Jurnal Perikanan Universitas Gadjah Mada 28 (1): 01-08 DOI 10.22146/jfs.110351

JPUGM
JURNAL PERIKANAN UNIVERSITAS GADJAH MADA
International Journal in Aquatic and Fisheries Sciences

ISSN: 2502-5066 (Online)
ISSN: 0853-6384 (Print)
Available online at
https://jurnal.ugm.ac.id/jfs

Early Detection of Heavy Metal Pollution with Biological Markers in Freshwater Clam (*Corbicula javanica*) in Maros River, Indonesia

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OPEN ACCESS

Article Info

Submitted	: 08 December 2025
Revised	: 26 January 2026
Accepted	: 28 January 2026
Published	: 10 March 2026
Available online	: 12 March 2026

Keywords:
Biomarker;
Corbicula javanica;
heavy metals;
metallothionein;
pollution

ABSTRACT

This study aims to identify suitable biomarkers for monitoring heavy metal pollution in the Maros River. The results showed that the livers of *Corbicula javanica* from the Maros River contained metallothionein Pb and metallothionein Cd. The average accumulation values of Pb and Cd that induced the appearance of metallothionein Pb were 0.001628 ppm and 0.004929 ppm, respectively. This study demonstrates that *Corbicula javanica* clams exhibit a significant biological response to exposure to the heavy metals Pb and Cd in the Maros River, as evidenced by high bioaccumulation levels, the presence of the molecular biomarker metallothionein, and disturbances in physiological parameters, including the Gonad Somatic Index (GSI). Cd exhibited higher and faster accumulation patterns and Bioaccumulation Factor (BCF) values compared to Pb, indicating more substantial toxicity potential and biological retention. The expression of Cd- and Pb-metallothionein in liver tissue, observed in the first and fifth weeks of exposure, demonstrated the high sensitivity of these biomarkers as early detection tools for heavy metal contamination. Conversely, although the GSI values of the river freshwater clam were significantly lower than those of the control group, this parameter proved less sensitive to specific metal types, making it unsuitable as a sole indicator for early detection of contamination. The positive correlation between metal concentrations in tissues and shifts in physiological distribution, as determined by Principal Component Analysis (PCA), further supports the finding that freshwater clams experience stress accumulation over time. Based on the results, Pb metallothionein and Cd metallothionein are biological markers (biomarkers) that can be used to detect water pollution early.

How to Cite this Article Indrawati, E., R. Ratnawati, H. Hadijah, R.A. Aldyan, J. Jalil & D. Dahlifah. 2026. Early detection of heavy metal pollution with biological markers in freshwater clam (*Corbicula javanica*) in Maros River, Indonesia. Jurnal Perikanan Universitas Gadjah Mada. 28 (1): 01-08. https://doi.org/10.22146/jfs.110351

INTRODUCTION

Water pollution may arise when industrial or household effluents are released into aquatic environments without adequate treatment, or when treated effluents retain pollutant concentrations exceeding specified quality limits (Novotny, 2022). Heavy metal pollutants are hazardous when they contaminate water bodies, as they are toxic, carcinogenic, bioaccumulative, and biomagnificative (Edo *et al.*, 2024; Sonone *et al.*, 2021). Among heavy metals, lead (Pb) and cadmium (Cd) are the most toxic. Pb and Cd pose health risks throughout the food chain. Animals absorb Pb and Cd from food, which then accumulate in tissues such as the kidneys, liver, and reproductive organs (Ako *et al.*, 2019; Latief *et al.*, 2020).

The Maros River functions as a critical water catchment area for the region. In addition to its hydrological role, the river serves as a transportation route for local communities and fishermen, providing access to the Maros Fish Landing Port (TPI Maros). The river's water is also utilised for multiple purposes, including agriculture, aquaculture, industry, and domestic needs. Communities residing near the Maros River, particularly in its downstream areas, frequently harvest freshwater clams (also known as mussels) from the river for both daily consumption and commercial sale. Research conducted by the South Sulawesi Environmental Agency has detected the presence of heavy metals, particularly lead (Pb) and cadmium (Cd), in the Jeneberang watershed, raising significant concerns that these contaminants may have also reached the Maros River (Indrawati, 2013).

