

Original Article

Metallothionein induction in bivalves exposed to heavy metals in sediment of the Balamban Coast, Cebu, Philippines

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Abstract: This study investigates heavy metal bioaccumulation and metallothionein (MT) responses in bivalve mollusks, *Dosinia histrio* (Gmelin, 1791), *Hiatula diphos* (Linnaeus, 1771), and *Septifer bilocularis* (Linnaeus, 1758), collected seasonally along the Balamban coastline in Cebu, Philippines, to assess their potential as bioindicators for evaluating sediment contamination levels. Tissue and sediment samples were analyzed for copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), and zinc (Zn) using flame atomic absorption spectrophotometry. The study also calculated biota-sediment accumulation factor, enrichment factors (EF), MT induction levels, and sediment pollution indices, including the geoaccumulation index, contamination factor, pollution load index (PLI), and potential ecological risk index. The results revealed that Zn and Cu were the predominant metals in mollusk tissues, while Cd and Pb levels were notably higher during the wet season, reflecting seasonal variability in metal bioavailability. The highest EF was recorded for Pb during the wet season (5.95), highlighting significant anthropogenic enrichment. The highest Zn accumulation (582 ± 32.0 mg/kg) was observed in *D. histrio* during the wet season. Sediment Pb levels exceeded FAO/WHO safety thresholds in both seasons, while PLI values above 1 indicated considerable pollution, with Pb and Cd as major ecological risk contributors. These findings emphasize the health risks of consuming these mollusks, particularly during the wet season. Regular monitoring is recommended to mitigate environmental and public health impacts in the Balamban coastal area.

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Introduction

Heavy metal contamination is a significant environmental concern in marine ecosystems, as these pollutants adversely affect marine life and can enter the food chain, ultimately impacting human health. Heavy metal exposure can alter an organism's physiology, biochemistry, molecular structure, and behavior (Shahjahan et al., 2022). While natural levels of heavy metals have historically posed little threat to health, increased industrial activities, such as airborne emissions, vehicular exhaust, industrial effluents, and solid waste disposal, have emerged as significant sources of heavy metal pollution in the environment (Bi et al., 2020; Alburo et al., 2023). Once these trace metals enter natural water bodies, they accumulate in sediments, redistribute throughout the aquatic system,

or are consumed by marine organisms. Such contamination can severely limit an organism's ability to survive, increasing its vulnerability to illness and injury (Lamine et al., 2024).

Marine invertebrates, particularly bivalves and univalves, are effective bioindicators of heavy metal pollution due to their roles as bottom dwellers and filter feeders, which makes them particularly susceptible to various environmental contaminants (Costello et al., 2021). Geolin et al. (2021) reported that mean metal concentrations in the univalve *Canarium labiatum* (Roding, 1798) from the Balamban coast followed the trend $Zn > Cu > Pb > Cr > Cd$, with no observed seasonal variation. This highlights the univalve's capability to accumulate high concentrations of metals in response to increased

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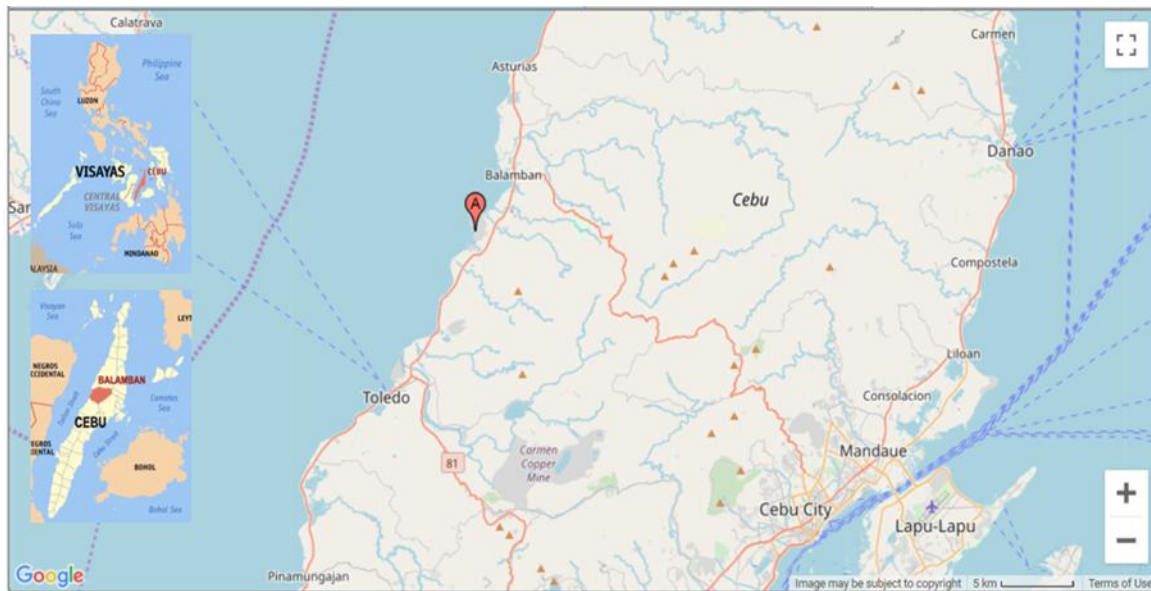


Figure 1. Location map of the sampling site in Balamban Shipyard (adapted from Geolin et al., 2022)

sediment metal levels.

