

Preliminary comparative bioconcentration of heavy metals in aquatic organisms from selected ecosystems in Terengganu and Kelantan, Malaysia

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ABSTRACT

Heavy metal contamination in aquatic ecosystems poses a significant threat to environmental health and food safety, particularly in biodiverse regions such as Terengganu and Kelantan, Malaysia. This study assessed the concentrations of eleven (11) heavy metals - silver (Ag), aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn) in eight (8) aquatic species from freshwater, brackish and marine habitats. The sampled organisms represented three major phyla: Chordata, Arthropoda, and Mollusca, collected from six study areas with different ecosystems. Samplings were conducted from March to April 2025. Heavy metals from the organisms' tissues were detected using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Results revealed significant interspecies and spatial variations in metal accumulation ($p < 0.05$; One-Way ANOVA), primarily influenced by habitat type, feeding strategy, and ecological niche rather than body size. Bivalve species (*Anadara pilula* and *Polymesoda expansa*) and the mud crab (*Scylla tranquebarica*) from Setiu Wetlands exhibited the highest metal concentrations, particularly for Zn (43.8 ± 3.88 , 37.12 ± 3.20 and 33.67 ± 2.85 mg/kg, respectively) and Fe (19.9 ± 1.77 , 75.92 ± 6.84 and 4.48 ± 0.39 mg/kg, respectively). In contrast, fish species of *Diagramma pictum* from Beting Lintang displayed significantly lower concentrations (Fe- 0.803 ± 0.07 mg/kg; and Zn- 0.816 ± 0.07 mg/kg). This study highlighted the importance of using benthic filter feeders for biomonitoring and provides a scientific basis for future research aimed at developing a bioaccumulation-based risk assessment model for sustainable aquatic resource management.

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1. INTRODUCTION

Heavy metals (HMs) are elements characterised by high atomic weight and density exceeding 5 g/cm^3 , known for their durability, solubility, and potential toxicity (Aji & Olufemi, 2024). These elements are categorised into essential (e.g., magnesium (Mg), nickel (Ni), sodium (Na), copper (Cu), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), potassium (K) and zinc (Zn)) and non-essential metals (e.g., silver (Ag), lead (Pb), mercury (Hg), and cadmium (Cd)) (Foley et al., 2021). While essential HMs act as micronutrients in metabolic activities such as redox reactions, osmoregulation, and enzymatic functions, they become toxic at elevated concentrations. Non-essential HMs, in contrast, lack biological importance and functions, and are harmful to organisms even at the lowest levels, leading to disruptions in membrane integrity, enzyme functions, and DNA structure (Bharti & Sharma, 2022).

Heavy metals originate from both natural (e.g., volcanic eruptions, forest fires, windblown dust) and anthropogenic sources, with industrial activities such as electroplating, mining, fossil fuel combustion, and agricultural

chemical use constituting the major contributors to environmental HM (Masindi et al., 2021). These pollutants, often transported over long distances via air or water, can pollute the environment and accumulate in ecosystems, posing persistent ecological and health threats. Globally, up to 90% of heavy metal effluents from industries are released into the atmosphere, causing mass water body contamination (Shankar et al., 2025). The contamination does not remain confined to the water column and sediment layers only. Instead, the pollutants are continuously being absorbed by aquatic organisms and interact together within the food chain, causing bioaccumulation and biomagnification (Saidon et al., 2024).

Aquatic organisms, particularly those in direct contact with contaminated water and sediments, such as filter feeders, benthic species, and fish, are subjected directly to constant exposure, leading to bioconcentration of heavy metals (Kolarova & Napiórkowski, 2021). Bioconcentration refers to the uptake of heavy metals from the surrounding environment into the living organisms, typically via dermal or respiratory pathways (Lipy et al., 2021). Following the bioconcentration process, bioaccumulation takes place,

where the rate of metal uptake exceeds the contaminant loss rate, leading to an increase in metal concentration within specific tissues. Metabolically active organs such as the gill, liver, kidney, and gut tend to accumulate higher levels compared to passive tissues like muscle or skin (Singh & Sharma, 2024). The elevated HM levels eventually disrupt ecological balance and the survival of sensitive organisms, while altering community structure and ecosystem function. In humans, the intake of contaminated fish and shellfish directly exposes them to the toxic metals such as mercury, cadmium and lead. The exposure comes together with established risks of neurological impairment, organ toxicity and carcinogenic effects.

In Malaysia, which is a country with a rich diversity of aquatic ecosystems, ranging from freshwater rivers and lakes to estuarine zones and extensive marine coastlines, understanding the dynamics of HM bioconcentration is crucial. These ecosystems are not only biodiversity hotspots but also support local livelihoods through fisheries, aquaculture, and tourism. The accumulation of HMs in aquatic organisms, especially sessile and benthic, makes them excellent bioindicators for environmental monitoring (Abdullah et al., 2025). The limited mobility and close interaction with sediments and surrounding water allow these organisms to absorb and retain contaminants over time, reflecting both chronic and episodic pollution events. The physiological and biochemical responses of the organisms to HM exposure can be quantitatively assessed, providing crucial data for early warning systems, risk assessments, and the formulation of mitigation strategies.

However, despite the excellent bioindicators of the sessile organisms, current studies in Malaysia remain fragmented, resulting in a lack of comparative, systematic understanding of the bioconcentration process across ecological gradients. It is presumed that the heavy metal accumulation in aquatic organisms varies significantly across habitat types of freshwater, marine and estuarine. This is possibly due to the differences in water chemistry, sediment characteristics, and pollutant exposure in the areas. Furthermore, it is hypothesised that organisms with prominent ecological roles of sessile and mobile will exhibit different bioconcentration patterns, reflecting the specific modes of exposure and physiological adaptations. Studying the bioconcentration and distribution of HMs in Malaysian aquatic ecosystems and organisms is necessary in elucidating ecological risk levels associated with anthropogenic pollution, contributing to the development of national environmental health policies, and providing scientific insights essential for effective ecosystem conservation and sustainable resource management. Applying the bioconcentration concept on

different organisms enhances the understanding of absorption, storage and detoxification mechanisms of the metals, hence, contributing to broader toxicodynamic knowledge.

