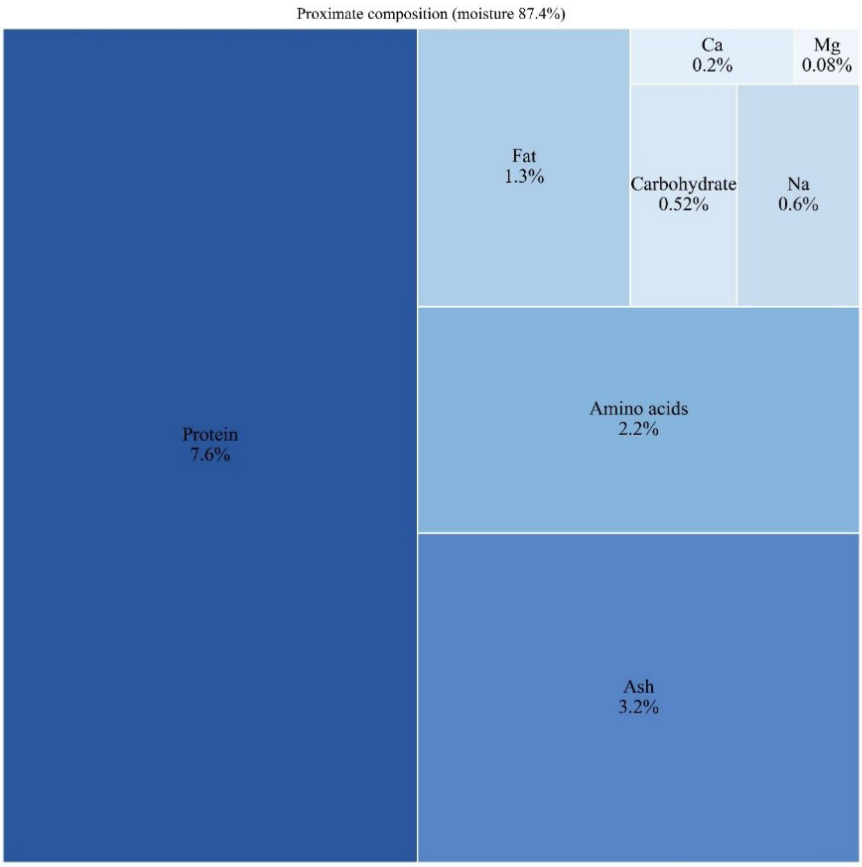

Figure 2. Treemap visualization of the proximate and mineral composition of *Carcinoscorpius rotundicauda* showing the proportional distribution of proximate nutrients (protein, carbohydrate, lipid, ash, moisture, and total amino acids) and major minerals, such as calcium (Ca), sodium (Na), and magnesium (Mg). The moisture value is displayed separately to avoid scale distortion. The size of each block corresponds to the relative percentage of each nutrient or mineral after moisture standardization.

and structural matrices, resulting in their enrichment in tissues and shells. This regulated accumulation, combined with environmental exposure, underpins the effectiveness of crustaceans as bioindicators of trace metal contamination.[37,41-43] Comparable suites of Cd, Pb, and Hg have been measured in the eggs of HSC and other estuarine invertebrates at the base of shorebird food webs, with concentrations showing geographic variation in response to local pollution pressures.[44,45]

Overall, the integrated proximate, mineral, and trace metal profile shows that *C. rotundicauda* exhibits a biochemical profile typical for benthic marine arthropods with high moisture content, a protein-dominated organic matter, and a mineral constituent with high Na and low Mg content. The presence of elevated levels of vital trace metals, especially Cu and Zn, together with relatively low concentrations of non-essential and toxic metals, principally Pb, Cd, and Hg, is indicative of the combined effects of HSCs' metal requirements and their close contact with sediment-bound contaminants. These results provide a biochemical and ecotoxicological baseline for *C. rotundicauda*, along with a nutritional evaluation and potential use as a sentinel species to assess metal exposure in coastal ecosystems under anthropogenic pressure.

3.2. Heavy metal concentrations and compliance assessment

The HM profile in *C. rotundicauda* suggests a burden due to both sediment interactions and physiological metal-handling processes (Figure 3). Among the quantified toxic metals, Pb had the highest concentration at 2.40 mg/kg. The FAO/WHO standards for HMs in seafood typically range from 0.3 mg/kg for Pb and 0.5 mg/kg Cd,[46] and 0.5 mg/kg for Hg, based on the species. Some studies have revealed that Cu and Zn are crucial metals that can be found in higher concentrations.[33,47] Specifically, Cu and Zn limits are respectively around 30 and 30 mg/kg.[48]

The Pb concentration exceeded the FAO/WHO permissible limit for crustaceans, confirming non-compliance with international food safety standards. Cd was assayed at 2.20 mg/kg, which is also above the FAO/WHO limit. In crustaceans, Cd often accumulates in metabolically active tissues, such as the hepatopancreas, an observation consistent with Cd absorption from sediment-associated sources. The extremely low Hg concentration (3.4×10^{-2} mg/kg) observed in this study is consistent with previously reported baseline levels in HSCs from relatively unpolluted coastal environments, indicating limited bioavailability and minimal Hg accumulation in this species, rather than an anomalous result.[49]

The profile of essential trace metals was characterized principally by Cu and Zn, with Cu at 149 mg/kg and Zn at 52.3 mg/kg. The elevated Cu levels are expected in HSCs because of the Cu-based hemocyanin component of arthropod hemolymph,[50] while Zn is a key component of enzymatic activity and structural integrity.[51,52] Although the two elements are recognized as essential micronutrients, at high concentrations they can serve as biomarkers of metal bioavailability in benthic ecosystems.

