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# Metallothionein in the Gills of *Anadara antiquata* as a Biomarker of Heavy-Metal Pollution in Jakarta Bay

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## Abstract

This study analyses Metallothionein (MT) expression in the gill tissues of the feathered clam (*A. antiquata*) from Jakarta Bay using CLSM method, and evaluation of its potential as a biological biomarker of heavy-metal pollution. A total of 90 samples were collected from Three sites representing different levels of anthropogenic contamination. Heavy-metal analysis revealed significant variation among sites, with the highest concentrations recorded at Muara Angke (Cd: 2.15 ± 0.32 mg·kg$^{-1}$; Pb: 3.84 ± 0.41 mg·kg$^{-1}$; Hg: 0.67 ± 0.08 mg·kg$^{-1}$), followed by Cilincing (Cd: 1.42 ± 0.21 mg·kg$^{-1}$; Pb: 2.11 ± 0.36 mg·kg$^{-1}$; Hg: 0.42 ± 0.05 mg·kg$^{-1}$), and Tanjung Priok (Cd: 0.84 ± 0.14 mg·kg$^{-1}$; Pb: 1.27 ± 0.22 mg·kg$^{-1}$; Hg: 0.21 ± 0.03 mg·kg$^{-1}$). Histological examination revealed progressive structural damage consistent with pollution levels: Intact secondary lamellae at Tanjung Priok, epithelial hypertrophy and hyperplasia with MT distribution at Cilincing, and severe lesions including oedema, lamellar fusion, epithelial desquamation, and cytoplasmic vacuolisation at Muara Angke. CLSM analysis confirmed significant differences in MT expression among sites, with Mean Fluorescence Intensity (MFI) lowest at Tanjung Priok (22.5 ± 3.3 a.u.), higher at Cilincing (39.7 ± 4.9 a.u.), and highest at Muara Angke (47.4 ± 5.1 a.u.) (ANOVA, $p < 0.05$). This study represents the first application of CLSM to quantify metallothionein expression in *A. antiquata* from Indonesian coastal ecosystems.

**Major Findings:** Overall, the results demonstrate a strong relationship between heavy-metal exposure, gill histopathology, and MT expression, underscoring the value of *A. antiquata* as a sensitive bioindicator for biomonitoring heavy-metal pollution in urban coastal ecosystems.

**Keywords:** *Anadara antiquata*, Biomarker, Heavy Metals, Immunohistochemistry, Jakarta Bay, Metallothionein

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## 1. Introduction

Heavy-metal pollution is a critical environmental issue in coastal urban ecosystems, including Jakarta Bay, which is heavily influenced by industrial, port, and residential activities. Metals such as Cadmium (Cd), Lead (Pb), and Mercury (Hg) are toxic, persistent, and bioaccumulative, posing risks to marine organisms and human health through food-web transfer[1,2]. The feathered clam (*A. antiquata*) is widely used as a bioindicator due to its filter-

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feeding behaviour, sedentary lifestyle, and high capacity to accumulate metals in its soft tissues[3]. Several studies have reported that bivalves from Jakarta Bay contain Cd, Pb, and Hg above safe consumption limits, highlighting risks to both ecosystem health and food safety monitoring in coastal communities[4-6].

MT are small (6–7 kDa), cysteine-rich cytoplasmic proteins that bind metal ions via thiol (-SH) groups, playing dual roles in essential metal homeostasis (Zn, Cu) and detoxification of toxic metals (Cd, Pb, Hg)[7,8]. MTs also act as endogenous antioxidants by scavenging Reactive Oxygen Species (ROS), thereby protecting cells from oxidative damage[8,9]. Their expression is strongly induced through the metal response element-binding transcription factor-1 (MTF-1) pathway under conditions of elevated metal exposure[10,11]. For these reasons, MT expression in aquatic organisms is recognised as a sensitive and specific biomarker of heavy-metal pollution[10].

Detection of MT expression can be achieved using several techniques, including quantitative Polymerase Chain Reaction (PCR), western blotting, and immunofluorescence microscopy[12]. Among these, CLSM offers key advantages: High-resolution three-dimensional visualisation, quantification of fluorescence intensity, and precise localisation of proteins within tissues[12,13]. In bivalves, gill tissue is a primary site of metal uptake and detoxification, making it a suitable organ for histopathological and molecular biomarker studies[13,14]. CLSM therefore provides an integrative view of both MT expression and its histolocalisation, linking molecular response to tissue-level pathology[12-15].