The present study aims to determine and compare the bioconcentration patterns of heavy metals in aquatic organism species, specifically mud crab (*Scylla tranquebarica*), various fish species (*Diagramma pictum*- painted sweetlip, *Osteochilus vittatus*- bony-lipped barb, *Pristolepis fasciatus*- Malayan leaf-fish, *Oreochromis mossambicus*- Tilapia, *Trichopodus trichopterus*- three-spot gourami), and bivalves (*Anadara pilula*- blood clams and *Polymesoda expansa*- mud clams) collected from diverse aquatic ecosystems in Terengganu and Kelantan. Heavy metal concentrations were quantitatively assessed in these organisms' tissues to understand the differences in metal accumulation. Sampling sites include a wide spectrum of freshwater, brackish, and coastal environments of Setiu Wetlands (SW), Beting Lintang coastal area (BLC), UniSZA Besut Lake (UBL), Besut Knowledge Park (BKP), Taman Rimba Ilmu Tanah BRIS (TRIBE) UniSZA, Terengganu and Pengkalan Chepa River, Kelantan. Furthermore, this study also assesses bioconcentration levels across spatial variations relative to site-specific potential anthropogenic pollution sources.

2. MATERIALS AND METHODS

2.1. Ethical Statement

All experimental procedures were conducted under the institutional guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the UniSZA Animal and Plant Research Ethics Committee (UAPREC), with reference number UAPREC/008/050, ensuring compliance with relevant national legislation.

2.2. Study Area

The sampling was conducted from March to April 2025 (inter-monsoon period), across six ecologically diverse aquatic environments within the states of Terengganu and Kelantan (Figure 1). The timing is ecologically significant as it follows the Northeast Monsoon season (November to February), where the rainfall is high and terrestrial runoff can elevate sediment and metal input into the aquatic ecosystem of estuarine, marine and freshwater. These sites were strategically selected to cover a range of habitat types (freshwater, brackish, and marine) and varying degrees of anthropogenic influence, providing a comparative framework for evaluating heavy metal bioconcentration in aquatic organisms.

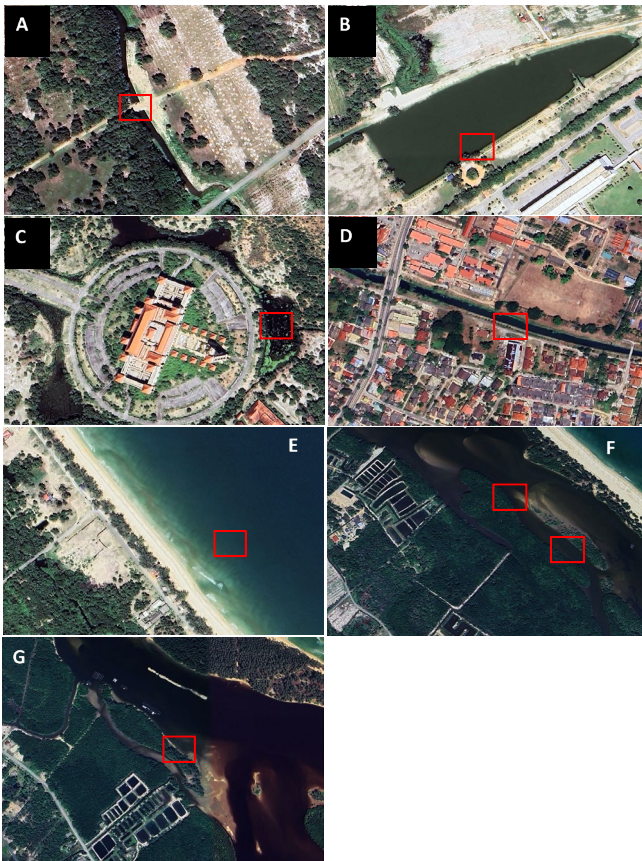


Figure 1: Study area. A. TRIBE. B. UniSZA Besut Lake (UBL). C. Besut Knowledge Park (BKP). D. Pengkalan Chepa River (PCR). E. Beting Lintang Coastal area (BLC). F and G. Setiu Wetlands (SW), Terengganu

Taman Rimba Ilmu Tanah Bris (TRIBE), UniSZA, Besut, Terengganu

The Taman Rimba Ilmu Tanah Bris (TRIBE) is a conservation-oriented ecological research area within the UniSZA Besut Campus (Figure 1A). This site has been chosen as it encompasses forested areas with small freshwater ponds and slow-flowing streams that collectively form a lowland freshwater ecosystem embedded in a secondary tropical rainforest matrix (Yaakub et al., 2022). The TRIBE hydrology is primarily fed by rain, with minimal artificial alteration, resulting in stable aquatic conditions with low sediment input and limited external nutrient loading. The aquatic habitats in TRIBE are largely undisturbed, protected by high canopy cover, intact riparian zones, and naturally regulated physicochemical parameters, supporting native flora and fauna (Yaakub et al., 2025). As a minimally impacted site, TRIBE serves as a vital control in ecological and bioconcentration studies to understand natural background levels of heavy metals in a freshwater ecosystem under conservation management.

UniSZA Besut Lake, Terengganu (UBL)

UniSZA Besut Lake (UBL) is an artificial freshwater lake within the Universiti Sultan Zainal Abidin (UniSZA), Besut campus, primarily used for aesthetic, educational and recreational purposes (FIK, 2025) (Figure 1B). The lake is centrally located in a landscaped academic setting, bordered by gently sloping banks with planted vegetation and walkways around, and receives input from rainwater and minor surface runoff. Ecologically, the lake acts as a lentic freshwater system with low to moderate water flow, supporting aquatic macrophytes, phytoplankton, and a variety of freshwater organisms such as fish and macroinvertebrates. The hydrology is primarily sustained by rainwater, surface runoff, and possibly groundwater seepage, with no significant inflow from natural rivers. Its semi-controlled environment and limited anthropogenic intrusion (e.g., from walkways and drains) make it suitable as a low-impact reference site for this bioconcentration study. It provides baseline data on heavy metal accumulation in freshwater species under minimal external influence.