Metallothionein (MT) assays play a crucial role in assessing metal exposure and serve as potential biomarkers for metal uptake (Frank et al., 2008). Various studies have demonstrated that different metals can induce MT production in the tissues of aquatic mollusks (Gillis et al., 2004). Elevated MT concentrations in bivalve tissues indicate a typical response to metal contamination, whether observed in field or laboratory settings (Geng et al., 2015). Consequently, MT induction has been incorporated into several monitoring programs as a reflection of metals' bioavailability and toxic effects (Ivankovic et al., 2010).

This study evaluated heavy metal bioaccumulation and metallothionein responses in bivalve mollusks of *Dosinia histrio* (Gmelin, 1791), *Hiatula diphos* (Linnaeus, 1771), and *Septifer bilocularis* (Linnaeus, 1758) collected seasonally along the Balamban coastline in Cebu, Philippines. The research assessed their potential as bioindicators and examined sediment contamination levels. Specifically, the study quantified the biota-sediment accumulation factor and MT induction in the bivalves while conducting a seasonal ecological risk assessment of the sediment. The findings established baseline trace metal levels in the Balamban shipyard area and evaluated these contaminants' risks to mollusks. The results are

expected to assist the Local Government Council in developing regulatory policies to mitigate contamination and protect marine ecosystems. Furthermore, the study aimed to enhance community awareness of the health risks of heavy metal exposure, including diseases and intoxication from consuming contaminated mollusks.

Materials and Methods

Sampling site: The sediment and shell samples were collected along Barangay Arpili, Balamban coastline with GPS coordinates of 10.47141 N, 123.69051 E (Fig. 1). The shipyard of Tsuneishi Heavy Industries (Cebu) Inc. (THICI) is situated nearby and recognized as a point source of industrial effluents, which are allegedly contaminating this section of the coastline.

Sample collection and identification: Approximately 1 kg of sediment samples were randomly collected (Fig. 2) from the sampling site where the mollusks were found along the coastline in April and November 2018 to represent the dry and wet seasons, respectively. Specifically, bivalves and sediments were dug up and collected along the intertidal zone of the coastline. The bivalves were cleaned and labeled, and both samples were placed in plastic bags and transported to the lab in ice chests. Before further processing, the shells were rewashed with distilled water, wrapped in aluminum foil, placed

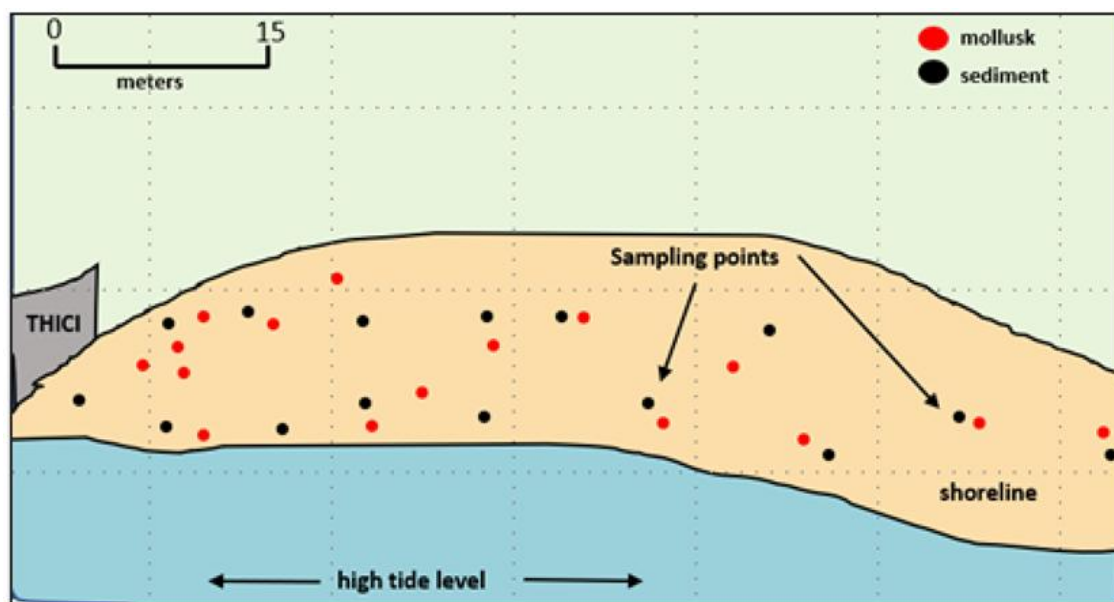


Figure 2. Sampling scheme for sediment and mollusk samples.



Figure 3. Bivalves collected from Balamban coastline, Cebu, Philippines: (A) *Dosinia histrio* (Gmelin, 1791), (B) *Septifer bilocularis* (Linnaeus, 1758), and (C) *Hiatula diphos* (Linnaeus, 1771).

in zip-top bags, and frozen at -10°C . Representative samples of each were then submitted to the curator of the marine biological collection at the University of San Carlos' Department of Biology for identification. The identified bivalves are shown in Figure 3.

Sample preparation and analysis

Mollusk: The method described by Chandurvelan et al. (2015) was used to prepare and analyze the mollusk samples. Preliminary cleaning of the specimens' shells was performed using distilled water. The shellfish tissues were detached from the shells with a stainless-steel knife and thoroughly rinsed multiple times with distilled water to remove all dirt. For each of the three experimental trials, five individual mollusk samples were combined to form a single pooled sample for

analysis. The samples were dried at 60°C until a constant weight was achieved. About 0.5 g of the dried samples were weighed and placed separately in acid-washed tubes and digested at 90°C for an hour using 5 ml of 50% HNO_3 . Once clear, the samples were diluted with 2% HNO_3 before being analyzed for metal content.