Despite this potential contamination, residents remain largely unaware that the water, sediments, and mussels collected from the river may contain hazardous levels of Pb and Cd. This poses serious health risks, as prolonged exposure through consumption can lead to carcinogenic effects and bioaccumulation in pond-raised aquaculture organisms using river water. Based on Government Regulation of the Republic of Indonesia Number 22 of 2021, the quality standards for lead (Pb) and cadmium (Cd) in class II surface water used for fish farming activities are set at 0.03 mg/L and 0.01 mg/L, respectively. As a mitigation and early prevention effort, a biological approach utilising biological markers (biomarkers) is crucial. Biomarkers enable the detection of heavy metals at the subcellular level before more widespread ecological damage occurs (Kadim & Risjani, 2022). One biomarker widely used is metallothionein, a metal-binding protein induced by heavy metals such as lead (Pb) and cadmium (Cd) (Yang, 2024). The presence and expression levels of metallothionein in freshwater clam liver tissue can serve as biological indicators of heavy metal exposure, even at concentrations below quality standards. Therefore, early detection of heavy metal contamination in *Corbicula javanica* in the Maros River using biomarkers is crucial, not only for environmental monitoring but also to protect public health and the sustainability of aquatic ecosystems.

MATERIALS AND METHODS

Research method

The study was arranged according to a quantitative approach, with periodic mussel sampling. This research was conducted in the Maros River using cages placed in the riverbed for three months. Control mussels were collected from the river, depurinated, and then reared in tanks to detect the presence of metallothionein in unexposed mussels. All mussels used came from the Maros River. Collected mussels were selected with shells measuring 4-5 cm in length. They were then placed in cages at a stocking density of 100 mussels. Ten mussels were collected for each sampling, with livers, gonads, and tissue samples collected. Subsequently, the tissues were analyzed for Pb and Cd heavy metal content, livers were examined for MT, and gonads were weighed. Accumulated Pb and Cd levels were analyzed using an Atomic Absorption Spectrophotometer (AAS), metallothionein levels using High Performance Liquid Chromatography (HPLC), and gonads were weighed to determine the Gonade Somatic Index. Sampling for GSI determination was also conducted seven times at one-week intervals.

Data analysis

The Bioconcentration Factor (BCF) and Gonadosomatic Index (GSI) were calculated using the following formulas (Connell *et al.*, 2009; Wootton, 2012):

$$BCF = \frac{Heavy\ metal\ concentration\ in\ the\ organism\ (mg/kg)}{Heavy\ metal\ concentration\ in\ the\ medium\ (mg/kg)}$$

$$GSI\ (\%) = \frac{Gonad\ weight\ (g)}{Total\ Gonad\ wight\ (g)} x\ 100$$

The effect of sampling time on heavy metal accumulation and the induction of Pb- and Cd-specific metallothioneins were analyzed using correlation tests in SPSS version 20. Additional data analyses were performed in RStudio using R version 4.2.2. Pearson correlation analysis was applied to evaluate the relationships between heavy metal concentrations (Pb and Cd) and the biological parameters GSI and BCF. Principal Component Analysis (PCA) was conducted to identify dominant patterns in metal accumulation and biomarker responses. Graphical visualizations, including correlation plots, GSI trend lines, and PCA biplots, were generated using RStudio and OriginPro 2022b.

RESULTS AND DISCUSSION

Results

Heavy metal accumulation and BCF values (Pb and Cd)

Indicators of heavy metal pollution in freshwater clams cultured in cages can be assessed through the accumulation levels of lead (Pb) and cadmium (Cd), as well as their corresponding bioconcentration factor (BCF) values. These quantitative indicators are presented in Table 1.