Collectively, most of the metals measured in the specimen exceeded the FAO/WHO permissible limits, indicating a need for immediate food safety measures. The results in terms of metal composition are characteristic of benthic marine invertebrates living in coastal systems and support the suitability of *C. rotundicauda* as a baseline reference species to assess metal exposure in estuarine environments.[53]

3.3. Relative metal burden structure

The metal–metal distribution profile shows the relative internal burden and compositional hierarchy of detected metals in *C. rotundicauda* (Figure 4). This analysis defines the proportional contributions and dominance structure of individual metals to the total metal load, as the internal metal fractions and their relative proportions better reflect ecotoxicological status than total concentrations.[54]

Among the detected metals, Cu accounted for the largest fraction of the total metal burden, followed by Zn. The pronounced predominance of Cu is compatible with the hemocyanin system of arthropods, where Cu is a key respiratory metal naturally found in hemolymph and associated tissues at high concentrations.[50,55,56] Zn,

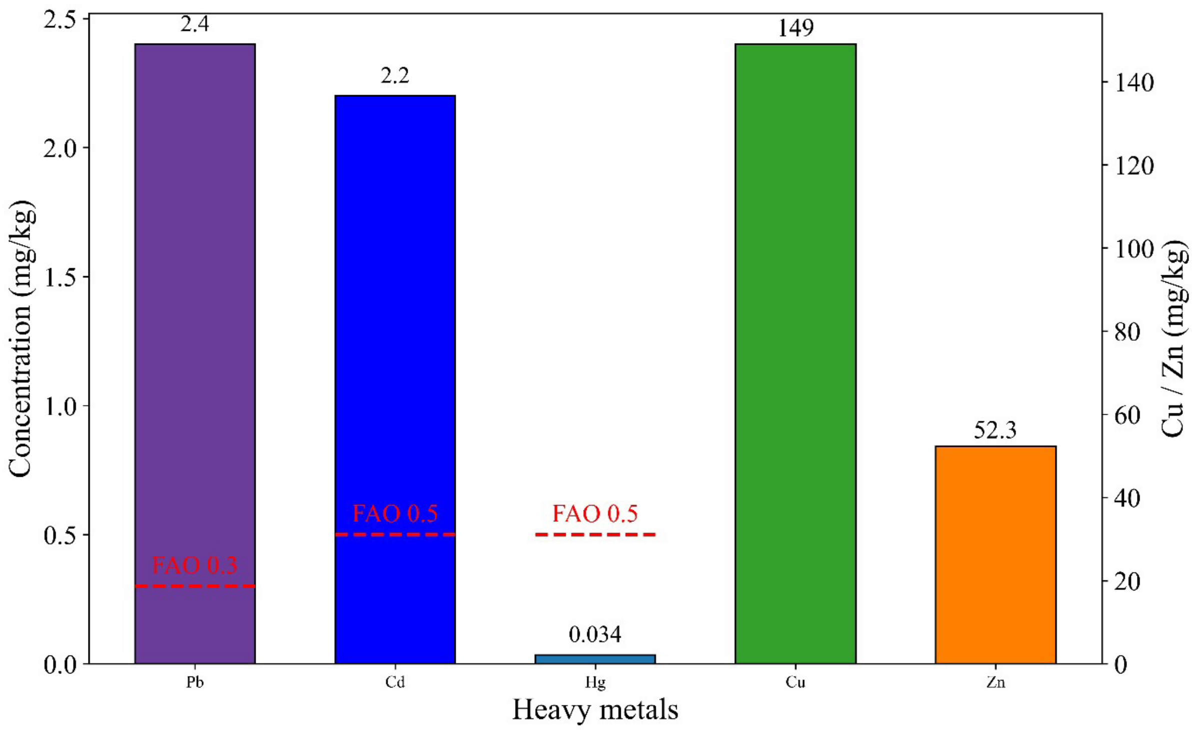

Figure 3. Heavy metal concentrations and compliance with the Food and Agriculture Organization (FAO)/World Health Organization (WHO) guidelines in *Carcinoscorpius rotundicauda*. Dual-axis bar chart showing the amount of toxic metals: lead (Pb), cadmium (Cd), and mercury (Hg), and essential trace metals: copper (Cu) and zinc (Zn). The FAO/WHO and European Union permissible limits for crustaceans are shown as red dashed reference lines. Essential metals were plotted on a secondary axis because of their higher magnitudes.