Sediments: In the laboratory, the sediments were air-dried, sieved through a $180\text{-}\mu\text{m}$ mesh, and homogenized using a ball mill. The resulting homogenized samples were dried in an oven at 105°C to constant weight for moisture determination, followed by digestion using US EPA Method 3050B. The concentrations of trace metals in the mollusks and sediments were analyzed using flame atomic

absorption spectrometry (FAAS) in triplicate, with the results reported as $\mu\text{g g}^{-1}$ of dry-weight tissue.

Quality control: For quality control, a blank sample was included in each metal analysis. Method reliability was evaluated using linearity, range, and recovery rates. The recovery percentages were as follows: 98.5-107% for Cd, 102-110% for Cr, 109-112% for Cu, 95-101% for Pb, and 110-115% for Zn. Linearity values ranged between 0.998 and 0.999 for Cd, 0.997 and 0.998 for Cr, 0.999 for Cu, 0.997-0.998 for Pb, and 0.997-0.999 for Zn across dry and wet seasons.

Biota-sediment accumulation factor (BSAF): BSAF were calculated using the mean metal concentration in bivalves and the corresponding metal concentrations in sediments based on the formula of $\text{BSAF} = (\text{Metal concentration in bivalve}) / (\text{metal concentration in sediments})$. A BSAF value > 1 is indicative of bioaccumulation (Cañete et al., 2014; Gao et al., 2015).

Metallothionein assay: The method of Linde and Garcia-Vazquez (2006) was used to quantify cellular MT. Bivalve tissue was weighed, homogenized in a buffer (0.5 M sucrose, 20 mM Tris-HCl at pH 8.6, and 0.01% β -mercaptoethanol), and centrifuged at 3,000 rpm for 20 minutes to isolate the MT-containing supernatant. After transferring the supernatant, cold ethanol and chloroform were added, followed by centrifugation at 6,000 rpm at 0-4°C. Samples were then stored at -20°C before further centrifugation. The pellet was washed with ethanol: chloroform (87:1:12), recentrifuged, air-dried, and resuspended in NaCl and HCl with EDTA. After vortexing, NaCl with DTNB was added, and samples were left at room temperature for 30 minutes. Absorbance was measured at 412 nm with a UV-Vis spectrophotometer, using GSH as a standard. The MT concentration was calculated by dividing the cysteine content by 20.

Sediment toxicity risk assessment: The degree of contamination of the heavy metals in the sediments was assessed by determining the geoaccumulation index (Igeo), Enrichment factor (EF), contamination factor (CF), pollution load index (PLI), and and

potential ecological risk index (PERI).

Geoaccumulation index: Igeo values were calculated using the equation of $I_{geo} = \log_2 (C_n / 1.5 B_n)$, where C_n is the measured concentration of metal n in the sediment and B_n is the geochemical background value of element n in the background sample (Gao et al., 2015; Islam et al., 2015).

Enrichment factor: Each element's EF value was calculated to estimate the anthropogenic impact on heavy metals in the sediment. In this study, Al was used for normalization to eliminate the grain size effect (Wang et al., 2017). The EF values of the heavy metals analyzed were calculated using the following formula (Rizabal et al., 2024): $\text{EF} = (C_n / C_{ref})_{\text{sample}} / (B_n / B_{ref})_{\text{background}}$. EF values exceeding 2 indicate that heavy metal contamination is likely not derived from the local sediment background. Instead, this contamination may be linked to long-range transportation from other natural and/or anthropogenic sources.

Contamination Factor: CF was calculated using the following equation, or by dividing the content of each metal by the background values in sediment (Suresh et al., 2012): $\text{CF}_{\text{metal}} = C_{\text{metal}} / C_{\text{background}}$

Pollution load index: To assess the sediment quality, an integrated approach of the pollution load index of the five metals is calculated according to Suresh et al. (2012). The PLI is defined as the nth root of the multiplications of the contamination factor of metals (CF), as shown in the equation of $\text{PLI} = (CF_1 \times CF_2 \dots CF_n)^{1/n}$ (5), where the PLI value of 0 is interpreted as unpolluted, PLI of 1 means polluted, and PLI > 1 is highly polluted.

Ecological risk index (Er): The ecological risk assessment was conducted using the method developed by Hakanson (1980), as cited by Williams and Antoine (2020), to evaluate the potential effect of the heavy metals in sediments on organisms within the marine ecosystem. It utilizes the toxicity coefficient and the contamination factor as shown in the equation: $\text{Er} = T_r \times \text{CF}$. The toxicity coefficients for each metal are Cu = 5, Pb = 5, Cd = 30, Zn = 1, and Cr = 2, where T_r is the toxicity coefficient and CF is the

Table 1. Environmental levels of Cd, Pb, Cu, Zn, and Cr found in mollusks from the Balamban Coastline, Cebu, Philippines.

Mean Metal Conc., mg kg ⁻¹	Bivalve samples						Standard FAO/WHO (2004)
	<i>Dosinia histrio</i>		<i>Haitula diphos</i>		<i>Septifer bilocularis</i>		
	Dry	Wet	Dry	Wet	Dry	Wet	
Cd	<i>bdl</i>	0.87±0.06	<i>bdl</i>	3.21±1.00	<i>bdl</i>	3.52±0.64	0.05
Cr	<i>bdl</i>	<i>bdl</i>	<i>bdl</i>	<i>bdl</i>	0.011±0.03	<i>bdl</i>	-
Cu	18.0±1.91	14.1±0.54	35.1±2.9	35.9±0.22	22.6±1.62	10.6±1.48	30
Pb	<i>bdl</i>	8.57±0.50	<i>bdl</i>	5.55±6.61	<i>bdl</i>	10.2±2.15	0.50
Zn	389.5±19.5	582.1±32.0	197.7±13.6	41.4±3.3	51.5±2.84	22.9±1.51	40

**bdl* = below detection limit

contamination factor.