Besut Knowledge Park (BKP)

The UniSZA Knowledge Park (BKP) consists of a network of artificial freshwater canals adjacent to a developing educational township (Figure 1C). This site has been chosen as the water bodies are shallow, with slow-moving or stagnant conditions depending on rainfall patterns and maintenance cycles. The habitat supports typical freshwater macroinvertebrates and fish species, which are hardy and tolerant to disturbed environments. The aquatic ecosystem is characterised by lentic freshwater dynamics, receiving periodic input from stormwater runoff, especially during the monsoon season. The runoff typically carries sediments, organic debris, nutrients (e.g., nitrogen and phosphorus), and possibly trace metals from nearby landscaped zones, paved surfaces, and ongoing construction activities. These anthropogenic inputs can influence the physico-chemical properties of the water. Given its proximity to construction and landscaped urban zones, the site makes a perfect location for studying early-stage urbanisation impacts on metal accumulation in aquatic organisms.

Pengkalan Chepa River, Kelantan (PCR)

Pengkalan Chepa River (PCR) is located near the Kelantan River estuary in the urban-industrial area of Kota Bharu, Kelantan (Figure 1D). It represents a transitional freshwater-to-estuarine ecosystem influenced by tidal fluctuations from the SCS and freshwater discharge from upstream regions (Ahmad et al., 2021). The estuarine

conditions fluctuate based on monsoonal rainfall and tidal amplitude, creating a dynamic mixing zone where both fluvial and marine processes interact. Pengkalan Chepa River has been chosen as the study site as it receives mixed discharges from domestic, industrial, and stormwater sources. The river is known to carry sediment-bound pollutants, such as trace metals, due to upstream agricultural, mining activities and the former Sabak dumpsite, which used to be the largest non-engineered landfill in Kelantan (Bouida et al., 2024). Its strategic location is subjected to multiple anthropogenic inputs, receiving untreated and partially treated discharges from domestic effluents, industrial discharges, stormwater runoff, and historical waste deposits. This site supports a mix of freshwater and estuarine biota adapted to fluctuating salinity and oxygen conditions, including benthic invertebrates and euryhaline fish species that serve as key bioindicators for contaminant exposure.

Beting Lintang coastal area, Besut, Terengganu (BLC)

Located on the northern coast of Terengganu, Beting Lintang beach is a coastal sandy area (Figure 1E) influenced by normal marine processes of erosion, transportation, and deposition of sediments, driven by waves, currents, and tides. Despite its popularity as a tourist location with a view of the South China Sea (SCS), the site is situated near traditional fishing villages, and often produces domestic waste, resembling an informal landfill site (Adlan, 2023). This beach receives runoff from nearby agricultural lands and minor rural discharges (Discovery Terengganu, 2025). It represents a shallow coastal marine ecosystem where the interaction of anthropogenic inputs, including those from the anchovy industry and natural marine processes, can influence metal distribution and bioavailability in aquatic biota. This site is especially relevant for assessing metal bioconcentration in fish, as the main protein source for human consumption, serving as a bioindicator of coastal contamination.

Setiu Wetlands, Setiu, Terengganu (SW)

Setiu Wetlands (SW) is the largest natural wetland on the east coast of Peninsular Malaysia (Lua et al., 2025). The ecologically sensitive area comprises a mosaic of nine interconnected ecosystems: sea, beach, mudflat, lagoon, estuary, river, islands, and coastal and mangrove forests (Mohd Salim et al., 2018) (Figure 1F, G). These ecosystems provide various ecological services for humans, reptiles, mammals, invertebrates and birds. The estuarine system specifically receives freshwater input from the Setiu River and is partially influenced by tidal seawater ingress. The chosen site for this study is subject to moderate anthropogenic activities such as aquaculture (brackish fish and shrimp farming), agriculture, and low-density rural settlements,

causing detrimental effects to the wetland ecosystems. These diverse ecological features make it ideal for assessing heavy metal dynamics across salinity gradients and habitat types.

2.3 Sample collection

This study encompasses eight aquatic species representing three different phyla: Arthropoda (mud crab– *Scylla tranquebarica*), Chordata (fish species including Painted Sweetlip– *Diagramma pictum*, Bony-lipped Barb– *Osteochilus vittatus*, Malayan Leafyfish– *Pristolepis fasciatus*, Tilapia– *Oreochromis mossambicus*, and Three-spot Gourami– *Trichopodus trichopterus*), and Mollusca (Blood cockles– *Anadara pilula* and Mud clams– *Polymesoda expansa*). Triplicate species samples were collected from various locations using species-specific sampling techniques (Table 1 and Figure 1) to facilitate subsequent bioconcentration analysis.

Table 1: Study area, geographic coordinates and collection methods of different aquatic organisms

No.	Species	Common name	Locations	Coordinates	Collection methods
1.	<i>Diagramma pictum</i>	Painted Sweetlip	Beting Lintang Coastal area (BLC)	5°44'24.89" N, 102°39'43.29" E	Fish net
2.	<i>Osteochilus vittatus</i>	Bony-lipped Barb	UnISZA Besut Lake (UBL)	5°45'24.3" N 102°37'43.4" E	Fish net
3.	<i>Pristolepis fasciatus</i>	Malayan Leafyfish	Besut Knowledge Park (BKP)	5°44'50.3" N 102°38'33.4" E	Rod and line fishing
4.	<i>Oreochromis mossambicus</i>	Tilapia	Pengkalan Chepa River (PCR)	6°09'16.7" N, 102°17'25.6" E	Rod and line fishing
5.	<i>Trichopodus trichopterus</i>	Three-spot Gourami	TRIBE, UnISZA	5°45'39.8" N 102°38'02.6" E	Fish net
6.	<i>Scylla tranquebarica</i>	Mud crab	Setiu Wetlands (SW)	5°41'12.29" N 102°42'20.76" E	Crab trap
7.	<i>Anadara pilula</i>	Blood cockles	Setiu Wetlands (SW)	5°41'08.7" N 102°42'25.1" E	Hand pick
8.	<i>Polymesoda expansa</i>	Mud clams	Setiu Wetlands (SW)	5°40'10.30" N 102°43'34.38" E	Hand pick