Previous studies have documented MT expression in bivalves such as *Crassostrea* spp., *Cerastoderma edule*, and *Mytilus* spp. exposed to heavy-metal pollution[15-17]. However, little is known about MT expression in *A. antiquata* from Jakarta Bay, despite its ecological role as a bioindicator and its importance as a seafood resource in Indonesia. Such information is crucial to support molecular-biomarker-based–based biomonitoring programs and to strengthen the one health perspective by linking environmental quality with food safety in contaminated coastal zones.

To date, no study has characterised metallothionein expression in *A. antiquata* from Jakarta Bay, despite its ecological and economic importance as a seafood bioindicator. This study, therefore, aimed to analyse MT expression in the gills of *A. antiquata* collected from three sites in Jakarta Bay with different levels of anthropogenic pressure, using CLSM. Specifically, we evaluated (i) the concentrations of Cd, Pb, and Hg in gill tissues, (ii) associated histopathological alterations, and (iii) quantitative variation in MT expression. The results provide new insights into the utility of *A. antiquata* as a sensitive bioindicator for heavy-metal pollution in tropical estuarine ecosystems. This study has limitations, including not measuring the activity of antioxidant enzymes involved in the defence mechanism under oxidative stress conditions caused by heavy metals, and only using negative controls, without including positive controls in the form of tissue from organisms originating from reference locations with very low levels of pollution.

## 2. Materials and Methods

### 2.1 Sites and Sampling

Feathered clams (*A. antiquata*) were collected from three stations in Jakarta Bay: (1) Muara Angke, (2) Cilincing, and (3) Tanjung Priok (Figure 1). These sites represent a gradient of anthropogenic activity, including port operations, dense settlements, and industrial/domestic effluent discharge. At each site, 30 adult individuals (shell length 4–5cm) were randomly hand-picked at low tide (total n = 90). Samples were transported to the laboratory in aerated seawater from the collection site to minimise stress before processing. Three technical replicates were analysed per individual for fluorescence quantification.

### 2.2 Heavy-Metal Analysis

Gill tissues (~0.5–1g) were digested using a microwave digestion system with $HNO_3$ (65%) and $H_2O_2$ (30%) at 180°C for 30 min until clear solutions were obtained. Digestates were diluted to 25mL with ultrapure water (Milli-Q). All glassware was pre-cleaned with acid to prevent cross-contamination.

Cadmium (Cd) and lead (Pb) were quantified by Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS), and mercury (Hg) by Cold-Vapour Atomic Absorption Spectrophotometry (CV-AAS). Calibration was performed with certified multi-element standards (five-point curves, $R^2 \geq 0.999$). Quality assurance included blanks, duplicates, spiked recoveries (90–110%), and Certified Reference Material (CRM). Metal concentrations are expressed as $mg \cdot kg^{-1}$ wet weight. Method detection limits (LOD) were 0.01 $mg \cdot kg^{-1}$ (Cd), 0.02 $mg \cdot kg^{-1}$ (Pb), and 0.005 $mg \cdot kg^{-1}$ (Hg).

