Distribution of Heavy Metals in Sediments and Soft Tissues of the *Cerithidea obtusa* from Sepang River, Malaysia

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Abstract: *The main purpose of the research was to analyze the distribution of Arsenic (As), Cadmium (Cd), Copper (Cu), Iron (Fe), Nickel (Ni), Cobalt (Co), Mangan (Mn), and Zinc (Zn) in soft tissues, shells, and associated surface sediments of* Cerithidea obtusa *(*C. obtusa*) mangrove snails collected from Sungai Besar Sepang. The concentration of iron (Fe) was found to be the highest in relation to other toxic elements in sediments, soft tissues, and shells of* C. obtusa*. The concentrations of Cu and Zn in soft tissues of* C. obtusa *were found to exceed the concentrations in sediments, indicating bioaccumulation of these metals. Metal pollution was assessed with the Enrichment Factor (EF), Geoaccumulation Index (I_{geo}), and Pollution Factor (CF). EF, I_{geo}, and CF were 0.34 to 22.41, -3.37 to 2.65, and 0.14 to 9.42, respectively. The results indicate that sediments in Sungai Besar Sepang are contaminated with As and Zn. According to the bivalve bioaccumulation results, the soft tissues of* C. obtusa *act as a macro-concentrator for Cu and Zn. As a result, it is suggested that ongoing monitoring of releases of heavy metals from anthropogenic sources and stricter environmental protection measures should be implemented.*

Keywords: *enrichment; bio-accumulation; trace elements; neutron activation analysis*

INTRODUCTION

Mollusks are invertebrates found in oceans, rivers, lakes, or even on land [1]. Mollusks are easily obtained from their habitat due to their wide distribution and abundance [2]. Mollusks have the great advantage of being able to be used as bioindicators based on minerals absorbed from their habitat environment [3]. Since these mollusks are one of the important food sources for various living things of beings, it is important to avoid these mollusks being exposed to harmful toxic compounds [4]. The toxic content of mollusks is elevated because of phytoplankton feeding or sediment filtration [5]. As reported in other studies, mollusks have been shown to store high levels of heavy metals in their tissues and shell and adapt to changing environmental conditions [6-7]. Consequently, these heavy metals are partially or fully used for the growth of shells and soft tissues of mollusks [8]. Recently, the accumulation of heavy metals in the shell and soft tissues of mollusks has received much attention [9]. The number of heavy metals in the shells and soft tissues of mollusks and in sediments can be used to assess the degree of contamination levels [3]. The type of diet they consume and how they live has an impact on their ability to deposit heavy metals into the shells and tissues of mollusks. These mollusks live by feeding or filtering sediment, exposing them to heavy metals consumed directly from the sediment. Therefore, thorough research is needed to determine the potential of this species to accumulate and store this toxic element in its shell and soft tissues from sediments in its natural habitat [10]. Numerous researches have been conducted to propose environmental analyses using elements found in sand and mollusks [3].

The purpose of this study was to determine the distribution of heavy metals in the shell and soft tissues of the *Cerithidea obtusa* mollusk, as well as sediments from their habitat. Furthermore, to investigate the potential application of this capability for pollution biomonitoring in research areas, as well as to compare heavy metal levels in the study environment with those in other parts of the world.

■ EXPERIMENTAL SECTION

Study Sites and Samples Collection

The research area of this study focused on the mouth of Sungai Sepang Besar (Sepang Besar River), which flows into the Straits of Malacca in Sepang District, Selangor Malaysia, at GPS coordinate: N 02° 56’ 16.9” and E 101° 45’ 9.4” as shown in Fig. 1. The study region has a tropical environment all year, with temperatures ranging from 27 to 34 °C and a moderately humid atmosphere. The amount of cloud cover is modest, and November is the wettest month, albeit rainfall is rare. Sepang District had rapid and significant growth from 1995 to 2015, owing to its proximity to Malaysia's administrative capital, Putrajaya. As a result, an expansion of urban growth has been found to impact Sungai Sepang Besar's ecosystems. The reserve forests and mangrove regions in Sepang District are quite modest, with mangrove forests covering around 546.7 hectares along the rivers Sungai Sepang Kecil and Sungai Sepang Besar. It has fish, crabs, and shrimp, which are all biodiversity components of the mangrove environment. Under the Sepang Local Plan 2025, the mangrove regions are designated as Level 1 Environmentally Sensitive Areas (ESA) (Sepang 2017) [11-12].