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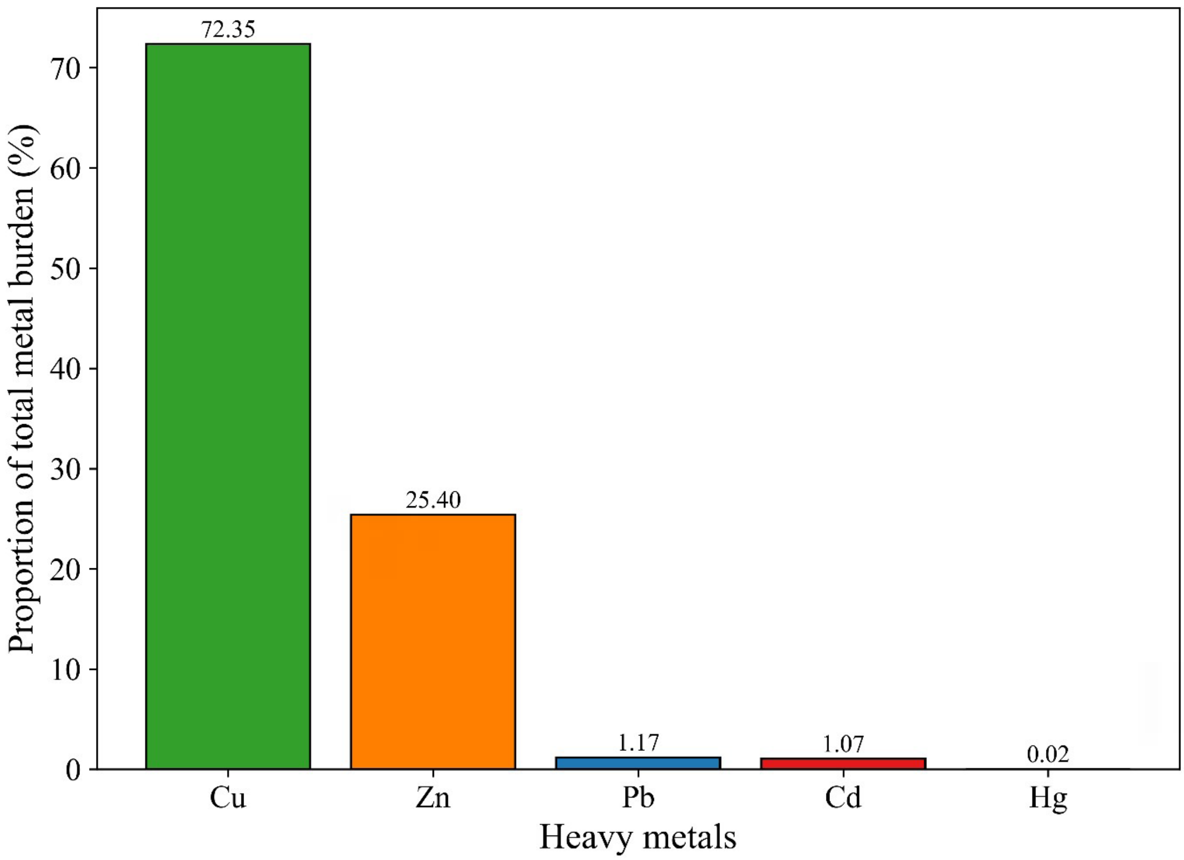

Figure 4. The metal–metal distribution defines the relative contribution of each of the metals detected to the aggregate internal metal burden in the specimen, highlighting a predominance of vital metals: copper (Cu) and zinc (Zn) over toxic metals: lead (Pb), cadmium (Cd), and mercury (Hg).

as the second-largest fraction, reflects its fundamental roles in enzymatic activity, protein stabilization, and shell mineralization.[56,57]

The relatively low contribution of non-essential and toxic metals such as Pb, Cd, and Hg to the total metal load reflects their lack of biological function and the presence of physiological mechanisms that limit their uptake and enhance detoxification and excretion, resulting in lower accumulation than that of essential trace metals.[51,58] Organisms generally keep them at low internal levels by reducing uptake and by detoxification/sequestration.[54,57] Pb and Cd were found at similar low concentrations, and Hg was present at trace levels, contributing insignificantly to the total metal burden. This metal hierarchy (Cu > Zn > Pb > Cd > Hg) indicates the predominance of essential trace metals in the internal metal profile. In summary, the metal–metal analysis provides a brief representation of the internal metal burden structure at the individual level, serving as a baseline for explaining patterns of metal dominance in benthic marine arthropods.

3.4. Nutrient–metal association patterns

The nutrient–metal association matrix obtained from *C. rotundicauda* reveals distinct co-occurrence patterns at the individual level (Figure 5). The analysis emphasizes the relative strength of the relationships among biochemical components using a normalized product-based formulation, highlighting the main pathways of metal binding and distribution.

In the analyzed specimen, the most significant correlations were observed with Cu and organic nutrient fractions, particularly protein, amino acids, ash, and lipids. The robust associations between Cu and proteins, as well as Cu and amino acids, align with the extensively recorded physiological function of Cu in arthropods, where it acts as a critical structural element of hemocyanin and exhibits a strong affinity for nitrogen-rich biomolecules and peptide complexes.[50,59] This association implies that Cu is predominantly bound to proteins and amino acids through specific biochemical interactions, reflecting its active involvement in metabolic processes and indicating that its distribution is controlled by physiological regulation rather than passive environmental accumulation. The relatively high Cu–ash association further indicates interaction with inorganic and mineralized tissue components, consistent with Cu partitioning between organic and inorganic compartments.