Post-capture, all samples were immediately stored in polyethylene bags, kept on ice, and transported back to the laboratory for further processing. Species were identified using standard relevant references, and biometric measurements, including total length and body weight, were recorded. For arthropod and molluscan species, carapace and shell widths were measured. Fish specimens were dissected to extract approximately 10 g of dorsal muscle tissue. In

molluscs and arthropods, soft tissues were carefully extracted from shells and carapaces using a ceramic knife and a scalpel. All tissues were oven-dried at 80 °C for 24 hours, cooled in a desiccator, and homogenised into fine powder using a ceramic mortar and pestle. The powdered samples were then stored in airtight containers for the acid digestion procedure.

2.4 Acid Digestion

Acid digestion samples were conducted in accordance with the guidelines set by the Association of Official Analytical Chemists (AOAC, 2016). Approximately 1.00 g of each homogenised tissue sample was transferred into a digestion vessel, followed by the addition of 10 mL of concentrated nitric acid (HNO₃, 60%). The mixture was digested using a microwave digester for 90 minutes under controlled temperature and pressure conditions, ensuring complete organic matter breakdown. Upon completion, 2mL of 30% hydrogen peroxide was added to the digested sample, cooled for 30 minutes in the fume hood at room temperature, filtered using 0.5 mm Whatman filter paper and subsequently transferred into 50 mL acid-washed volumetric flasks. The filtrates were made up to 50 ml volume with deionised water and transferred into clean, labelled Falcon tubes. Heavy metal detection of each sample was conducted using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES), ensuring high sensitivity and precision for multielement detection. The quality control included procedural analytical blanks and standard reference materials (SRM 2976), with recovery rates between 90% and 116%. Glassware was acid-washed (20% HNO₃) to avoid possible contamination.

2.5 Metal calculation

The concentration of heavy metals in arthropods, chordata and molluscs' tissues was calculated using the following formula APHA (2017):

$$C = (A \times B) / M \text{ (mg/kg)}$$

Where,

C = concentration of test samples (mg/kg)

A = ICP-OES readings (mg/L)

B = Volume of digested samples (L)

M = Weight of dried samples (kg)

2.6 Statistical Analysis

Data normality was assessed using both the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results indicated that the concentrations of all heavy metal types were normally distributed ($p > 0.05$). Therefore, a One-Way ANOVA was conducted to examine the statistical differences in heavy

metal concentrations across the different species and sampling stations. A Tukey's Honestly Significant Difference (HSD) post-hoc test was performed, where statistically significant differences were observed ($p < 0.05$), to identify specific pairwise differences in heavy metal concentrations among the species and stations.

3. RESULT AND DISCUSSION

The present study revealed the concentration of 11 heavy metals (Ag, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in eight aquatic species collected from various freshwater, marine and brackish water habitats in Terengganu and Kelantan (Table 2). These species represented three major phyla: Chordata, Arthropoda, and Mollusca. Heavy metal accumulation varied considerably across species, likely influenced by habitat type, feeding behaviour, and body morphology.

3.1 Bioconcentration Patterns of Heavy Metals in Aquatic Species

Among the fish species, *Oreochromis mossambicus* (Tilapia), *Pristolepis fasciatus* (Malayan Leaf-fish), and *Osteochilus vittatus* (Bony-lipped Barb) exhibited relatively high concentrations of Zn (20.5 ± 1.85 mg/kg, 23.61 ± 2.08 mg/kg, and 39.01 mg/kg, respectively, with the Zn values are statistically different among all sampling stations and species ($p = 3.4152 \times 10^{-40}$; $p < 0.05$) (Table 2, Figure 2). These higher Zn fish species were collected from freshwater bodies of PCR, BKP, and UBL, respectively and may have been exposed to anthropogenic inputs such as agricultural runoff or aquaculture discharge, which are common sources of Zn (Dawood et al., 2022; Emenike et al., 2022). Higher Al and Fe levels were also recorded in Tilapia (20.5 ± 1.80 mg/kg and 16.9 ± 1.50 mg/kg, respectively). These Al and Fe values were found to be statistically significantly different among stations and species ($p = 7.283 \times 10^{-38}$ and 2.331×10^{-27} , respectively; $p < 0.05$), further supporting the observed spatial and biological variation in metal bioaccumulation.

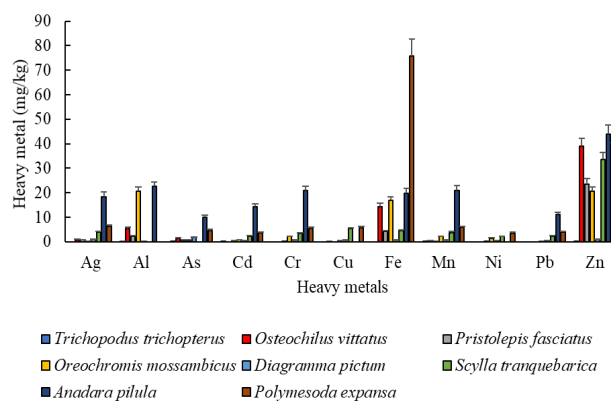


Figure 2: Heavy metal content in different aquatic species

Table 2: Morphometric data and heavy metal concentrations in selected aquatic fauna from various ecosystems. ND – Not detected