Bivalve mollusks (*C. obtusa*) were selected to assess the bioaccumulation of heavy metals in their shell and soft tissues because of their wide dispersion and abundance in the study area. This species has been identified in the same manner as in previous studies. Only commercially recognized normal sizes for each species were compiled to avoid changes in metal content related to its size or reproductive stage [13-14]. For this study, 40–50 individual snails of similar length (3.5 to 5.0 cm) and mangrove sediments weighing approximately 500–600 g were randomly collected at a depth of 3.0-5.0 cm by scraping the surface layer with a plastic spoon [15-16]. At the laboratory, all samples were rinsed off with tap water to remove the soil attached to the snails and placed in a clean plastic bag with a label. As shown in Fig. 2(a) and 2(b), the soft tissue is then removed from the shell by fine-crushing the shell without damaging the soft tissue. Each sedimentary, shell,

Fig 1. Map of Sepang Besar River

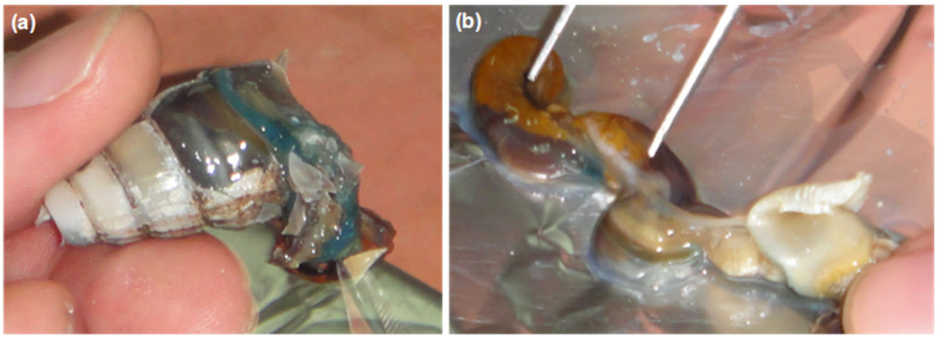

Fig 2. (a) Removing the shell, (b) Soft tissue

and tissue sample was packaged in a polyethylene plastic bag and stored in an icebox [17]. To remove moisture, sediments, shells, and soft tissues are dried in an oven at 85 °C for at least 72 h until a dry weight is produced. With glass mortar, each sediment sample, soft tissue, and shell of *C. obtusa* were individually crushed into a powder form. Then the powder is filtered through an opening made of stainless steel of 63 µm. Samples were stored in plastic pillboxes after being stirred vigorously and ready for further analysis [18].

Instrumentation

Neutron activation analysis (NAA) method was used to determine the concentration of elements. The powdered samples were separated into four subsamples of 150 and 200 mg each and stored individually in heat-sealed polyethylene vials for short and long radiation. Half-lives and gamma energies from radionuclides, along with a comparison approach, were used to determine element concentrations [19-20]. IAEA SL–1 (Lake Sediment)) and SRM 1566b/SRM 2976 were used as multi-element comparators for sediment and soft tissue bivalve mollusk, respectively.