Zinc showed moderate associations with protein, amino acids, and ash, indicating its involvement in enzymatic and structural functions in living organisms.[51,52] Relative to Cu, Zn showed a more distributed association profile, suggesting that a greater number of biochemical pools were involved and it was not dominated by a single fraction. Associations between Zn and lipids or carbohydrates were comparatively low, reflecting poor interaction with either nutrient reservoir. Pb, Cd, and Hg had uniformly low association scores across all nutrient classes. This pattern indicates poor biochemical integration of these metals into the measured nutrient fractions and is consistent with their relatively low concentrations in the specimen.[54,57] Of the nutrients tested, Na and Ca showed little effect on any metal, indicating a poor role of ionic balance/electrolyte pools in metal binding under the physiological conditions observed.

In summary, metal–nutrient interactions among *C. rotundicauda* are mostly determined by essential metal physiology, with Cu-driven associations at the center of the internal interaction network. The low association scores for toxic metals highlight a metal-specific biochemical handling strategy in the organism. These findings provide a baseline of nutrient-metal co-occurrence at the individual level and support the usefulness of *C. rotundicauda* as a model organism for research on physiologically mediated metal distribution in benthic marine arthropods.

3.5. Regional comparison of heavy metal accumulation in horseshoe crabs

The levels of HMs observed in the *C. rotundicauda* in the present study were compared with previous reports from other geographical areas to assess regional variability and tissue-specific patterns of HM accumulation (Table 1). The findings of the current research are reported on a wet-weight scale, whereas most published research reports concentrations on both a dry-weight and a wet-weight scale. The concentrations of the essential metals Cu (149 mg/kg)

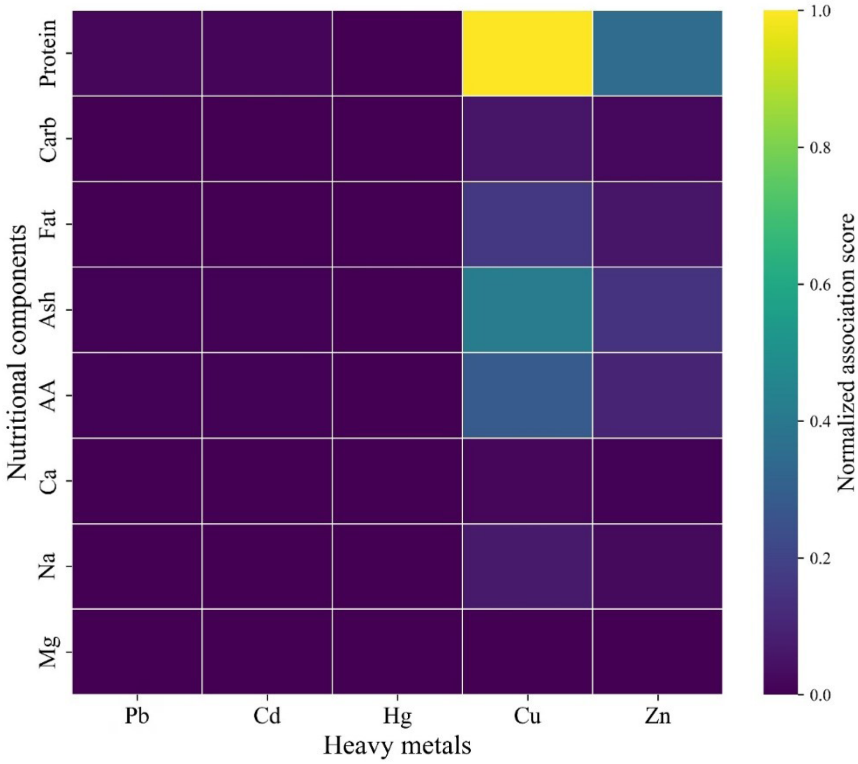

Figure 5. Nutrient–metal association matrix for *Carcinoscorpius rotundicauda*. Normalized nutrient–metal association matrices illustrating co-occurrence patterns between nutrients, such as protein, fat, carbohydrate (Carb), ash, amino acids (AA), calcium (Ca), sodium (Na), magnesium (Mg), and heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), copper (Cu), and zinc (Zn). Associations were computed using min–max normalization, followed by a normalized product model, yielding a 0–1-scaled interaction score. Lighter shades indicate a stronger nutrient–metal co-occurrence. The matrices reveal specimen-specific biochemical pathways that influence metal uptake and distribution.

and Zn (52.3 mg/kg) were relatively high in the present study. Similar or even greater levels of these metals have been noted in Malaysian populations of *C. rotundicauda* and *T. gigas*, especially in digestive organs, gills, abdominal muscle, operculum, and eggs, with the Cu concentration often exceeding 100 mg/kg and the Zn concentration often several hundred mg/kg on a dry-weight basis.[60,61]