Potential ecological risk: PERI is also introduced to assess the degree of contamination of heavy metals in the present sediments. Equation of $PERI = \sum RI = \sum (T_{rf} \times CF)$ was used to calculate the PERI proposed by Gao et al. (2015). PERI is the sum of RI, representing the biological community's sensitivity to the toxic substance, and illustrating the potential ecological risk caused by overall contamination.

Statistical analysis: Means and standard deviations were calculated using Excel. A two-way Analysis of Variance (ANOVA) was performed in GraphPad Prism 6.0 to assess significant differences between the data collected in April and November 2018. This analysis was conducted after confirming that the data sets were normally distributed and homogeneous. Pearson's correlation coefficient was used to assess the relationship between heavy metal accumulation and the metallothionein response in bivalves.

Results and Discussions

Heavy metal concentrations in bivalves: Table 1 presents the heavy metal concentrations (Cd, Cu, Pb, and Zn) in mollusk samples. The general trend of metal accumulation in mollusk soft tissues during dry and wet seasons was as follows: Zn > Cu > Pb > Cd > Cr. Cadmium and lead were detected exclusively during the wet season, while Cr was only found in *S. bilocularis* during the dry season. Cd concentrations ranged from 0.87 to 3.52 mg/kg, and Pb was observed in *D. histrio* (8.57±0.50 mg/kg) and *S. bilocularis* (10.2 mg/kg), both exceeding the FAO

and WHO standard limits for Pb and Cd of 0.50 and 0.05 mg/kg, respectively. Copper levels in *H. diphos* (35 mg/kg in the dry season and 36 mg/kg in the wet season) also surpassed the FAO/WHO limit of 30 mg/kg for Cu. Except for *S. bilocularis* in the wet season, all bivalves exceeded the FAO/WHO permissible limit of 40 mg/kg for Zn in both seasons. The results indicated that only Zn levels in bivalves showed significant seasonal variation.

Table 1 reveals distinct seasonal and species-specific patterns in metal accumulation across mollusks, with the order Zn > Cu > Pb > Cd observed in dry and wet seasons. Cr was largely undetected, except in *S. bilocularis* during the dry season, similar to patterns observed in *Senilia senilis* in the Atlantic (Dike et al., 2022). Cd and Pb were detected only during the wet season at levels exceeding FAO/WHO (2004) safety limits. Pb levels in this study were notably higher than 0.006 and 0.011 mg/kg reported in *Corbicula fluminea* and *Mytilus edulis* from Cagayan Valley, Philippines (Raju, 2021), and ranged between 0.17 and 0.30 mg/kg in *C. fluminea* from Laguna de Bay (Diwa et al., 2022). Lower Pb concentrations of 1-2 mg/kg were noted in *Polymesoda erosa* from India's Zuari Estuary (Cruz et al., 2020), with similar values observed in various bivalves from the Karnafully River estuary (Niloy et al., 2024). Elevated Cd levels also exceeded those reported by other studies (Raju, 2021; Diwa et al., 2022; Niloy et al., 2024), indicating possible anthropogenic sources such as antifouling paint residues and manufacturing processes (Morrow, 2003; Youravong and Teanpaisan, 2015; Pereira et al., 2018).

Table 2. Mean environmental concentrations of Cd, Pb, Cu, Zn and Cr of sediments in Balamban Coastline, Cebu, Philippines.

Name of River/Country	Mean Metal concentrations, mg/kg					References
	Cd	Cr	Cu	Pb	Zn	
Balamban Coast April 2018	0.470	10.21	20.89	11.23	26.14	This study
Balamban Coast November 2018	0.432	11.71	22.24	13.74	30.44	
World Health Organization	6.00	25.0	25.0	10.0	123	WHO, 2001
Toxicity Reference Values	1.20	81.0	34.0	46.7	150	USEPA, 1999

Copper in *H. diphos* exceeded the FAO/WHO limit of 30 mg/kg in both seasons, aligning with high sediment Cu concentrations (Geolin et al., 2021). These concentrations were higher than the 1.13-14.0 mg/kg Cu found in bivalves in Niloy et al. (2024) and the 0.3182 mg/kg in *Pharella* sp. from Quezon, Philippines (Sua et al., 2015) but lower than levels in *Batissa violacea* from the Danshuei Riverine Mangrove in Taiwan (Yam et al., 2020) and Taft River in the Philippines (Cabahug et al., 2023). Other studies reported Cu concentrations of 7.0 mg/kg along the French coastline (Barrera et al., 2024) and 26.4 mg/kg in *Mytilus edulis* from Korea (Jeong et al., 2024). Elevated Cu in Guajar Bay, Brazil, has been attributed to antifouling paint residues, especially cuprous oxide (CuO) (Guimares et al., 2022).

In the current study, Zn concentrations also exceeded the 40 mg/kg limit in all bivalves, except for *S. bicularis* during the wet season, with values notably higher than the 116 mg/kg Zn in French bivalves (Barrera et al., 2024), 43.33 mg/kg in *M. mercenaria* (Cabahug et al., 2022), and up to 110 mg/kg in *M. galloprovincialis* from Germein Bay, Australia (Bain et al., 2024). Zn persistence in sediments is often linked to anti-fouling paints used in shipyards (Paradas and Amado, 2007).