For quality assurance, blank samples, standard reference material, IAEA-SL-1, and SRM 1566b/SRM 2976 were all irradiated simultaneously in the pneumatic transport facility at the 750 kW (MINT TRIGA) research reactor with a thermal neutron flux of 4.0 Tcm^{-2}s^{-1}. The samples were radiated for 1 min and then counted for 5 and 20 min after cooling for 20 min and 24 h, respectively, under brief radiation. For extended radiation, the samples were irradiated for 6 h and then counted for 1 h after cooling for 3–4 and 21–28 days, respectively. The distance between the detector and the sample was kept between 12 cm (short radiation) and 2 cm (long radiation). A calibrated high-resolution HPGe detector was used to count radiated samples. The components present in the sample, as well as their concentration, were identified using the specific energy of delayed gamma rays. All the countings were done in conditions where the dead time was less than 10% [19].

Prior to the use of an Atomic Absorption Spectroscopy (AAS), a sediment sample of approximately 500 mg was digested in a 4:1 mixture of nitric acid and perchloric acid in powder form (BDH Analar grade). For the first hour, all digestive tubes were placed in a digestion block at 40 °C and then digested entirely at 140 °C for at least 3 h [8]. Forty milliliters of double distilled water was added to dilute the resultant solution. The solution was then filtered into a polyethylene container using a WhatmanTM No. 1 filter. The concentrations of the elements were determined using a Perkin Elmer Model Analyst 800 and an air-acetylene AAS in three replicates on all of the samples [8].

Procedure

The status of enrichment of heavy metals in sediments can be assessed using pollution tools such as enrichment factor, contamination factor, and geo-accumulation index. On the other hand, the bioaccumulation factor was used for the bioaccumulation of sediment-associated organic compounds or metals into the tissues of ecological.

Enrichment factor (EF)

The EF was used to assess the size of anthropogenic metal inputs. Metals in sediment with values ranging between 0.5 and 1.5 have a lithospheric or crustal origin, whereas values greater than 1.5 (EF > 1.5) have an anthropogenic origin [21]. Eq. (1) is used to determine the EF:

$$EF = \frac{(M / Fe)_{sample}}{(M / Fe)_{shale}} \quad (1)$$

where M and Fe are the metal concentrations in the sample and shale be averaged [22-23]. The EF values were interpreted as shown in Table 1.

Geo-accumulation index (I_{geo})

Müller created the geo-accumulation index (I_{geo}), which is a reliable instrument for calculating a system's contaminated condition [24]. It can be calculated using Eq. (2):

$$I_{geo} = \log_2\left[\left(C_N / 1.5B_N\right)\right] \quad (2)$$

where C_N denotes the metal concentration in the sample, and B_N denotes the average shale background metal concentration [25]. The background matrix correction factor of 1.5 is used to minimize variance owing to lithogenic influences. Muller divided the I_{geo} value into seven categories, as shown in Table 2 [26].

Contamination factor (CF)

The amount of metal contamination in sediment is frequently described as a contamination factor (CF). It calculates the amount of pollution caused by pollutants in an ecological system and can be calculated by using Eq. (3):

$$CF = \left(\frac{C_N}{B_N}\right) \quad (3)$$

where C_N denotes the metal content in the sample and B_N denotes the background metal concentration in typical shale [25]. The CF readings were interpreted as shown in Table 3 [27].

Table 1. Pollution classification of the enrichment factor (EF)

	Range	Status
Enrichment Factor (EF)	1	no enrichment
	3–5	minor enrichment
	5–10	moderately severe enrichment
	10–25	severe enrichment
	25–50	very severe enrichment, and
	> 50	extremely severe enrichment

Table 2. Pollution classification of the geo-accumulation index (I_{geo})

	Range	Status
Geo-accumulation (I_{geo})	< 0	virtually unpolluted
	0–1	unpolluted to moderately polluted
	1–2	moderately polluted
	2–3	moderately to highly polluted
	3–4	strongly polluted
	4–5	strongly to severely polluted
	> 5	extremely contaminated

Table 3. Pollution classification of the contamination factor (CF)

	Range	Status
Contamination Factor (CF)	CF < 1	low contamination
	1 ≤ CF ≤ 3	moderate contamination
	3 ≤ CF ≤ 6	considerable contamination
	CF > 6	very high contaminations

Biota-sediment accumulation factor (BSAF)

For selected metals, the BSAF was estimated using Eq. (4) [28]:

$$BSAF = \frac{C_x}{C_s} \quad (4)$$

where C_x and C_s are the mean metal concentrations in various snail parts (tissues and shell) and in sediment, respectively. BSAF values can be classified into three groups, as indicated in Table 4 [29].