The comparatively elevated Cd concentration observed in the Bangladesh specimen (2.2 mg/kg wet weight) warrants careful interpretation in the context of previous studies. Reported Cd concentrations in Malaysian HSCs were generally lower in several tissues. However, relatively elevated levels were also found in eggs, the telson, the abdomen muscle, and the operculum, depending on locality and species.[60,62] This demonstrates that Cd accumulation in HSCs is highly tissue-specific and region-dependent. Direct numerical comparison should therefore be made cautiously, as the present study analyzed muscle tissue on a wet-weight basis, whereas many previous studies examined multiple organs and often reported results on a dry-weight basis. Nevertheless, the Bangladesh value remains notable and may indicate stronger local Cd exposure in estuarine habitats influenced by sediment-associated contamination. Similar regional and tissue-specific differences in Cd accumulation have also been reported from Atlantic HSC populations.[63-65]

HSC tissues have also been found to exhibit significant regional and tissue-specific differences in Cd accumulation, with elevated levels reported in tissues of both species in Malaysia and in eggs and appendages along the Atlantic coast of the United States.[63-65] The levels of Pb in the current research (2.4 mg/kg) were higher than those detected in HSC eggs and muscle tissues from various regions, such as Japan, Vietnam, and portions of the United States, where levels were lower.[49,63,66] On the other hand, in HSC eggs and terminal appendages, Pb has also been reported to be higher in some coastal areas of the United States, suggesting large geographical differences in Pb levels.[64,65]

The Hg levels in *C. rotundicauda* in Bangladesh were very low (3.4×10^{-2} mg/kg) compared to other Hg measurements in *Limulus polyphemus* from the Atlantic coast of the United States, which showed a wide range of values across tissues and sites.[64,65] In Japan and Vietnam, studies tended to find lower Hg levels in the tissues of the HSCs, especially in muscle and egg tissues, of the species *T. tridentatus*,[49,66] again demonstrating regional variations in accumulation patterns of Hg. Generally, comparative studies indicate significant regional and tissue-specific variations in HM accumulation across HSC species, as evidenced by published data from South and Southeast Asia, East Asia, and North America.

3.6. Integrated interpretation and ecological significance

The combined analysis of proximate composition, mineral distribution, HM load, and nutrient–metal relationships provides a consistent description of the biochemical condition and ecological environment of *C. rotundicauda* in coastal environments. The visual data, derived from treemap images, metal burden, and nutrient–metal correlation matrices, elucidate the complex interrelations between an organism's physiology (sedimentary exposure), environmental metal availability, and sedimentary exposure. The nutritional plasticity of the genus *C. rotundicauda* is evident in the variation of the corresponding concentrations of proteins, carbohydrates, lipids, and minerals. This fluctuation aligns with the differences in feeding history, metabolic condition, molting phase, and habitat preferences.[67] A relatively higher carbohydrate content than lipid may reflect short-term metabolic status, recent feeding history, or species-specific nutrient allocation, but it should not be interpreted as direct evidence of recent energy expenditure or dietary change in the absence of controlled physiological data. On the other hand, high levels of Ca and Mg ions likely indicate that the body is performing functions such as repairing and mineralizing the exoskeleton.[68,69]

The heterogeneity of exposure, as reflected in patterns of HM accumulation, is further emphasized. Variation in the comparative abundance of Pb, Cd, Cu, and Zn can be used to depict the non-uniform distribution of sediment-bound contaminants, to show how benthic arthropods are sensitive to microscale changes in substrate contact, and to illustrate how foraging behavior is influenced by these changes. Similar effects have been found with other sediment-dwelling crustaceans, in which local gradients of exposure are converted into quantifiable variations in internal metal loads.[70] These observations are synthesized into nutrient–metal association matrices that show the mediation of biochemical composition in mediating metal partitioning in the organism. Close associations of the key metals with protein- and ash-rich fractions indicate that their binding is physiologically controlled, whereas weak associations of the non-essential ones indicate that few metals are integrated into the biochemical sphere and could be detoxified or stored.[71-73] Collectively, these interactions are of ecological interest because they affect metal storage, maximum detoxification, and trophic transfer potential, which supports the usefulness of using the ecotoxicological assessment model at the individual scale in coastal ecosystems within the context of the ecotoxicology approach.[74,75]

Table 1. Comparison of heavy metal concentrations (mg/kg) in *Carcinoscorpius rotundicauda* with international findings