The results of the study indicate that Corbicula from the Maros River have accumulated heavy metals Pb and Cd in their livers and tissues, with Pb concentrations ranging from 0.001595 to 0.224966 ppm and Cd reaching up to 0.009050 ppm. Pb BCF values varied, with an extreme peak in the fifth measurement, while Cd BCF was generally low. Molecular analysis showed that the Cd metallothionein biomarker was detected in the first week, while Pb metallothionein appeared in the fifth week of exposure. Conversely, freshwater clams in the control group did not show metallothionein expression, indicating that heavy metal exposure occurred only in freshwater clams from the Maros River environment. The

Table 1. Heavy metal accumulation and BCF values (Pb and Cd) in Maros River.

Sampling time	Metal concentration (ppm)		BCF of metal	
	Pb	Pb	Cd	Cd
I	0.000160	0.001595	0.668331	0.004011
II	0.002250	0.224966	0.419332	0.002515
III	0.001060	0.105990	0.441501	0.002650
IV	0.001712	0.071152	0.353666	0.002123
V	0.003140	0.002350	1.508500	0.009050
VI	0.003450	0.072445	1.700920	0.009230
Mean	0.001628	0.079749	1.000000	0.004929

average metal accumulation inducing the appearance of Cd metallothionein was 0.004929 ± 0.003323 ppm, while Pb metallothionein was 0.001628 ± 0.000872 ppm. The BCF values at the onset of metallothionein Pb were recorded as 0.079749 ± 0.082465, and for Cd as 1.0 ± 1.70092. These results indicate that although accumulation has occurred, the liver tissue's ability to absorb heavy metals remains relatively low and has not yet reached a steady state, likely due to the relatively short exposure duration.

Trends in heavy metal accumulation and BCF values (Pb and Cd)

To understand the dynamics of heavy metal bioaccumulation on Corbicula, trends in the concentrations of Pb and Cd accumulated in liver tissue were observed, and Bioaccumulation Factor (BCF) values were calculated at six observation times The measurement results indicate variations in the accumulation levels and BCF values of each metal, reflecting the complex interactions between water environmental conditions and the physiological capacity of the freshwater clam to absorb and tolerate these heavy metals. The following graphs present the trends in metal concentration changes and BCF values over time, as shown in Figures 1 and 2 below.

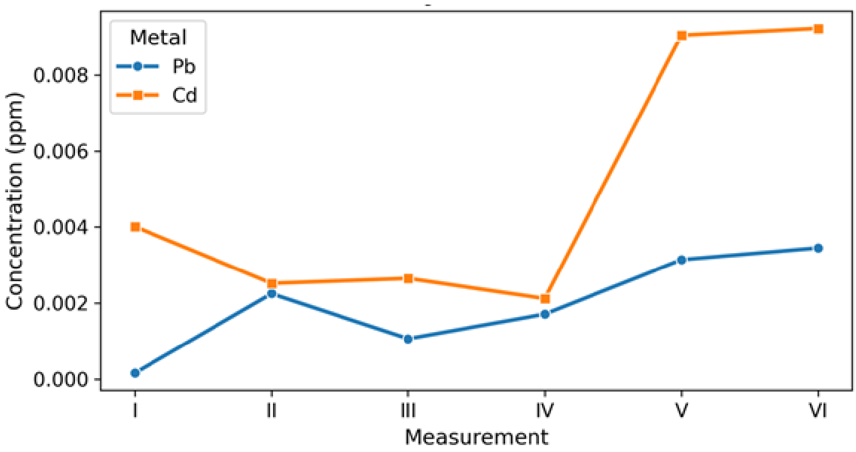

Figure 1. Trends in the accumulation of heavy metals Pb and Cd on corbicula tissue.