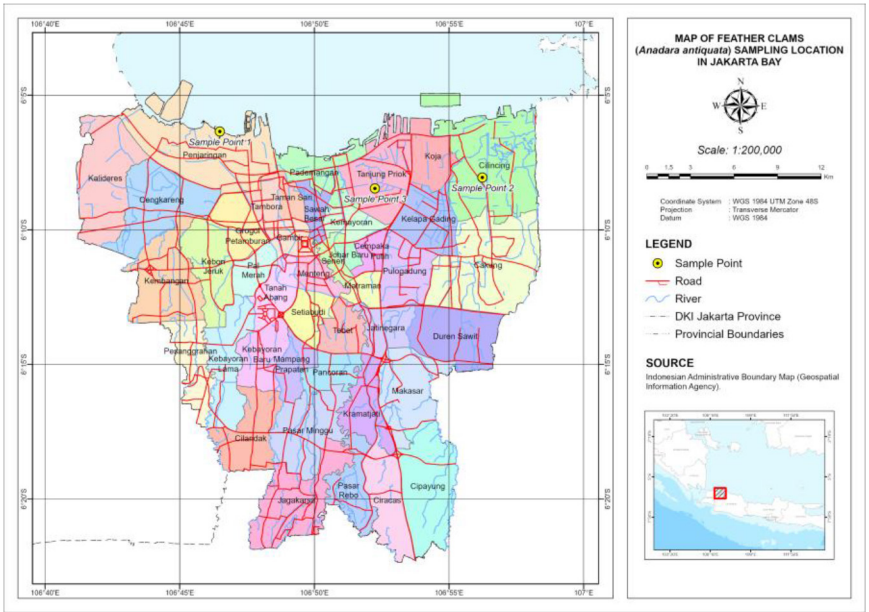



**Figure 1.** Map of sampling sites for feather clams (*A. antiquata*) in Jakarta Bay.

## 2.3 Histological Preparation

Gill tissue was selected as the target organ because of its critical role in metabolism and heavy-metal detoxification, making it representative for detecting both metal accumulation and histopathological alterations. Immediately after dissection, tissues were fixed to prevent autolysis and degradation. Fixation was performed in 10% Neutral Buffered Formalin (NBF; 4% formaldehyde in phosphate buffer, pH 7.0–7.4) at a tissue-to-fixative volume ratio of at least 1:10. Samples were fixed for 24 h at room temperature in formalin-resistant plastic containers (Kartell).

Following fixation, tissues were dehydrated in a graded ethanol series (Merck, ≥96% purity): 70% (2 h), 80% (2 h), 90% (2 h), 96% (2 h), and 100% (twice, 2 h each). This step was designed to remove residual water from the tissues. Dehydrated samples were cleared in xylene (pro analysis, Merck) for 2–3 changes, 30–60 min each, until the tissues became transparent. Samples were then embedded in paraffin wax (Leica Biosystems; melting point 56–58°C) using a tissue embedding station (e.g., Leica EG1150) at 58–60°C, producing paraffin blocks suitable for sectioning. Blocks were sectioned at 4µm thickness using a rotary microtome (Leica RM2235). Sections were carefully mounted on microscope slides coated with poly-L-lysine (Sigma-Aldrich) to improve adhesion. Finally, slides were dried in a histology incubator oven (Memmert) at 60°C for 1–2 h to allow a thin layer of paraffin to melt, ensuring firm attachment of tissue sections to the slides before staining.

## 2.4 Immunofluorescence and Confocal Microscopy

Paraffin sections were deparaffinised in xylene, rehydrated through descending ethanol series, and subjected to antigen retrieval in 10mM sodium citrate buffer (pH 6.0) at 95°C for 20 min. Sections were permeabilised with 0.3% Triton X-100, and non-specific binding was blocked with 5% normal goat serum (Sigma-Aldrich) for 1 h at room temperature.

Slides were incubated overnight at 4°C with mouse monoclonal anti-metallothionein antibody (Abcam, clone E9, Cat. No. ab12228; dilution 1:100). After Phosphate Buffered Saline (PBS) washing, slides were incubated for 1 h at room temperature with rhodamine-conjugated goat anti-mouse IgG secondary antibody (Rockland, Cat. No. 610-1002; 1:200 in PBS with 2% Bovin Serum Albumin (BSA). Nuclei were counterstained with DAPI (1 µg/mL, Sigma-Aldrich) for 5 min.

Negative controls included (i) Omission of primary antibody, (ii) Isotype-matched IgG control, and (iii) Autofluorescence control (unstained tissue). Imaging was performed on an Olympus FluoView FV1000 confocal laser scanning microscope with a 60× oil immersion objective (NA 1.4). Fluorescence channels were set to Rhodamine (λ_ex 543 nm; λ_em 560–620 nm) and DAPI

(λ_ex 405 nm; λ_em 450–470 nm). Laser power, gain, and offset were kept constant across samples. Each individual was imaged at 10 randomly selected fields of view.

### 2.5 Image Analysis

Digital images were analysed using ImageJ software (NIH, USA). For each clam, three Regions of Interest (ROIs) were drawn on secondary gill lamellae (excluding nuclei and lumen). Background fluorescence was subtracted from all measurements. MFI per individual was calculated as the average of all ROIs, yielding one biological replicate per animal (n = 30 per site).

### 2.6 Statistical Analysis

Metal concentrations and MFI values are reported as mean ± Standard Deviation (SD). Data were tested for normality (Shapiro–Wilk) and homogeneity of variance (Levene's test). One-way ANOVA was used to assess differences among sites, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test ($p < 0.05$). Effect sizes ($\eta^2$) and 95% Confidence Intervals (CI) were calculated. Correlations between individual metal concentrations and MFI values were examined using Pearson's correlation analysis. Statistical analyses were performed with Statistical Process and Service Solutions (SPSS) v.26 (IBM, USA).