	<i>Trichopodus trichopterus</i>	<i>Osteochilus vittatus</i>	<i>Pristolepis fasciatus</i>	<i>Oreochromis mossambicus</i>	<i>Diagramma pictum</i>	<i>Scylla tranquebarica</i>	<i>Anadara pilula</i>	<i>Polymesoda expansa</i>		
Length (cm)	7.4 ± 0.5	11.0 ± 3.61	11.9 ± 0.57	9 ± 1.5	20 ± 10	Carapace width: 11 ± 2.0	50 mm in length, 45 mm in width	60 mm in length; 80 mm in width		
Weight (kg)	3.89 ± 0.92	29 ± 3.61	48 ± 6	20 ± 5	75 ± 5	89 ± 5	Dry weight: 1 g	Dry weight: 1.403 ± 0.36	Permissible limit (mg/kg)	
Feeding strategy	Omnivore	Herbivore	Omnivore	Omnivore	Carnivore	Scavenger	Filter feeder	Filter/ deposit feeder		
	Heavy metals (mg/kg)								(FAO, 2000)	WHO (2017)
Ag	ND	0.94 ± 0.10	0.66 ± 0.08	0.23 ± 0.03	0.821 ± 0.07	3.88 ± 0.45	18.31 ± 2.10	6.14 ± 0.62	1.0	0.1
Al	0.089 ± 0.01	5.34 ± 0.54	2.34 ± 0.21	20.5 ± 1.80	ND	ND	22.57 ± 1.90	ND	40	150
As	0.003 ± 0.0005	1.4 ± 0.15	0.62 ± 0.06	0.61 ± 0.05	1.8 ± 0.17	ND	9.87 ± 0.88	4.62 ± 0.41	0.1	0.1
Cd	0.001 ± 0.0001	ND	0.43 ± 0.05	0.83 ± 0.09	0.482 ± 0.04	2.27 ± 0.19	14.41 ± 1.12	3.69 ± 0.32	0.5	0.5
Cr	ND	ND	0.07 ± 0.01	2.15 ± 0.20	0.713 ± 0.06	3.4 ± 0.33	20.9 ± 1.85	5.43 ± 0.45	55	30
Cu	ND	0.28 ± 0.03	ND	0.34 ± 0.04	0.717 ± 0.06	5.34 ± 0.47	ND	5.65 ± 0.52	0.05	0.05
Fe	ND	14.32 ± 1.30	4.19 ± 0.38	16.9 ± 1.50	0.803 ± 0.07	4.48 ± 0.39	19.9 ± 1.77	75.92 ± 6.84	0.8	2.0
Mn	0.022 ± 0.002	0.35 ± 0.04	0.2 ± 0.03	2.13 ± 0.20	0.727 ± 0.06	3.78 ± 0.33	21.02 ± 1.80	5.88 ± 0.51	30	30
Ni	ND	ND	0.03 ± 0.004	1.49 ± 0.13	0.462 ± 0.04	2.19 ± 0.19	ND	3.51 ± 0.31	2	2
Pb	ND	ND	ND	0.13 ± 0.02	0.492 ± 0.04	2.34 ± 0.20	11.17 ± 0.98	3.85 ± 0.34	0.2	0.3
Zn	0.052 ± 0.006	39.01 ± 3.21	23.61 ± 2.08	20.5 ± 1.85	0.816 ± 0.07	33.67 ± 2.85	43.8 ± 3.88	37.12 ± 3.20	0.02	0.02

The various species sizes indicate that biological differences (species and feeding strategies) affect more variation than spatial differences. Post-hoc analysis confirmed that filter-feeding bivalves (*Anadara pilula*, *Polymesoda expansa*) accumulated significantly higher levels of metals compared to fish and crustaceans. These findings statistically validate the observed spatial and biological variation in metal bioaccumulation, further supporting the role of feeding strategies in driving exposure risk. Importantly, several values exceeded FAO/WHO permissible limits, underscoring potential ecological and human health concerns.

Post-hoc analysis using Tukey's HSD test also revealed that aquatic organisms of *T. trichopterus*, *D. pictum*, *S. tranquebarica*, and *P. expansa* have no statistically significant differences in Al concentrations ($p > 0.05$), suggesting that the feeding strategy of those species does not strongly influence Al uptake. In contrast, the remaining organisms (*O. vittatus*, *P. fasciatus*, *O. mossambicus*, and *Anadara pilula*) documented significantly different Al accumulation levels. For Fe, *T. trichopterus* and *D. pictum* were grouped into one homogeneous subset, indicating similar Fe concentrations between them. Similarly, *P. fasciatus* and *S. tranquebarica* formed another subset. The remaining aquatic organisms each exhibited significantly different Fe concentrations and were assigned to separate subsets, highlighting the variability in Fe accumulation across species.

Conversely, *Diagramma pictum* (Painted Sweetlip), a marine/brackish species with the largest body size among the fish (20 ± 10 cm; 75 ± 5 g), exhibited relatively lower concentrations of most metals, possibly due to the dilution effect associated with larger biomass and reduced exposure in less polluted open waters (Balzani et al., 2022; Jiang et al., 2021). The larger biomass of *D. pictum* likely contributes to a dilution effect, where accumulated metal concentrations are distributed across a greater tissue volume, thus reducing detectable concentrations per unit weight (Singh & Sharma, 2024). Additionally, its habitat preference for less polluted, more open marine or brackish waters may result in lower exposure to contaminated sediments and runoff compared to species inhabiting more confined or freshwater environments (Tansel, 2024).