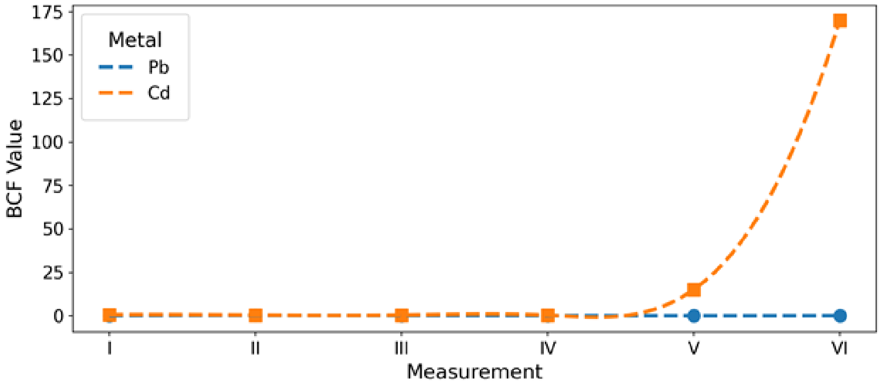

Figure 2. Trends in BCF value of Pb and Cd on corbicula tissue.

Figure 1 illustrates the pattern of heavy metal accumulation of Pb and Cd in Corbicula's liver tissue over time. Both metals exhibit increasing accumulation over time; however, Cd shows a sharp spike from the fifth to the sixth measurement. At that point, Cd concentrations were nearly double those of previous measurements, while Pb showed a more moderate increase. This trend highlights the greater potential risk to organisms from Cd and underscores the role of freshwater clams as effective bioindicators for detecting elevated Cd levels in aquatic environments. Figure 2 shows the trend of BCF values for heavy metals Pb and Cd in *Corbicula javanica* freshwater clam over six measurement times. Pb BCF values tended to be constant and low in all measurements, indicating that the accumulation capacity of Pb by tissues is relatively stable. In contrast, Cd BCF values increased sharply, particularly at the sixth measurement, exceeding 170, indicating significant Cd accumulation in the tissues. This surge suggests that freshwater clams actively accumulate Cd from the environment and have likely exceeded their detoxification capacity threshold, reinforcing Cd as the dominant contaminant at the site.

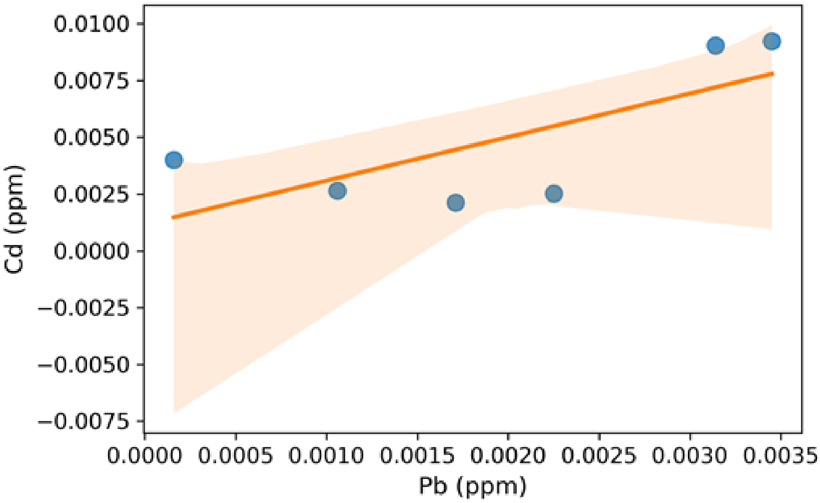

Figure 3. Correlation between heavy metals Pb and Cd on Corbicula tissue.

Figure 3 shows a linear relationship between Pb and Cd concentrations in Corbicula tissue. There is a positive correlation between the two metals, indicating that an increase in Pb levels tends to be followed by an increase in Cd levels. However, the correlation is not very strong. This trend suggests that the source of contamination likely originates from the same activity or location or that the two metals share similar pathways and biological distribution within the Corbicula tissue. This correlation indicates the potential for synergistic effects from dual heavy metal exposure on organisms, which could exacerbate their toxicological impacts.