Species/ Guideline	Body parts	Weight base	Copper	Zinc	Cadmium	Lead	Mercury	Geographical regions	References
Carcinoscorpius rotundicauda	Muscle	Wet	149	52.3	2.2	2.4	3.4×10^{-2}	Bangladesh (Cox's Bazar)	This study
	Eggs	Dry	112	164	5.32	–	–	Malaysia (Kg. Pasir Puteh)	60
	Prosomatic carapace	Dry	8.22–7.48	25.7–21.8	0.78–1.82	–	–	Malaysia (KP Puteh & BTPoh)	60
	Opisthosomatic carapace	Dry	19.2–16.7	21.4–18.1	1.51–1.85	–	–	Malaysia (KP Puteh & BTPoh)	60
	Telson	Dry	9.53–14.9	11.9–21.4	5.77–5.64	–	–	Malaysia (KP Puteh & BTPoh)	60
	Eggs	Dry	112–52.1	164–136	5.32–2.15	–	–	Malaysia (KP Puteh & BTPoh)	60
	Gills	Dry	97.2–82.4	111–82.5	1.43–1.59	–	–	Malaysia (KP Puteh & BTPoh)	60
	Operculum	Dry	39.5–56.7	77–57.9	4.22–3.12	–	–	Malaysia (KP Puteh & BTPoh)	60
	Abdomen muscle	Dry	65.2–58.8	406–473	5.71–5.98	–	–	Malaysia (KP Puteh & BTPoh)	60
	Digestive organs	Dry	184–89.6	227–240	1.40–2.07	–	–	Malaysia (KP Puteh & BTPoh)	60
	Remainder of the shell	Dry	40.4–34.5	77–82.7	1.54–1.38	–	–	Malaysia (KP Puteh & BTPoh)	60
	Remainder of soft tissues	Dry	96.9–303	223–34	1.93–3.53	–	–	Malaysia (KP Puteh & BTPoh)	60
Tachypleus gigas	Prosomatic carapace	Dry	22.4–8.2	17.9–27.4	1.08–1.78	–	–	Malaysia (KP Puteh & LKTengar)	60
	Opisthosomatic carapace	Dry	24.2–12.5	19.0–15.9	0.89–1.14	–	–	Malaysia (KP Puteh & LKTengar)	60
	Telson	Dry	19.2–18.6	9.28–13.6	6.03–6.13	–	–	Malaysia (KP Puteh & LKTengar)	60
	Eggs	Dry	89.9–49.4	126–146	5.41–5.00	–	–	Malaysia (KP Puteh & LKTengar)	60
	Gills	Dry	107–80.6	87.7–73.8	1.22–1.97	–	–	Malaysia (KP Puteh & LKTengar)	60
	Operculum	Dry	58.2–28.2	52.6–41.6	4.06–3.98	–	–	Malaysia (KP Puteh & LKTengar)	60
	Abdomen muscle	Dry	37.9–36.4	471–375	5.99–2.18	–	–	Malaysia (KP Puteh & LKTengar)	60
	Digestive organs	Dry	151–52.3	295–343	1.71–1.50	–	–	Malaysia (KP Puteh & LKTengar)	60
	Remainder of the shell	Dry	26.1–22.5	56.9–56.1	4.11–3.50	–	–	Malaysia (KP Puteh & LKTengar)	60
	Remainder of soft tissues	Dry	146–57.1	237–254	3.95–6.00	–	–	Malaysia (KP Puteh & LKTengar)	60
	Apodeme (flesh)	Dry	129.95	921.11	4.16	–	–	Malaysia (Pekan, Pahang)	61

(*cont'd...*)

Table 1. (*Continued*)

Species/Guideline	Body parts	Weight base	Copper	Zinc	Cadmium	Lead	Mercury	Geographical regions	References
	Gill	Dry	102.22	912.68	3.70	–	–	Malaysia (Pekan, Pahang)	61
	Gut	Dry	183.42	606.44	3.80	–	–	Malaysia (Pekan, Pahang)	61
	Leg	Dry	60.85	615.01	2.12	–	–	Malaysia (Pekan, Pahang)	61
	Mouth	Dry	97.10	605.36	2.59	–	–	Malaysia (Pekan, Pahang)	61
	Eggs	Wet	43.3–70.02	53.08–71.62	0.02–0.52	10–25.84	–	Malaysia (Port Dickson)	62
	Eggs	Wet	30.54–120.32	46.34–150.53	1.38–4.11	–	–	Malaysia (Johor Bharu)	62
Carcinoscorpius rotundicauda	Eggs	Wet	18.84–65.44	34.65–104.08	0.52–3.64	–	–	Malaysia (Johor Bharu)	62
Limulus polyphemus	Eggs	Dry	–	–	0.01–0.04	0.01–0.10	0.05–0.13	USA (Jamaica Bay)	63
	Eggs	Dry	–	–	0.01–0.03	0.01–0.14	0.04–0.09	USA (West Jones Beach)	63
	Eggs	Dry	–	–	0.01–0.13	0.02–0.12	0.04–0.16	USA (Manhasset Bay)	63
	Eggs	Dry	–	–	0.01–0.17	0.02–0.22	0.04–0.09	USA (Oyster Bay)	63
	Eggs	Dry	–	–	0.01–0.07	0.01–0.07	0.04–0.11	USA (Westhampton Bay)	63
	Eggs	Wet	–	–	16–44	20–55	10–21	USA (New Jersey)	64
	Eggs	Wet	–	–	16–22	24–55	10–19	USA (Delaware Bay)	64
	Eggs	Wet	–	–	7–78	6–73	0.2–26	USA (Atlantic coast)	65
	Legs	Wet	–	–	6–107	2–57	0.3–115	USA (Atlantic coast)	65
Tachypleus tridentatus	Muscle	Wet	0.037	0.610	–	–	–	Vietnam	66
	Eggs	Wet	0.093	0.076	–	–	–	Vietnam	66
	Hepatopancreas	Wet	0–120	53–113	0.96–1.64	0.12–0.24	0.14–0.20	Japan (Hakata Bay)	49
	Hepatopancreas	Wet	33–117	0–259	0.31–1.27	0.27–0.49	0.05–0.11	Japan (Habu Bay)	49
	Eggs	Wet	16.6–23.4	28.6–35.4	0.004–0.016	–	0.04–0.06	Japan (Hakata Bay)	49
	Eggs	Wet	17.3–22.7	28.8–35.2	0.01	–	0.01–0.03	Japan (Habu Bay)	49
	Muscle	Wet	2.9–16.9	101–147	0.01–0.03	–	0.07–0.09	Japan (Hakata Bay)	49
	Muscle	Wet	8.1–15.9	111–133	0.007–0.013	–	0.03–0.05	Japan (Habu Bay)	49
FAO/WHO and European Commission Guideline	–	–	100	30	0.05	0.3	0.5	–	33, 46-48

Abbreviations: FAO: Food and Agriculture Organization; USA: United States of America; WHO: World Health Organization.