Statistical analysis

All of the findings were statistically analyzed with SPSS version 22.0. The difference between groups (i.e., sample locations) and the association between environmental parameters and accumulation were determined using Pearson's correlation coefficient test. MS Excel was used to calculate the mean and standard deviation (SD).

■ RESULTS AND DISCUSSION

The concentrations of eight elements were determined from four replicates across all samples and certified reference material (CRM), as shown in Table 5. The recovery percentage for all elements of the SL-1 and SRM 1566b CRMs of the IAEA ranges from 81.2% to 144.6% and 84.6% to 145%, respectively. The determined values corresponded well to their certified values.

The mean concentration of the target element in sediment, soft tissue, and shell of *C. obtusa*, along with their standard deviation, are presented in Table 6. The concentration of metals for this study was calculated based on the average values of all the analyzed samples and the standard error (SE) of total metal concentrations (in mg/kg dry weight basis). A total of eight elements were identified and quantified using the Instrumentation Neutron Activation Analysis (INAA) and alternative technique, i.e., the AAS method. The mean concentration of elements in the sediments was Fe > Mn > Zn > Ni > As > Cu > Co. Fe was found most abundant among all monitored toxic elements at trace levels in sediment. The mean concentration of elements in sediments is higher, suggesting the tendency of studied elements to accumulate in sediments as well as the role of sediments as a receptor of heavy metals. This is consistent with the studies in Qeshm Island, Persian Gulf of United Arab Emirates [30], and Sepang River, Malaysia [31-32]. Based on the results, the high concentration of elements in sediments can be due to the impact of a common resource (natural or anthropogenic) in the study area. Sediment quality guides and other studies results are provided in Table 7. The comparison of the results of the sediment sample of this study against the relevant guide suggests that the

Table 4. Pollution classification of the BSAF

	Range	Status
Biota-Sediment Accumulation Factor (BSAF)	> 2	macroconcentrators
	1–2	microconcentrators
	< 1	deconcentrators

Table 5. Mean of measured and certified values of CRM (mg/kg dry weight)

	SL-1			SRM1566b/SRM2976		
	Standard value	Measured value	Recovery (%)	Standard value	Measured value	Recovery (%)
Fe	67400	68716	102.0	205.8	278.6	135.4
As	27.6	22.4	81.2	7.65	6.47	84.6
Mn	3460	3178.1	91.9	18.5	16.61	89.8
Zn	223	257.3	115.4	1424	1386.4	97.4
Co	19.8	21.3	107.6	0.371	0.538	145.0
Cd	1.3	1.88	144.6	0.82	0.85	103.7
Ni	26	28.15	108.3	0.93	0.86	92.5
Cu	11	11.13	101.2	71.6	70.1	97.9

Table 6. Concentrations of heavy metals (mg/kg dry weight) in sediment, soft tissue, and shell of *C. obtusa*