No significant correlation ($P>0.05$) was found between heavy metal concentrations across mollusk samples, likely indicating the influence of unaccounted environmental factors or diverse pollution sources. Seasonal variation was significant only for Zn, with Cd and Pb appearing exclusively in the wet season, suggesting that rainfall and runoff are major factors in anthropogenic heavy metal introduction. This seasonal pattern aligns with other

studies (Tayone et al., 2020; Raju, 2021), though Cd and Pb levels here are higher than those found in Davao Oriental and Cagayan Valley, respectively.

Heavy metal concentrations in sediments: The heavy metal concentrations in sediment samples had the accumulation trend of Zn > Cu > Pb > Cr > Cd (Table 2). The average concentrations of heavy metals detected in sediments were within the standard limits set by the World Health Organization (WHO) and the toxicity reference values established by the United Nations Environmental Protection Agency (USEPA, 1999), except for Pb, which exceeded these limits in both seasons. Pb concentrations, however, consistently exceeded these thresholds, averaging 12.49 ± 1.77 mg/kg. This elevated Pb level likely originates from local sources, such as leaded paint, gasoline, and emissions from manufacturing and recycling. Similar pollution sources, including atmospheric lead deposition from industrial activities and aging paint, have been noted in other studies (Oquiena-Paler and Ancog, 2014). Since fishing is a key livelihood for surrounding communities, these Pb levels present potential environmental and health risks, particularly through bioaccumulation in local marine species. Metal concentrations were generally higher in the wet season than in the dry season, except for Cd. The results showed no significant seasonal differences for any of the metal concentrations.

Biota-sediment accumulation factor: Figure 4 illustrates the BSAF values of cadmium, copper, lead, and zinc in bivalves collected from the Balamban coast relative to sediment concentrations. Zinc consistently showed the highest BSAF in both seasons in *D. histrio* and *H. diphos*. Among the species, *S. bicularis* displayed the highest BSAF for Cd,

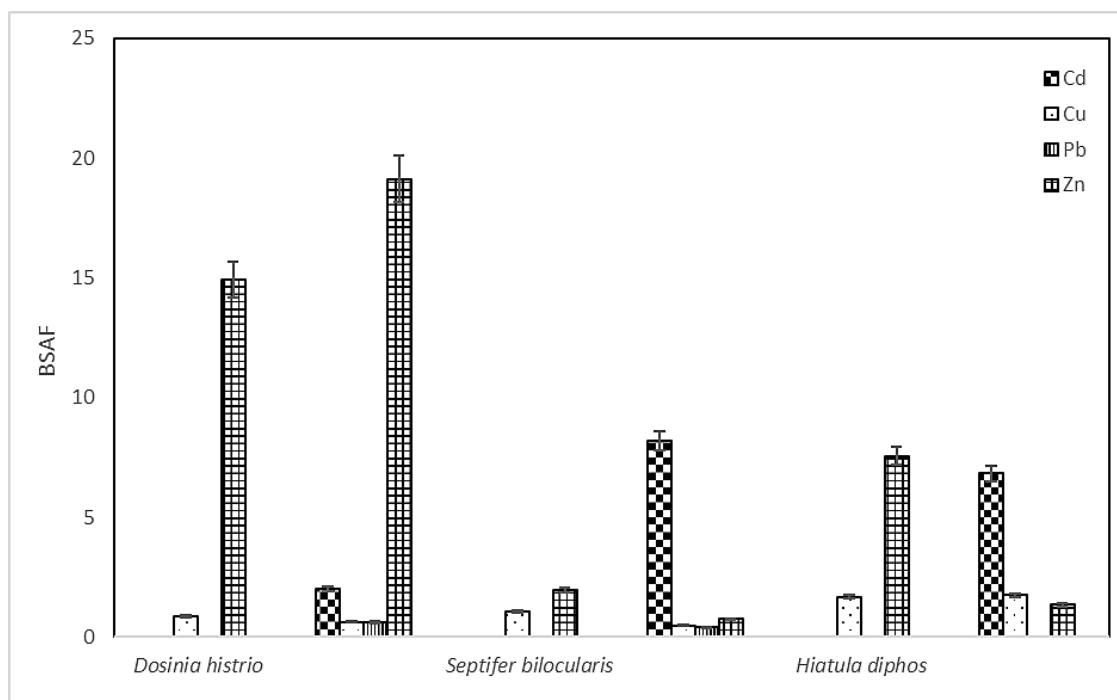


Figure 4. BSAF values in bivalves concerning the sediments of the Balamban coast.

particularly in the wet season; however, *H. diphos* and *D. histrio* showed lower but still considerable values. Cu showed a BSAF value greater than 1 in *H. diphos* during both seasons and *S. bilocularis* during the dry season, indicating its significant accumulation potential. In contrast, only Pb had BSAF values below 1 for *D. histrio* and *S. bilocularis* in the wet season, suggesting lower accumulation levels. Overall, the elevated BSAF values, particularly for Zn and Cd, indicate a substantial bioaccumulation of these metals in the soft tissues of the bivalves.

The mean BSAF values generally follow the trend $Zn > Cd > Cu$. The Zn BSAF in *D. histrio* in this study was higher than the 7.63 and 8.34 reported for *Polymesoda erosa* (Cruz et al., 2020) but is comparable to *H. diphos* in the dry season. *Septifer bilocularis* displayed the highest accumulation for Cd, followed by *H. diphos* and *D. histrio*, suggesting a strong affinity for this toxic metal, consistent with Cd BSAF values of 0.31 to 6.01 in *C. fluminea* as reported by Diwa et al. (2022). In contrast, Cu showed variable BSAF values, exceeding 1 in *H. diphos* during both seasons and *S. bilocularis* during the dry season, indicating these species may serve as reliable bioindicators of copper pollution. Diwa et al. (2022)

reported lower Cu BSAF values of 0.32-0.67 in *C. fluminea*, highlighting the bivalves in Balamban as superior bioaccumulators.