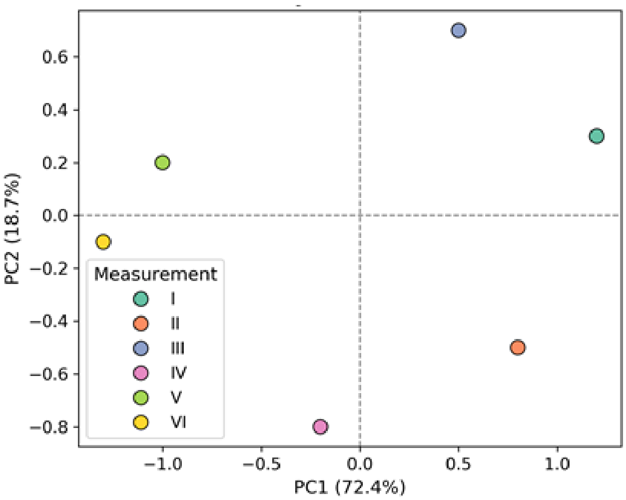

Figure 4. PCA graph of heavy metal accumulation variability distribution.

Figure 4 illustrates the distribution of heavy metal accumulation variability based on six measurement times. The first principal component (PC1) accounts for 72.4% of the total variation, while PC2 accounts for 18.7%. Points from different measurement times are scattered across four quadrants, indicating significant differences in metal accumulation patterns between each time point. The fifth and sixth measurements are located far from the initial measurements, indicating a shift in environmental conditions or increased pressure from heavy metal contamination during the final period. This supports previous findings that freshwater clams experience cumulative stress over exposure.

Gonade somatic index (GSI) biomarker

In addition to indicating heavy metal pollution biomarkers, such as accumulation and BCF values, Corbicula cultivated in the Maros River also showed Gonadal Somatic Index (GSI) values that were compared with those of controls. The GSI values of Corbicula in the control and river environments are presented in Table 2 below.

Table 2. GSI values of *Corbicula javanica* in the control environment and Maros River.

Sampling time	GSI value	
	Control	River
I	0.23	0.10
II	0.27	0.13
III	0.23	0.15
IV	0.19	0.13
V	0.23	0.17
VI	0.23	0.13
VII	0.22	0.16
VIII	0.21	0.14
Mean	0.22625	0.13875
SD	0.02263	0.02167

The results of Gonadal Somatic Index (GSI) measurements showed significant differences between Corbicula in the control condition, without exposure to heavy metals, compared to the environment in the Maros River. The average GSI value for the control was recorded as 0.22625 ± 0.02263, while the GSI value in the Maros River was approximately 0.13875 ± 0.02167.

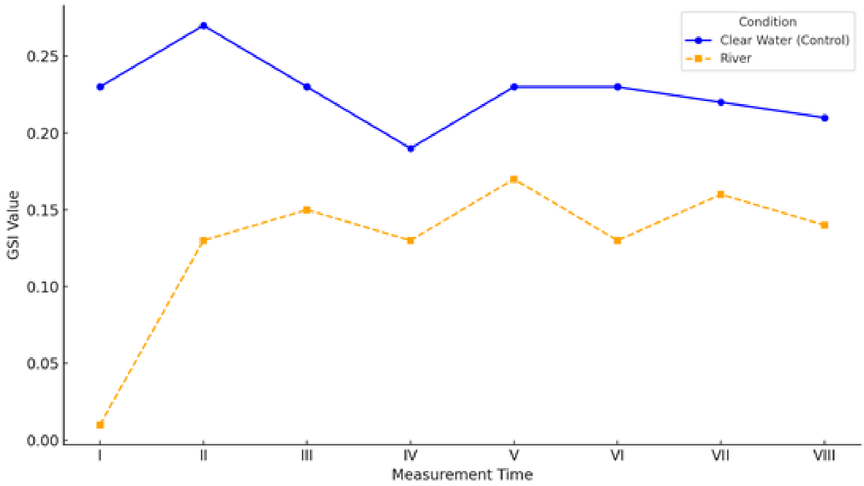

Figure 5. Trends in GSI value of control and river environment.

Figure 5 compares Corbicula GSI values in control (no heavy metals) and Maros River conditions over eight observation periods. Control group GSI remained stable and high (0.19-0.27), indicating normal gonadal development. In contrast, river GSI values fluctuated more and were always lower (0.01-0.17), starting very low (0.01) in the first observation, suggesting acute stress from initial heavy metal exposure. While there were slight increases at later points, river GSI never matched control levels.