		min	max	average	Std. dev.
Fe	Assoc.sediments	21874	25674	23659	1910
	Soft tissues	224	4822	2230	2236
	shell	286.64	345.32	315.9	41.5
As	Assoc.sediments	14.57	18.34	16.95	1.68
	Soft tissues	9.46	16.41	12.06	3.03
	shell	0.453	0.602	0.552	0.068
Mn	Assoc.sediments	123.45	152.39	137.68	12.15
	Soft tissues	53.85	184.08	134.55	70.49
	shell	9.35	15.23	12.33	2.58
Zn	Assoc.sediments	85.08	101.35	91.78	7.24
	Soft tissues	67.72	110.51	92.34	18
	shell	8.26	10.39	9.29	0.87
Co	Assoc.sediments	5.68	6.59	7.33	0.72
	Soft tissues	0.91	8.19	4.06	3.74
	shell	0.13	0.58	0.31	0.24
Cd	Assoc.sediments	nd	nd	nd	nd
	Soft tissues	0.640	0.904	0.773	0.132
	shell	0.311	0.336	0.327	0.014
Ni	Assoc.sediments	17.23	28.20	22.72	5.48
	Soft tissues	17.97	18.42	18.19	0.22
	shell	11.98	17.78	14.88	2.90
Cu	Assoc.sediments	11.11	11.19	11.14	0.04
	Soft tissues	90.59	103.88	97.24	9.40
	shell	1.73	2.50	2.11	0.54

nd – not detected

Table 7. Comparison of Mn, As, Zn, Fe, Cu, Ni, and Cd concentrations (mg/kg dry weight basis) with other studies and guideline

Guideline	Mn	Cu	As	Zn	Cd	Ni	Fe	Ref.
NOAA Guidelines								
ERL (Effects Range Low)	na	34	8.2	150	1.2	20.9	na	[44]
ERM (Effects Range Median)	na	270	70	410	9.6	51.6	na	
Canadian Guideline								
TEL (Threshold Effect Level)	na	18.7	7.24	124	0.7	15.9	na	[43]
PEL (Probable Effect Level)	na	108	41.6	271	4.2	42.8	na	
USEPA standards limits harbor sediments		25–50	3–8	90–200	6	20–50		[41]
(Mean current study)	137.68	11.14	16.95	91.78	-	22.7	23659	
Sepang River, Malaysia	na	77.17±0.25		246.38±0.22	1.15±0.04	26.05±0.08	na	[42]
Peninsular Malaysia	na	1.63–150.81		23.70–609.20	1.63–150.81	2.41–36.29	na	[31]
Qeshm Island, Persian Gulf	na	31.58–127.86		61.80–159.22	0.11–0.16	27.35–109.46	na	[30]

na – not available

concentration of other measurable elements was lower compared to the presented guide except for As and Ni. Also, in the sediment sample, As and Ni has been larger than ERL, TEL, and USEPA standards. These findings show that human activities such as urban, residential, and industrial wastewater, agriculture, shipping and transportation, coastal activities (i.e., marinas, jetties, ports, and harbors), and mining operations may have an impact on this area with high As and Ni concentrations. These findings are consistent with those of prior investigations [30,33]. Quick environmental changes altered the components in water, whereas sediments preserved the history of environmental changes [34].

The abundance order of target elements in soft tissue and shell of *C. obtusa* was found as Fe > Mn > Cu > Zn > Ni > As > Co > Cd and Fe > Ni > Mn > Zn > Cu > As > Cd > Co respectively. The heavy metal concentrations in the shells show a similar pattern of accumulation when compared with soft tissues of *C. obtusa*. The levels of As, Mn, Zn, Co, and Cu in the shells of *C. obtusa* were significantly lower than in the soft tissues. The accumulation of the studied elements except for the Ni, Co, and Cd in the soft tissue was far higher than that in the shell. This accumulation was obtained with a significant difference for Fe, Cu, Zn, and Mn compared to the shell. Different elements have various roles and functions, which lead to differences in the accumulation of elements in the tissues of the mollusk. The higher values of Cu and Zn in soft tissues can be due to the tendency of *C. obtusa* to accumulate these essential elements for cell growth and metabolism. In mollusks, Zn is used in the structure of many enzymes and the synthesis of hematocyanin [35]. Cu's role in the metabolism of molecular oxygen in mollusks is biochemically similar to, if not identical, that of Fe because the oxygen-carrying pigment of mollusk's blood is Cu-containing cuproprotein, hemocyanin, rather than hemoglobin [36]. Furthermore, Cd binds to low-molecular-weight proteins called metallothionein, which reduces its toxicity [37]. So, by applying the mechanism of detoxification, the soft tissue of oysters can accumulate more contents of this element [38] suggesting that mollusks may accumulate a portion of absorbed heavy metals in their shell. This issue can be a part of the detoxification process of over-absorbed essential elements and unnecessary elements in mollusks.