4. Discussion

The present study provides one of the first integrated biochemical and ecotoxicological assessments of *C. rotundicauda* from the northern Bay of Bengal, offering important insights into its nutritional composition, metal burden, and ecological significance. The proximate composition observed is consistent with previously reported ranges for marine arthropods and HSCs, in which high moisture and moderate protein content predominate in soft tissues.[37-40] This protein-dominated, low-lipid profile is also comparable to that of other species, such as *T. gigas*, suggesting conserved biochemical traits across HSC taxa.[39,41]

The macro-mineral composition, characterized by relatively elevated Na with moderate Ca and Mg, reflects the osmotic and ionic regulation typical of estuarine invertebrates exposed to fluctuating salinity conditions.[38,39] Such mineral patterns are frequently observed in marine crustaceans and indicate physiological adaptation to dynamic coastal environments.

A major finding of this study is the predominance of essential trace metals, particularly Cu and Zn. Elevated Cu concentrations are biologically expected due to its structural role in hemocyanin, the oxygen-transport protein in arthropods.[50,56] Similarly, Zn plays a central role in enzymatic activity and structural integrity. As an essential trace element, Zn functions as a cofactor for numerous enzymes involved in metabolic regulation, antioxidant defence, protein synthesis, and cellular repair. It also contributes to the stabilization of proteins and membranes and may support shell mineralization and other structural processes in marine arthropods. Therefore, the relatively high Zn concentration observed in *C. rotundicauda* is consistent with its broad physiological importance and regulated biological accumulation rather than solely passive environmental exposure.[52,53,58] The observed dominance hierarchy (Cu > Zn > Pb > Cd > Hg) aligns with previous findings in benthic invertebrates and reflects regulated physiological accumulation of essential metals rather than passive environmental uptake.[37,41]

Despite this physiological regulation, the elevated concentrations of Pb and Cd exceeding FAO/WHO permissible limits raise important food safety concerns.[33,46] These elevated levels likely originate from sediment-associated contamination driven by anthropogenic activities such as industrial discharge, aquaculture, and urban runoff in coastal Bangladesh.[15,16] The accumulation of these toxic metals in a benthic organism highlights the critical role of sediment as a contamination reservoir and supports earlier findings that benthic feeders are particularly vulnerable to bioaccumulation.[17,18]

The extremely low Hg concentration observed is consistent with previous studies indicating limited bioaccumulation of Hg in HSCs due to their feeding ecology and reduced trophic transfer of methylmercury.[45,49] This further supports the species-specific differences in metal accumulation pathways across marine organisms.

The nutrient–metal association analysis further strengthens the interpretation of physiologically mediated metal distribution. Strong associations between Cu and protein, amino acids, and ash reflect its affinity for nitrogen-rich biomolecules and structural integration into hemocyanin and related complexes.[50,59] In contrast, Zn exhibited more distributed associations, consistent with its role in diverse enzymatic systems.[52,53] The weak associations observed for Pb, Cd, and Hg suggest limited biochemical integration and possible detoxification or sequestration mechanisms, as reported in other marine invertebrates.[55,58]

A comparative analysis with global datasets reveals significant regional variability in metal accumulation. The Cu and Zn levels observed in this study fall within the range reported for Southeast Asian populations, particularly in Malaysian HSCs, where tissue-specific accumulation can be substantial.[60,61] However, the comparatively higher Cd and Pb levels in the present study suggest localized environmental pressure on the Bangladesh coast. Similar geographic variability has been documented across regions such as Japan, Vietnam, and the United States, emphasizing the influence of environmental conditions and pollution sources on metal bioaccumulation.[63,64,66]

From an ecological perspective, these findings reinforce the suitability of *C. rotundicauda* as a bioindicator species. Its benthic feeding behavior, sediment interaction, and ability to accumulate both essential and toxic metals make it an effective sentinel for monitoring coastal pollution.[53] Moreover, the integration of visualization techniques, such as nutrient–metal interaction matrices and metal burden distributions, provides a more comprehensive understanding of complex biochemical relationships compared to conventional descriptive methods.[26,27]

Nevertheless, the study is constrained by the analysis of a single specimen, limiting statistical inference and generalization. While descriptive and visualization-based approaches offer valuable baseline insights, future research should incorporate larger sample sizes, seasonal variation, and tissue-specific analyses to validate these findings and improve ecological interpretation.