Discussion

The results showed that Corbicula from the Maros River accumulated heavy metals Pb and Cd in their liver tissue, with Pb concentrations ranging from 0.001595 to 0.224966 ppm and Cd reaching up to 0.009050 ppm. Pb BCF values varied, with an extreme peak in the fifth measurement, while Cd BCF values were generally low. Molecular analysis showed (Figure 5) that the Cd metallothionein biomarker was detected in the first week, while Pb metallothionein appeared in the fifth week of exposure. Conversely, Corbicula in the control condition did not exhibit metallothionein expression, indicating that heavy metal exposure was limited to freshwater clams in the Maros River environment. These results indicate that although accumulation has occurred, their liver tissue still has a low ability to absorb heavy metals and has not yet reached a steady state, likely due to the relatively short exposure duration. Significant differences in GSI values between the control and river indicate that the presence of Pb and Cd in the Maros River water inhibits the development of Corbicula gonads. Lower GSI values in exposed Corbicula suggest that reproductive processes and gonadal maturation are disrupted, likely due to physiological stress caused by heavy metal accumulation (Taslim *et al.*, 2022). Heavy metals such as Pb and Cd are known to penetrate the gill membrane, enter the bloodstream, and then distribute to various target organs, including the liver, gonads, kidneys, muscles, and mantle (Pavlova *et al.*, 2024). This process can disrupt metabolism and endocrine function, which are important for gonad formation and maturation (Gautam *et al.*, 2024; Socha *et al.*, 2024). These findings align with the principles of ecotoxicology, which state that reproductive disruption is one of the most sensitive indicators of heavy metal contamination in aquatic environments. Thus, GSI values can be used as a biological indicator to assess the impact of heavy metal pollution on the reproductive health of aquatic organisms.

The accumulation of heavy metals in the gonads causes damage to the gonadal tissue, leading to gonadal degeneration, reduced size, and impaired reproductive capacity (Bera *et al.*, 2022). According to Wuertz *et al.* (2024) and Prakash (2022), the presence of organic pollutants in water can inhibit sexual maturation, causing small gonads and low GSI values. Furthermore, according to Dane & Şişman (2023), organic pollutants in water can reduce gonadal size in fish and lower GSI values. The results showed that the Corbicula biomarker, metallothionein, was detected after isolation. Meanwhile, Corbicula, kept under control of laboratory conditions, did not show metallothionein in its liver tissue. The presence of Pb metallothionein and Cd metallothionein in the samples is due to the presence of metal-binding proteins (metal binding protein) that play a role in the binding of heavy metals Pb and Cd in the Corbicula liver, such as metallothionein formed from thionine. After proteins bind Pb and Cd, these heavy metals induce the synthesis of Pb metallothionein and Cd metallothionein (Tamás *et al.*, 2014). Based on these findings, it can be inferred that Pb-induced metallothionein differs from Cd-induced metallothionein due to the metal-specific binding affinities of thionine components, which form distinct metallothionein complexes depending on the type of metal involved.

The bioaccumulation trends and physiological responses of Corbicula to heavy metal exposure (Pb and Cd) in the Maros River show complex interactions. Environmental stress and biological defense mechanisms interact at the sub-organism level. The study found that Cd accumulates faster and increases the Bioaccumulation Factor (BCF) more sharply than Pb, especially in the sixth week. This suggests the biological detoxification system may saturate with Cd. Previous studies also indicate that Cd binds more strongly to aquatic tissues than Pb and is harder to excrete (Haseeb *et al.*, 2022). Additionally, the expression of metallothionein (MT) proteins supports these findings. MT-Cd is detected in the first week, while MT-Pb appears in the fifth week. This indicates a faster molecular defense response to Cd exposure. No MT expression was detected in the control group, confirming that the protein responds only to heavy metals. A significant decrease in the Gonad Somatic Index (GSI) in the river group compared with controls indicates reproductive system disruption. This is likely due to metal accumulation in gonadal tissue, which interrupts gametogenesis. Earlier studies show that heavy metals such as Cd and Pb can damage gonadal structure and inhibit gamete development in mollusks (Marinaro *et al.*, 2024; Nkinda *et al.*, 2021).