Several environmental indicators (EF, I_{geo}, and CF) were monitored to determine the pollutant level in the sediments. Table 8 shows the mean of the EF, I_{geo}, and CF of target elements in sediment, as well as their standard deviation. The Sepang river site was extremely severely enriched with As (10 < EF < 25) and minor enriched with Zn (3 < EF < 5), according to the results of enrichment factor data shown in Table 8. The study area was extremely heavily contaminated with As (CF > 6) and moderately contaminated with Zn (CF = 1–3), based on the contamination factor. According to Hakanson, Mn, Fe, Co, Cd, and Ni had low contamination in all investigated heavy metals (CF < 1). The geo-accumulation index (I_{geo}) showed that the mangrove environment of Sungai Besar Sepang was polluted with As but relatively unpolluted (I_{geo} < 0) for other elements in this study which is consistent with findings from prior studies in the area [32]. Bioaccumulation is the process of a chemical migrating from the external environment into the organism through all possible exposure channels, which is evaluated by a BSAF [39]. In this regard, the soft tissue

Table 8. The calculated value of enrichment factor (EF), geoaccumualtion index (I_{geo}), and contamination factor (CF)

	Enrichment Factor (EF)				Geo-accumulation (I_{geo})				Contamination Factor (CF)			
	min	max	average	Std dev	min	max	average	Std dev	min	max	average	Std dev
Fe	-	-	-	-	-1.95	-1.72	-1.84	0.12	0.39	0.46	0.42	0.03
As	20.83	22.34	22.41	0.89	2.43	2.76	2.65	0.17	8.09	10.19	9.42	1.06
Mn	0.33	0.35	0.34	0.01	-3.53	-3.23	-3.37	0.15	0.13	0.16	0.14	0.02
Zn	3.13	3.17	3.12	0.03	-0.30	-0.05	-0.19	0.13	1.22	1.45	1.31	0.12
Co	0.58	0.58	0.70	0.07	-2.72	-2.51	-2.36	0.18	0.23	0.26	0.29	0.03
Ni	0.53	0.59	0.80	0.14	-2.87	-2.47	-2.16	0.36	0.21	0.27	0.34	0.07
Cu	0.48	0.41	0.44	0.03	-3.02	-3.01	-3.01	0.01	0.19	0.19	0.19	0.00

shows larger BSAF values than the shell, indicating the greater tendency of elements to accumulate in the soft tissue than in the shell. Based on data presented in Table 9, the Cu and Zn concentrations in the soft tissue of *C. obtusa* are several times greater than their concentration in the sediments. This suggests the ability of the soft tissue to accumulate metals several times as large as the environment. The soft tissue of the studied *C. obtusa* acts as a macroconcentrator for the elements of Cu and Zn, and its shell functions similarly for Cd, according to the classification presented for the BSAF factor, which is similar to the findings of similar bivalve and which have presented *C. obtusa* as a macroconcentrator of metals [40]. The lower values of the BSAF factor for elements such as Co, Ni, and As in the soft tissue can be due to the fact that they are not biologically needed, which corresponds to the other study findings on bivalve mollusks [30]. The concentration of elements in the oyster shell is less influenced by the physicochemical conditions of the environment. The lower values of coefficient variation indicate greater accuracy in determining the biomonitor organism for the studied metals. According to the studies [3], the lower coefficient of variation for the concentration of metals in a particular tissue suggests the accuracy and validity of that tissue being used as a biomonitor of metals. Thus, this factor can be effective in choosing a living organism as a biomonitor.