This study establishes a foundational biochemical and ecotoxicological baseline for *C. rotundicauda* in coastal Bangladesh. The species demonstrates a nutritionally valuable yet environmentally responsive profile, where physiological metal regulation coexists with evidence of anthropogenic contamination. This dual role highlights its importance not only as a potential food resource but also as a critical bioindicator for assessing coastal ecosystem health under increasing environmental stress.

A key limitation of this study relates to the stochastic intrinsic uncertainty associated with single-specimen analysis. Given that the results are derived from a single specimen, the observed biochemical composition and metal concentrations may reflect individual-specific variability rather than population-level trends. In benthic estuarine organisms such as *C. rotundicauda*, metal accumulation and nutrient composition are inherently influenced by stochastic factors including microhabitat heterogeneity, feeding history, molt stage, and physiological condition. These factors introduce random variability in both exposure and internal partitioning of metals, which cannot be quantified in the absence of replication. Furthermore, sediment-associated metal availability is spatially heterogeneous at fine scales, leading to probabilistic and non-uniform uptake pathways. Consequently, the measured metal burden and nutrient–metal associations may partly represent localized and transient conditions rather than stable ecological patterns. Although the use of descriptive and visualization-based approaches provides a transparent baseline, the lack of statistical replication prevents estimation of variance and uncertainty bounds. Therefore, the findings should be interpreted as indicative rather than definitive, and future studies incorporating larger sample sizes and spatial replication are necessary to constrain stochastic variability and validate the observed patterns.

Several recommendations for future work can be derived from this study:

(i) Multi-individual and tissue-specific sampling of seasonal and coastal gradient intervals in Bangladesh will confirm the biochemical and metal baseline developed at the single-specimen level.
(ii) Using HSCs as sentinel organisms in routine coastal studies will be especially relevant in sediment-related metals (Pb, Cd, Cu, Zn) in estuarine and mangrove ecosystems.
(iii) The use of specific food safety warnings and regulatory controls will deter human use of the HSCs that grow in polluted coastal areas where the concentration of Pb and Cd is more than the internationally accepted limit of the levels of use.
(iv) To reinforce ecotoxicology risk assessment, future analysis needs to include more pollutants (e.g., As species, organic pollutants) and biomarkers of oxidative stress to support future analyses.
(v) The use of uniform wet and dry reporting systems will enhance the comparativeness of the metal-accumulation data of various regions and studies.
(vi) Integrating biochemical profiling of populations with population ecology and conservation research will enable an evaluation of the effects of metal exposure due to pollution on reproductive biology and long-term population sustainability of the species.

5. Conclusion

The study is an integrated biochemical and ecotoxicological analysis of *C. rotundicauda* from coastal Bangladesh. The baseline was established based on an overall examination of a single specimen. The proximate composition, including high moisture content and an organic fraction based on protein, agreed with the physiological profiles of benthic marine arthropods, but the mineral composition showed high Na and moderate Ca levels, as is characteristic of estuarine invertebrates. Physiological needs strongly influenced the metal burden, with the essential trace metals, especially Cu and Zn, accounting for the largest share of the internal metal load, thereby indicating their roles in hemocyanin and enzyme activities. On the contrary, the proportion of non-essential and toxic metals (Pb, Cd, and Hg) was lower; however, the levels of Pb and Cd in the seafood exceeded the permissible levels set by the FAO/WHO and the European Union, indicating possible food safety risks and localized environmental pollution.

Metal distribution analyses in nutrient/metal association showed that organic nutrient pools, particularly protein- and ash-rich fractions, generally mediate nutrient/metal associations, thereby again supporting the idea of physiologically controlled metal partitioning rather than stochastic accumulation. The relatively low absorption of toxic metals into nutrient stores suggests that active processes are in place to control or store them, even when the environment is contaminated. Despite the limitations of the study due to a small sample size, it establishes a reference framework for the biochemical composition and metal hierarchy of the understudied region for *C. rotundicauda* research. These findings highlight the two-fold importance of this species as a valuable nutritional source and a sensitive bioindicator of metal pollution at the coastline. As a whole, the research suggests that further population-scale research is needed to better address spatial variability, ecological risk, and the population health consequences of metal exposure in *C. rotundicauda* populations from Bangladesh's coastal ecosystems.

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Conflict of interest

The authors declare no financial or personal conflicts of interest that could have influenced this work.

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Availability of data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Appendix

Table A1. Conditions for analyzing heavy metals in a sample solution using atomic absorption spectrometry

Metals	Hollow cathode lamp condition		
	Lamp current (mA)	Wavelength (nm)	Slit width (nm)
Calcium	10/0	422.7	0.5
Copper	6/0	324.8	0.5
Magnesium	8/0	285.2	0.5
Sodium	10/0	589.0	0.2
Zinc	8/0	213.9	0.5

Table A2. Certified reference material for heavy metals and minerals

Metals and minerals	Limit of detection	Limit of quantification	Recovery percentage
Calcium	0.02	0.08	95–105%
Cadmium	0.5	1.60	95–105%
Copper	0.02	0.09	95–105%
Mercury	2.3	4.6	95–105%
Magnesium	0.01	0.06	95–105%
Sodium	0.01	0.03	95–105%
Lead	3.5	7.1	95–105%
Zinc	0.01	0.005	95–105%