Correlation analysis showed a positive relationship between Pb and Cd levels in Corbicula liver. This suggests these metals may come from the same pollution source or share similar accumulation pathways. Such correlations matter because dual exposure can cause toxic effects stronger than single exposure (Jomová *et al.*, 2024; Wu *et al.*, 2025). Principal Component Analysis (PCA) also revealed a clear separation among observation times. PC1 explained 72.4% of the variation, and PC2 explained 18.7%. This indicates physiological shifts due to metal buildup over time. The week 5 and 6 observation points were far from the initial group. This reflects growing biological stress and increased environmental pressure. PCA is often used in ecotoxicology to track changes and identify key biological indicators (Bhagat *et al.*, 2021).

This study shows that Corbicula clams are effective bioindicators for detecting heavy metal pollution in freshwater. Using molecular (metallothionein), physiological, and multivariate parameters provides a complete picture of the impact of heavy metals on health and ecosystem conditions (Gagné *et al.*, 2008; Karlsson *et al.*, 2022). The findings support the use of biological biomarkers as an early warning system in water quality programs. Metallothionein found in mussel livers can act as a biomarker for heavy metal pollution in rivers. Metallothionein indicates specific exposure to certain heavy metals (Filipoiu *et al.*, 2022). Certain metals bind proteins to form specific metallothioneins. Lead metallothionein is formed when protein and lead bind; cadmium metallothionein is formed when protein and cadmium bind. Metallothionein binds metals very strongly, but metal exchange can occur easily (Chatterjee *et al.*, 2020; Wang *et al.*, 2014). Even low levels of Pb and Cd can induce metallothionein synthesis in liver tissue. This results in the production of metallothionein, which detoxifies these heavy metals.

CONCLUSION AND RECOMMENDATION

Conclusion

The most appropriate biomarkers for the early detection of heavy metal contamination in the Maros River are Pb- and Cd- induced metallothioneins. These biomarkers were identified in the liver tissue of the freshwater bivalve *Corbicula javanica*. Both proteins exhibit high sensitivity to heavy metal exposure and trigger rapid molecular responses, even at low metal concentrations. In contrast, the Gonadosomatic Index (GSI), when used as a single indicator, shows low sensitivity to Pb and Cd, rendering it insufficient for independent early detection. GSI is more appropriately used as a supporting parameter within a broader biological monitoring framework. Overall, integrating bioaccumulation indicators, metallothionein biomarker expression, and physiological parameters such as the Gonadosomatic Index (GSI) reinforces *Corbicula javanica* status as a reliable and effective bioindicator for early detection of heavy metal contamination in freshwater ecosystems. These findings provide a strong basis for developing more responsive, adaptive, and biomarker-driven strategies for environmental monitoring in the river.

Recommendation

Pb- and Cd- induced metallothioneins in *Corbicula javanica* show strong potential as standard biomarkers for early detection of heavy metal contamination. Future studies should validate their applicability across diverse freshwater systems, integrate advanced molecular detection methods, and explore multi-metal interactions. Combining biomarker data with spatial mapping and predictive models could enable rapid, accurate, and proactive river pollution monitoring.

AUTHORS' CONTRIBUTIONS

EI: conceptualized and designed the study, acquired funding, supervised the research, and drafted the manuscript. R: generated the data, conducted laboratory analyses, contributed to manuscript drafting. H: collected samples, processed data, performed preliminary analyses. RAA: carried out statistical analyses, interpreted the data, and revised the manuscript. J: assisted with field sampling, measured environmental parameters. D: curated the data, conducted the literature review, contributed to manuscript revisions.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Directorate General of Higher Education Indonesia, through the DIPA scheme, Program of Bosowa University, Indonesia, for the financial support provided for the implementation of this research and the preparation of this manuscript

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