The mud crab (*S. tranquebarica*), collected from Setiu wetlands, exhibited moderate to high levels of several metals, including Cu (5.34 ± 0.47 mg/kg), Mn (3.78 ± 0.33 mg/kg), and Cd (2.27 ± 0.19 mg/kg) (Figure 3). These metal concentrations were statistically significantly different among other stations and species (3.097×10^{-19} , 1.976×10^{-34} , 2.215×10^{-38} ; $p < 0.05$), highlighting site-specific accumulation patterns. These results are consistent with the

crab's benthic and scavenging lifestyle, which increases exposure to sediment-bound contaminants (Lordan & Zabetakis, 2022; Waqas et al., 2024). For Cu, *T. trichopterus*, *O. vittatus*, *P. fasciatus*, and *A. pilula* have no statistically significant differences in Al concentrations ($p > 0.05$), indicating similar Al levels among them. Fishes of *O. vittatus* and *O. mossambicus* also documented similar Cu accumulation levels. In contrast, the remaining organisms documented significantly different Cu accumulation levels. For Cd, *T. trichopterus* and *O. vittatus* are assigned to one subset, indicating no difference in Cd concentration. Similarly, *P. fasciatus* and *D. pictum* show statistically similar Cd concentrations.

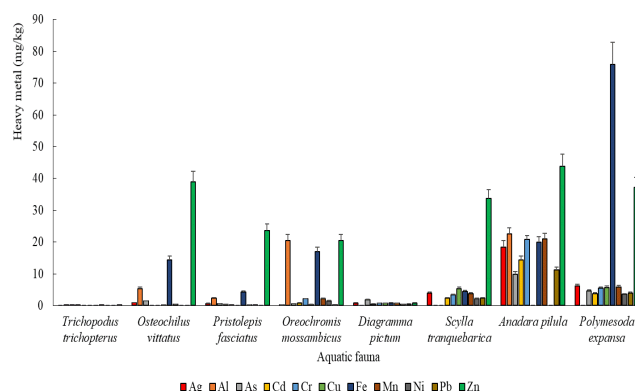


Figure 3: Bioconcentration of heavy metals in different aquatic species

Similarly, the molluscan species (*A. pilula* and *P. expansa*) displayed the highest concentrations of nearly all heavy metals, particularly Zn (43.8 ± 3.88 mg/kg and 37.12 ± 3.20 mg/kg), Fe (19.9 ± 1.77 mg/kg and 75.92 ± 6.84 mg/kg), Mn (21.02 ± 1.80 mg/kg and 5.88 ± 0.51 mg/kg), Cd (14.41 ± 1.12 mg/kg and 3.69 ± 0.32 mg/kg), and Ag (18.31 ± 2.10 mg/kg and 6.14 ± 0.62 mg/kg), respectively. The higher values can be attributed to their filter-feeding behaviour and close interaction with sediment in estuarine environments (Singh & Gupta, 2021; Morankar & Kurhe, 2023). The exceptionally high Fe concentration in *P. expansa* suggests site-specific enrichment or a high bioaccumulation capacity for this element.

In comparison with the World Health Organization (WHO, 2017) and Food and Agriculture Organization (FAO, 2000), Ag levels were generally within WHO's limit (0.1 mg/kg), except in *S. tranquebarica* (3.88 ± 0.45 mg/kg), *A. pilula* (18.31 ± 0.45 mg/kg), and *P. expansa* (6.14 ± 0.62 mg/kg) from Setiu Wetlands, Terengganu, all of which significantly exceeded both FAO (1.0 mg/kg) and WHO thresholds. These findings raise serious concerns about potential Ag bioaccumulation, particularly in molluscs and crustaceans. Aluminium concentrations were the highest in *O.*

mossambicus (20.5 ± 1.80 mg/kg) and *A. pilula* (22.57 ± 1.90 mg/kg), but remained below both FAO (40 mg/kg) and WHO (150 mg/kg) permissible limits. Nevertheless, the elevated levels suggest possible environmental contamination from industrial or agricultural sources (Botté et al., 2022). Arsenic levels in *O. vittatus* (1.4 ± 0.15 mg/kg), *D. pictum* (1.8 ± 0.17 mg/kg), *A. pilula* (9.87 ± 0.88 mg/kg), and *P. expansa* (4.62 ± 0.41 mg/kg) were alarmingly high, exceeding both FAO and WHO limits of 0.1 mg/kg by wide margins. The high bioconcentration level of As represents a significant toxicological risk, especially in *A. pilula*, where levels were nearly 100 times the safe limit.

Cadmium concentrations breached safe limits (0.5 mg/kg) in *P. fasciatus* (0.43 ± 0.05 mg/kg), *O. mossambicus* (0.83 ± 0.09 mg/kg), *D. pictum* (0.482 ± 0.04 mg/kg), *S. tranquebarica* (2.27 ± 0.19 mg/kg), *A. pilula* (14.41 ± 1.12 mg/kg), and *P. expansa* (3.69 ± 0.32 mg/kg), highlighting the strong bioaccumulation potential for heavy metals. The potential sources of Cd included sewage sludge, farmyard manure and commercial fertilisers used by farmers (Wang et al., 2023). Chromium was higher in *O. mossambicus* (2.15 ± 0.20 mg/kg), *S. tranquebarica* (3.4 ± 0.33 mg/kg), *A. pilula* (20.9 ± 1.85 mg/kg), and *P. expansa* (5.43 ± 0.45 mg/kg). Although these values fall within FAO (55 mg/kg) and WHO (30 mg/kg) limits, the concentration in *A. pilula* is considerably higher. It suggests substantial exposure, with the sources of Cr that might come from burning coal and oil, released to the environment through drainage and fertilisers (Kolarova & Napiórkowski, 2021). Every species where copper was detected exceeded both FAO and WHO limits, with *S. tranquebarica* and *P. expansa* showing extremely hazardous levels (5.34 ± 0.47 and 5.65 ± 0.52 , respectively).