According to Romero-Isart and Vasák (2002), metallothioneins preferentially bind d10 metal ions such as Zn(II) and Cd(II) via cysteine clusters, explaining the significant bioaccumulation of Zn and Cd in these bivalves. Similarly, Nordberg et al. (2009) observed that clam metallothioneins bind both “physiologic” metals (e.g., Zn and Cu) and “xenobiotic” metals (e.g., Cd). Conversely, Pb BSAF values were below 1 for both *D. histrio* and *S. bilocularis*, indicating limited Pb accumulation, which may be due to low bioavailability or effective detoxification. This is consistent with Cruz et al. (2020), who reported Pb BSAF values of 0.04 and 0.06.

The seasonal variations in BSAF values suggest continued monitoring, as factors like temperature and sediment composition can impact metal bioavailability and accumulation. Additionally, a weak correlation between sediment metal levels and mollusk tissue concentrations suggests that bioaccumulation is influenced by environmental metal levels and factors such as species-specific traits,

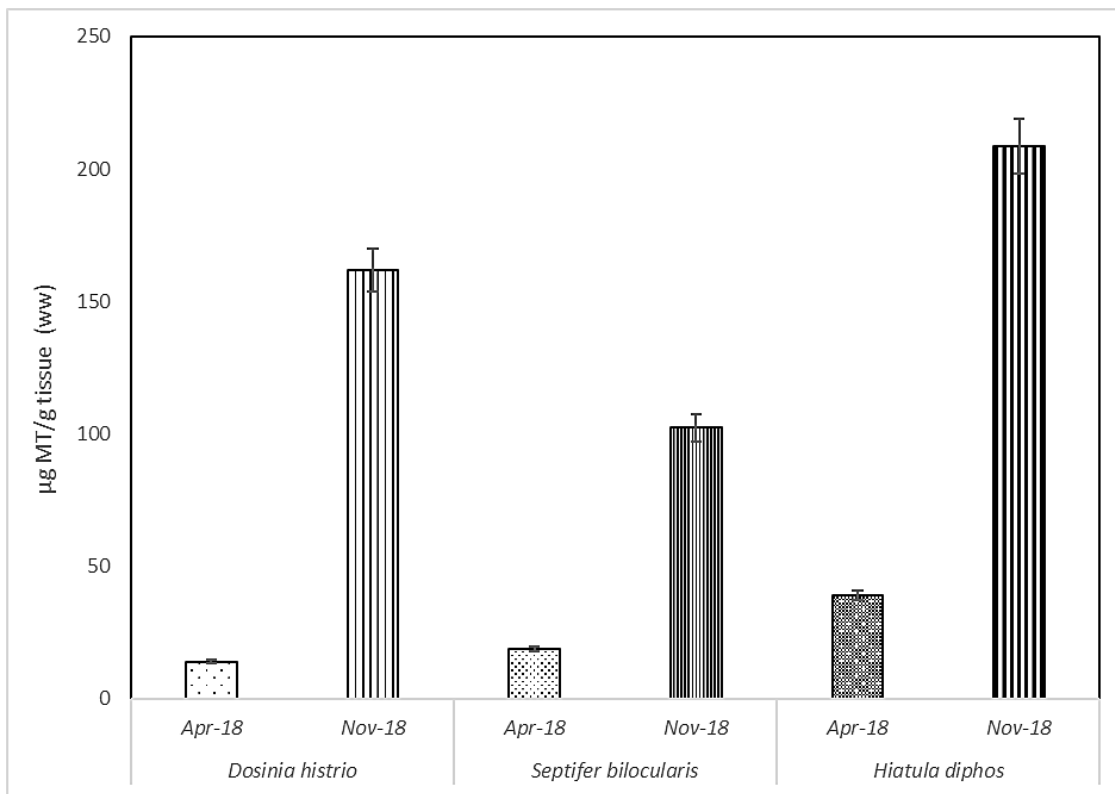


Figure 5. MT levels of bivalves in two seasons. Data are expressed in µg/g.

feeding habits, and organism maturity (Tanhan et al., 2022). The high variability in tissue metal concentrations observed in this small sample size may also contribute to the limited correlation.

Metallothionein assay: Figure 5 illustrates MT levels in all bivalves during the wet season. The highest concentration, 209 µg/g, was recorded in *H. diphos* in November 2018, while the lowest concentration of 14.0 µg/g was detected in *D. histrio* in April 2018. The significant rise in MT levels across all bivalve species during the wet season indicates a strong physiological response to increased metal exposure. This seasonal trend likely reflects higher metal concentrations in sediments during rainfall and runoff periods, enhancing metal bioavailability in the aquatic environment. This aligns with findings by Lee et al. (2016), which suggest that seasonal factors and environmental conditions, such as temperature, nutritional status, salinity, and hypoxia, influence MT induction. The highest MT concentration of 209 µg/g recorded in *H. diphos* during the wet season, compared to a minimum of 14.0 µg/g in *D. histrio*

during the dry season, underscores *H. diphos*'s strong metal-binding capacity. Notably, *D. histrio*'s dry-season level is lower than those reported by Oaten et al. (2016) for *F. spiralis* (20.8 µg/g) and by Buzzi and Marcovecchio (2016) for *N. granulata* (88.2 µg/g). MT synthesis in these bivalves serves as a defense mechanism, sequestering excess metals and reducing cellular damage. These results support MT as a sensitive biomarker for assessing metal pollution and the ecological impact of human activities on coastal ecosystems.