Table 9. BSAF of shell and soft tissue of *C. obtusa* in the whole area investigated

		min	max	average	Std dev
Fe	muscle	0.01	0.19	0.10	0.09
	shell	0.01	0.01	0.01	-
As	muscle	0.65	0.89	0.71	0.13
	shell	0.03	0.03	0.03	-
Mn	muscle	0.44	1.21	0.98	0.40
	shell	0.08	0.10	0.09	0.01
Zn	muscle	0.80	1.09	1.01	0.15
	shell	0.12	0.09	0.10	0.01
Co	muscle	0.16	1.24	0.55	0.55
	shell	0.02	0.09	0.04	0.03
Ni	muscle	1.04	0.81	0.65	0.20
	shell	0.70	0.78	0.53	0.13
Cu	muscle	8.15	8.69	9.32	0.59
	shell	0.16	0.22	0.19	0.03

Table 10. Relations between the heavy metals concentrations in sediment and those in the soft tissue and shell of *C. obtusa*

Fe	sediment	Soft tissues	shell
sediment	1		
Soft tissues	0.994**	1	
shell	0.356	0.449	1

As	sediment	Soft tissues	shell
sediment	1		
muscle	0.294	1	
shell	0.857	0.550	1

Mn	sediment	Soft tissues	shell
sediment	1		
Soft tissues	-0.241	1	
shell	-0.990**	0.316	1

Zn	sediment	Soft tissues	shell
sediment	1		
muscle	-0.609	1	
shell	0.039	0.246	1

Co	sediment	Soft tissues	shell
sediment	1		
Soft tissues	0.692	1	
shell	0.156	-0.550	1

Cu	sediment	Soft tissues	shell
sediment	1		
muscle	0.488	1	
shell	0.401	0.995**	1

Cu	sediment	Soft tissues	shell
sediment	1		
muscle	0.488	1	
shell	0.401	0.995**	1

Correlation is significant at the 0.01 level

The Pearson's correlation coefficients in Table 10 illustrate the correlation of metals between soft tissues, the shell of *C. obtusa*, and sediments. The concentration of all the heavy metal (except for Mn and Zn) in surface sediments were positively and strongly correlated with respective heavy metals in soft tissues and the shell of *C. obtusa*. The correlations of Fe, As, Co, Ni, and Cu concentrations in soft tissue and habitat sediments showed the ability of bivalve mollusks to reflect their individual metal levels in their environment. For As, Ni, and Cu, the shells revealed substantial relationships with the surface sediments. This showed that the *C. obtusa* shell could be used as a biomonitor for the three metals.

■ CONCLUSION

The variations in metal distribution could be attributed to variances in tissue physiology as well as metal handling, storage, and detoxification procedures, according to the current findings. Cu and Zn concentrations in soft tissues were substantially greater than in sediments. According to the pollution index, the area is severely enriched with As and Zn, possibly as a result of anthropogenic activity near the sampling point. *C. obtusa* soft tissues are macroconcentrators and could be employed as biomonitors for metal buildup. As a result, our findings are useful for future ecotoxicological investigations aiming at establishing *C. obtusa* as a good heavy metal biomonitor and bioindicator species. Stricter environmental protection measures should be implemented to control the discharge of heavy metals from anthropogenic sources. In order to preserve sustainable development in the Sungai Besar Sepang river, it is also critical to raise public knowledge about maritime environmental preservation.

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■ AUTHOR CONTRIBUTIONS

Elias B. Saion and Yap CK are the team leader who proposed the work and designed the experiment. Kumar Krishnan, Cheng WH, Prakash Balu, and Chong MY executed the experiments and analyzed the results, manuscript writing, revised and verified the manuscript and the results obtained from the experiment. All authors read and approved the manuscript. All playing roles as the main contribution to this study due to their expertise and knowledge.

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