Iron concentrations were particularly high in *P. expansa* (75.92 ± 6.84 mg/kg), and exceeded the WHO limit (2.0 mg/kg) and FAO limit (0.8 mg/kg). The extreme Fe level may not pose acute toxicity, but could indicate an environmental abnormality. *Osteochilus vittatus* and *O. mossambicus* also exceeded FAO's stricter limit, with 14.32 ± 1.30 and 16.9 ± 1.50 , respectively. Manganese levels were all within the FAO/WHO limits of 30 mg/kg, though *A. pilula* (21.02 ± 1.80 mg/kg) and *P. expansa* (5.88 ± 0.51 mg/kg) again showed higher concentrations. Nickel was the highest in *P. expansa* (3.51 ± 0.31 mg/kg), surpassing the permissible limit of 2 mg/kg. *Oreochromis mossambicus* (1.49 ± 0.13 mg/kg) and *S. tranquebarica* (2.19 ± 0.19 mg/kg) were close to or slightly above the limits, indicating concern for nickel toxicity.

Lead was not detected in most species except in *O. mossambicus* (0.13 ± 0.02 mg/kg), *D. pictum* (0.492 ± 0.04 mg/kg), *S. tranquebarica* (2.34 ± 0.20 mg/kg), *A. pilula* (11.17

± 0.98 mg/kg), and *P. expansa* (3.85 ± 0.34 mg/kg), which exceeded both WHO (0.3 mg/kg) and FAO (0.2 mg/kg) limits, suggesting serious contamination risks.

Zinc was particularly higher across most species, especially *O. vittatus* (39.01 ± 3.21 mg/kg), *S. tranquebarica* (33.67 ± 2.85 mg/kg), *A. pilula* (43.8 ± 3.88 mg/kg), and *P. expansa* (37.12 ± 3.20 mg/kg), greatly exceeding FAO and WHO limits (0.02 mg/kg). While Zn is essential, such high concentrations may indicate industrial runoff or other anthropogenic sources (Alengebawy et al., 2021).

Anadara pilula and *P. expansa* from Setiu Wetlands, Terengganu, showed the most consistent and extreme exceedance of heavy metal limits, followed by *S. tranquebarica* and *O. mossambicus*. These findings indicate a significant risk of heavy metal bioaccumulation in the aquatic food chain, posing hazards for both ecological systems and human consumption (Morankar & Kurhe, 2023; Singh & Gupta, 2021).

3.2 Spatial Bioconcentration Pattern of Heavy Metals

Overall, the spatial distribution of heavy metal concentrations showed statistically significant differences across the study sites ($p < 0.05$). The Setiu wetlands (SW) are an estuarine ecosystem exhibiting the highest pollution level and emerged as a hotspot for heavy metal accumulation (in mud crab, blood cockle, and mud clam) (Figures 4 and 5), likely due to sedimentation of metals from upstream sources or localised anthropogenic activities (Li et al., 2023). Pengkalan Chepa River (PCR), a flowing freshwater system, also revealed significant contamination with certain heavy metal concentrations are higher in *O. mossambicus*- Al (20.5 ± 1.80 mg/kg), Fe (16.9 ± 1.50 mg/kg) and Zn (20.5 ± 1.85 mg/kg), possibly stemming from upstream industrial effluents, urban runoff, or historical contamination, as rivers often act as conduits for pollutants (Maliki, 2020; Ahmad et al., 2021).

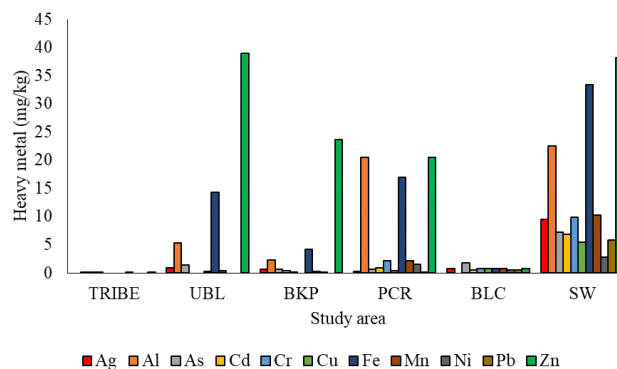


Figure 4: Spatial heavy metal concentrations in aquatic organisms. TRIBE- Taman Rimba Ilmu Beris, UBL- UniSA Besut Lake, BKP- Besut Knowledge Park, PCR- Pengkalan Chepa River, BLC- Beting Lintang Coast, SW- Setiu Wetlands.

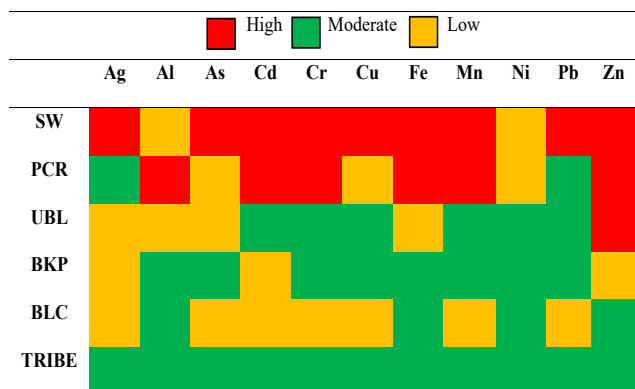


Figure 5: Infographic spatial distribution of heavy metal pollution levels. Red box = high pollution levels, yellow box = moderate pollution levels, and green box = low pollution levels. TRIBE- Taman Rimba Ilmu Beris, UBL- UniSZA Besut Lake, BKP- Besut Knowledge Park, PCR- Pengkalan Chepa River, BLC- Beting Lintang Coast, SW- Setiu Wetlands.

In contrast, UniSZA Besut Lakes (UBL) and Besut Knowledge Park (BKP) with freshwater ecosystems displayed moderate levels of pollution of heavy metals like Zn, Fe, and Al, indicative of semi-urban and localised agricultural influences (Sobri et al., 2021). Beting Lintang coastal area (BLC) showed lower metal concentrations in *D. pictum*, reinforcing the lower pollution exposure in marine zones, consistent with the dilution effect of seawater and reduced direct anthropogenic input (Kuznetsova & Timerbaev 2021). TRIBE, as UniSZA research hub, revealed the lowest heavy metal concentration and remains near-background levels in *T. trichopterus*, possibly due to its habitat, which is a conservation and protected area, and vegetated freshwater ecosystems, where limited industrial discharge and natural phytoremediation reduce metal bioavailability (Yaakub et al., 2025).