Sediment toxicity risk assessment: Geoaccumulation Index (Igeo) (Muller and Suess, 1979) is a qualitative metric for assessing the intensity of pollution in sediments and was applied to evaluate anthropogenic enrichment of heavy metals in this study. Sediment metal concentrations from Balamban were compared against background levels from Nug-as Cave, a site within the Nug-as Forest Reserve in Barangay Nug-as, Alcoy, Cebu. Nug-as Cave was chosen for its remoteness and minimal human activity, making it an ideal reference point with negligible

Table 3. Enrichment factor of sediments in Balamban coastline.

Metal	Enrichment factor, EF				
	This study		Kahal et al., 2020	Pooveneswary et al., 2020	Jahromi et al., 2021
	Balamban Coastline		Red Sea Saudi Arabia	Kelantan nearshore, Malaysia	Persian Gulf, Hormozgan, Iran
	Apr-18	Nov-18			
Cd	3.86	4.13	7.66	93.54	4.48
Cr	0.164	0.188	3.93	1.95	5.72
Cu	2.04	2.11	4.73	1.70	2.51
Pb	4.86	5.95	5.58	24.94	3.42
Zn	2.77	3.80	3.32	2.78	2.68

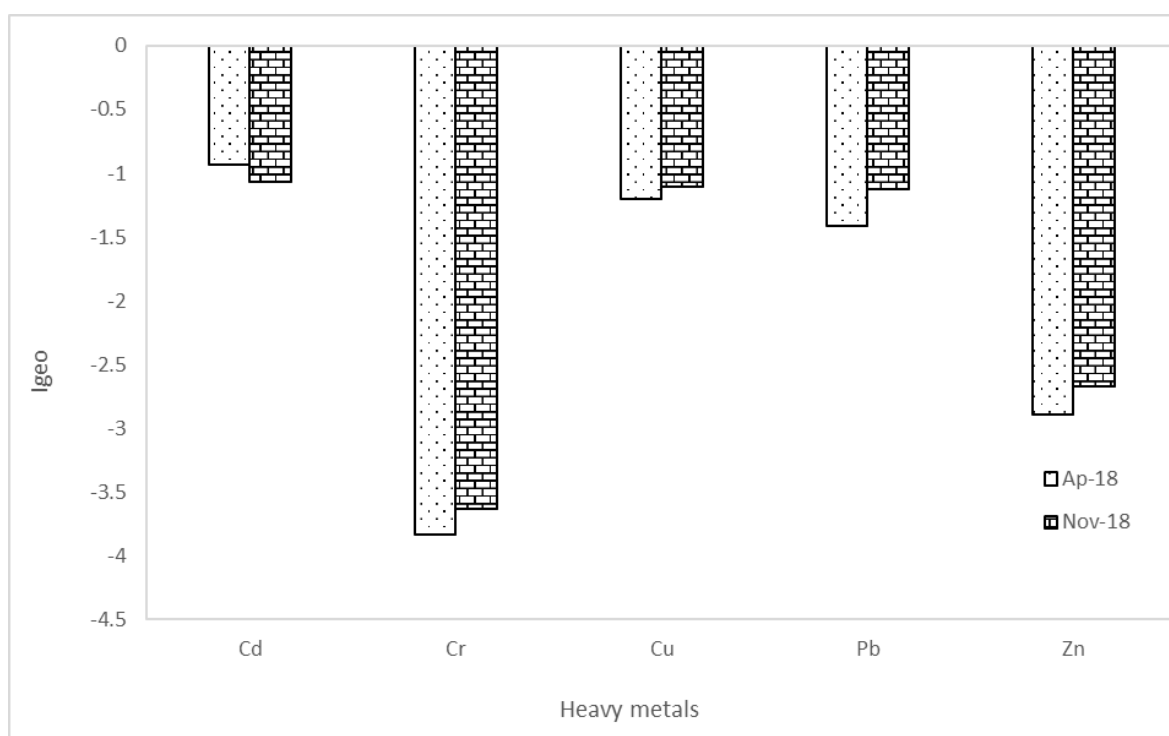


Figure 6. Geo-accumulation index (Igeo) values of heavy metals in Balamban coast sediments.

anthropogenic influence. As shown in Figure 6, all metals exhibited negative Igeo values, indicating the absence of anthropogenic enrichment compared to background levels. Based on the Igeo classification, the sediment samples from Balamban are categorized as "uncontaminated" for all metals. This observation is consistent with the findings of Ali et al. (2022), who reported negative Igeo values for V, Mn, Fe, Co, and As, as well as those of Zhang et al. (2022), who observed negative Igeo values for Cd, Cu, Cr, Pb, and Zn in sediments from the western Sunda Shelf in the South China Sea. Both studies similarly concluded a lack of anthropogenic enrichment in the analyzed

sediments.

Enrichment factor: The average EF for heavy metals followed a decreasing trend: Pb > Cd > Zn > Cu > Cr (Table 3). The highest EF value was observed for Pb during the wet season (5.95), while Cr consistently displayed the lowest EF in both seasons. Compared to other coastal regions, the heavy metal concentrations in this study fall within the ranges reported in similar research. Notably, Pb levels were lower than those recorded near the Kelantan nearshore in Malaysia (Pooveneswary et al., 2020) but higher than those reported in the Persian Gulf, Iran. Kahal et al. (2020) pointed out that metal enrichment is primarily due to

Table 4. Evaluation on potential ecological risk of heavy metals pollution in sediments from Lahug River.