Overall, the present study suggests that metal accumulation is more strongly associated with habitat type and feeding strategy than with body size alone. While larger organisms like painted sweetlip (*D. pictum*) and mud crab (*S. tranquebarica*) showed moderate levels of heavy metals, smaller filter feeders such as blood cockles (*A. pilula*) and mud clams (*P. expansa*) accumulated much higher concentrations on a per-gram basis, highlighting the crucial role of species-specific physiology, feeding strategies, and ecological niches in bioconcentration patterns (Moniruzzaman et al., 2023). Besides being actively mobile (swimming), larger aquatic organisms, represented by fishes, typically have greater biomass, which can result in a dilution effect, whereby accumulated metals are distributed across more tissue mass, leading to lower concentrations per gram (Jiang et al., 2021; Balzani et al., 2022).

For instance, the painted sweetlip, despite its substantial size (20 ± 10 cm, 75 ± 5 g), showed relatively low levels of Zn (0.816 ± 0.07 mg/kg), Pb (0.492 ± 0.04 mg/kg), and other metals compared to smaller species. Similarly, although the mud crab is a benthic scavenger exposed to sediment-associated contaminants, its moderate accumulation levels may reflect both its size and potentially a more selective diet or slower metal uptake rate (Singh & Gupta, 2021; Morankar & Kurhe, 2023).

In contrast, filter-feeding molluscs, such as blood cockles and mud clams, are in constant contact with the surrounding water and sediment, actively filtering large volumes to obtain nutrients (Singh & Gupta, 2021; Morankar & Kurhe, 2023). This feeding behaviour exposes them to continuous ingestion of particulate-bound metals, especially those adsorbed to suspended solids or organic matter. As a consequence, metals penetrate and accumulate more in whole-body tissues than in the environment, leading to the replacement of essential elements with toxic ones, causing a negative effect on the organisms (Berezina et al., 2023). Moreover, molluscs possess simpler detoxification systems compared to vertebrates, making them more prone to bioaccumulation of non-essential and toxic metals such as Cd, Pb, and As (Deidda et al., 2021). The high metal accumulation may disrupt key biological functions, including enzymatic activities, reproductive capabilities and cellular homeostasis of aquatic organisms. The data supports the present study in which blood cockles exhibited extremely high levels of Cd (14.41 ± 1.12 mg/kg), Ag (18.31 ± 2.10 mg/kg), and Zn (43.8 ± 3.88 mg/kg), while mud clams had the highest recorded Fe level (75.92 ± 6.84 mg/kg), along with substantial concentrations of other metals like Mn (5.88 ± 0.51 mg/kg) and Cr (5.43 ± 0.45 mg/kg).

The observed patterns suggest that body size alone does not serve as a reliable predictor of metal accumulation in aquatic organisms. Instead, factors such as trophic level, habitat type, and specific biological traits, particularly filtration behaviour and proximity to sediments, play a more critical role in influencing the metal accumulation. The present study evidenced an important role in environmental monitoring, highlighting the value of using smaller, benthic filter feeders as sentinel species or bioindicators in heavy metal pollution studies. The higher heavy metal concentration in tissues reflects local contamination more sensitively and rapidly than larger, higher-trophic species.

The present study also highlighted important implications for fisheries management and environmental policy in Malaysia. The detection of heavy metals, including Cd, Pb, Zn, and As, which exceed FAO/WHO permissible limits in *A. pilula*, *P. expansa* and in *S. tranquebarica*, revealed

potential food safety risks for consumers. As these species are economically harvested for human consumption, stricter enforcement of water quality standards and regular biomonitoring programs in estuarine and coastal environments are highly needed. The significant species-specific variation in metal bioaccumulation also suggests that feeding strategies affected contaminant uptake, and thus should be integrated into risk assessment models. From a management aspect, restricting shellfish harvesting in contaminated areas and increasing community awareness on safe seafood consumption should be included in policies.

4. CONCLUSION

This study provides a comprehensive assessment of heavy metal contamination in various aquatic species from freshwater, marine and brackish environments in Terengganu and Kelantan. The data demonstrate that heavy metal accumulation is strongly influenced by ecological and biological traits such as habitat type, feeding behaviour, and proximity to sediments rather than body size alone. Molluscs such as *A. pilula* and *P. expansa* from Setiu Wetlands consistently exhibited the highest concentrations of toxic metals, including As, Cd, and Ag, often exceeding WHO 2017 and FAO 2000 safety thresholds by significant margins. These findings highlight their vulnerability and support their suitability as sentinel species for environmental monitoring.

Spatial analysis revealed that estuarine areas such as the Setiu Wetlands are hotspots of contamination, likely due to the cumulative effects of upstream anthropogenic discharges and sedimentation. The hydrodynamic conditions of estuaries with tidal mixing, slower water circulation, and high sedimentation rates encourage the trapping and persistence of heavy metals. Estuarine contamination in the Setiu Wetlands revealed a significant policy gap, as land-use planning and current environmental regulations fail to notice the cumulative downstream impacts of upstream discharges and anthropogenic activities. Addressing this issue necessitates strengthening integrated river basin and coastal zone management policies, with stricter enforcement and monitoring, to protect the biodiversity, fisheries, and community livelihoods.

Conversely, marine species like *D. pictum* from Beting Lintang and freshwater species from the TRIBE site exhibited lower contaminant levels, affirming the influence of habitat conditions and water dynamics on pollutant distribution. Importantly, the study emphasises that smaller, benthic filter-feeding molluscs offer a more sensitive indicator of localised pollution due to their limited mobility and constant exposure to sediment-bound metals. These understandings are critical for developing targeted biomonitoring strategies

and informing policy decisions on future pollution control and aquatic ecosystem health.

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STATEMENTS AND DECLARATIONS

The authors declare no conflict of interest.

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