Seasons	Risk Index (Eir)					Potential Ecological Risk Index (PERI)	Risk Grade (Hakanson, 1980)
	Cd	Cr	Cu	Pb	Zn		
	$C_f^i \times T_f^i$	$C_f^i \times T_f^i$	$C_f^i \times T_f^i$	$C_f^i \times T_f^i$	$C_f^i \times T_f^i$		
Dry	23.5	0.21	2.81	3.26	0.203	30.0	Low ecological risk
Wet	21.5	0.24	3.43	3.48	0.236	28.9	Low ecological risk

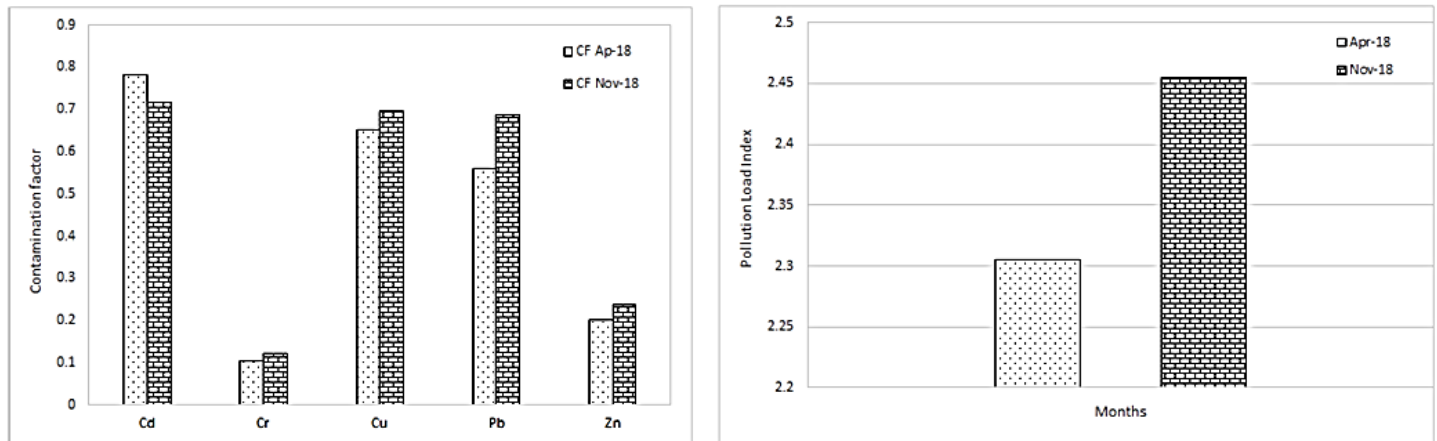


Figure 7. Contamination factor and Pollution Load Index values of heavy metals in Balamban coast sediments.

anthropogenic activities, including urban and agricultural wastewater discharge from surrounding areas. Similarly, this study aligns with Zhang et al. (2021), who reported significant EF values in the South China Sea despite negative Igeo values. This suggests that localized sources may contribute to metal enrichment, even when overall contamination levels, as measured by Igeo, do not indicate widespread anthropogenic impact.

In the case of the Balamban coast, the potential runoff or direct discharge of industrial wastewater from the nearby THIC shipbuilding facility likely played a significant role in the elevated concentrations of heavy metals. This localized industrial activity emerges as a critical factor driving metal enrichment. These findings underscore the need for ongoing monitoring and implementing targeted mitigation measures to effectively manage and reduce contamination in the area.

Contamination factor and pollution load index: Figure 7 reveals that although individual heavy metal concentrations in the sediments of the Balamban coast fall below contamination thresholds, the combined

PLI exceeds 1. This suggests a measurable cumulative pollution load despite individual metals' low CF. The interplay between the CF and PLI indexes indicates that while no single metal poses a critical threat, but their combined ecological impact could be significant. **Potential ecological risk index:** To validate the Igeo, CF, and PLI evaluations, the ecological risk index and the PERI were calculated, with the results summarized in Table 4. The ranking of potential ecological risk coefficients for heavy metals in the Balamban coast sediments was $Cd > Cu > Pb > Cr = Zn$. All potential ecological risk coefficients were below 40, indicating a low ecological risk classification.

The ecological risk assessment aligns with the Igeo and PLI findings, emphasizing that the metals pose minimal immediate ecological threats. However, Cd was identified as the highest-risk metal among those analyzed, with EF values of 2.86 and 3.13, BSAF values ranging from 2.02 to 8.20, and risk indices of 21.5 and 23.5 across seasons. Lead was followed by EF values of 4.86 and 5.95, BSAF values of 0.403 to 0.623, and risk indices of 3.26 and 3.48.

Despite the low overall ecological risk

classification, the anthropogenic origins of these metals and their inherent toxicity and mobility in sediments could elevate the ecological threat over time. Similar observations were noted by Kahal et al. (2020) in the Jazan coastal area of the Red Sea, where long-term exposure and accumulation amplified risks. Therefore, regular monitoring is essential to address any future ecological impacts preemptively.

Conclusion

The study reveals that the three mollusk species from the Balamban coastline contain elevated levels of Zn, Cu, Pb, and Cd, often exceeding safe limits for human consumption, which poses potential health risks to the local community. The seasonal variability, with Pb and Cd peaking during the wet season, suggests sensitivity to changes in metal bioavailability due to environmental factors, such as industrial discharges or runoff. This makes them reliable indicators of temporal fluctuations in contamination levels. Notably, due to their high metal accumulation, *H. diphos* and other bivalves could serve as bioindicators. However, the weak correlation between sediment and tissue metal levels indicates that complex environmental factors may also influence bioaccumulation. To mitigate these risks, stricter monitoring by government agencies, especially during wet seasons, alongside a more extensive ecological risk assessment, is recommended to safeguard the environment and public health.

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