## 3. Results and Discussion

### 3.1 Heavy-Metal Concentrations in Gill Tissues of *A. antiquata*

Analysis of heavy metals in *A. antiquata* gills revealed significant spatial variation across Jakarta Bay (Table 1). The highest levels were recorded at Muara Angke (Cd: 2.15 ± 0.32 mg·kg$^{-1}$; Pb: 3.84 ± 0.41 mg·kg$^{-1}$; Hg: 0.67 ± 0.08 mg·kg$^{-1}$), followed by Cilincing (Cd: 1.42 ± 0.21 mg·kg$^{-1}$; Pb: 2.11 ± 0.36 mg·kg$^{-1}$; Hg: 0.42 ± 0.05 mg·kg$^{-1}$), and the lowest at Tanjung Priok (Cd: 0.84 ± 0.14 mg·kg$^{-1}$; Pb: 1.27 ± 0.22 mg·kg$^{-1}$; Hg: 0.21 ± 0.03 mg·kg$^{-1}$). This gradient reflects differences in anthropogenic activity, with Muara Angke receiving intensive input from domestic waste, small-scale industry, and port activities, while Tanjung Priok is comparatively less impacted.

Notably, Pb concentrations at Muara Angke exceeded international food safety limits for bivalves (Food and Agriculture Organisation/ World Health Organisation (FAO/WHO) Codex Alimentarius: 0.5–2.0 mg·kg$^{-1}$), and Cd levels at all sites were close to or above long-term safety thresholds. Although Hg levels were lower, its bioaccumulative and biomagnifying properties remain a concern for seafood consumers. These findings align with previous studies reporting elevated Pb and Cd in bivalves from Jakarta Bay and other polluted estuaries in Indonesia[1,5,23,24].

The elevated concentrations of heavy metals at Muara Angke can be attributed to the high intensity of anthropogenic activities in the area, including domestic wastewater discharge, small-scale industries, as well as port and fisheries operations[18]. Waste inputs into coastal waters carry heavy-metal particulates that accumulate in sediments and subsequently enter the food chain through filter-feeding organisms such as *A. antiquata*[19]. Differences in metal concentrations among sites also reflect varying levels of pollutant exposure: Cilincing showed intermediate concentrations, while Tanjung Priok recorded the lowest. These variations are likely influenced by differences in pollution sources, hydrodynamic conditions, and sedimentation rates at each location[4].

When compared with previous studies, the present findings are consistent with reports of elevated Cd and Pb accumulation in green mussels (*P. viridis*) from Jakarta Bay, driven by massive inputs of domestic and industrial effluents[5]. Similar patterns were also observed along the Semarang coast, Central Java, where Pb and Cd concentrations in *P. viridis* were relatively high compared to areas with lower anthropogenic activity[20]. The same study further documented ultrastructural damage in *P. viridis* from Jakarta Bay, characterised by reduced microvilli, as a consequence of chronic metal accumulation[4]. These results confirm that the presence of ports, industrial zones, and dense human activity are key factors aggravating heavy-metal accumulation in filter-feeding bivalves.

From a food-safety perspective, the Pb concentration detected at Muara Angke (3.84 mg·kg$^{-1}$) exceeds the

**Table 1.** Concentrations of Cd, Pb, and Hg (mg·kg$^{-1}$ wet weight) in gill tissues of *A. antiquata* from Jakarta Bay

| Location | Cd (mg·kg$^{-1}$) | Pb (mg·kg$^{-1}$) | Hg (mg·kg$^{-1}$) |
|---|---|---|---|
| Muara Angke | 2.15 ± 0.32 | 3.84 ± 0.41 | 0.67 ± 0.08 |
| Cilincing | 1.42 ± 0.21 | 2.11 ± 0.36 | 0.42 ± 0.05 |
| Tanjung Priok | 0.84 ± 0.14 | 1.27 ± 0.22 | 0.21 ± 0.03 |

international safety threshold for bivalves (FAO/WHO Codex Alimentarius: 0.5–2.0 mg·kg$^{-1}$). Likewise, elevated Cd levels across all sites indicate long-term risks, given that Cd is recognised as both carcinogenic and nephrotoxic. Although Hg concentrations were lower than Cd and Pb, the strong bioaccumulative and biomagnifying nature of this element still poses potential health risks for consumers. These findings are consistent with reports from Vietnam, where consumption of green mussels (*P. viridis*) collected from heavy-metal–contaminated waters has been linked to chronic human exposure risks[21].

## 3.2 Histopathological Alterations of Metallothionein on *A. antiquata* gills

Histological examination of gill tissues revealed progressive structural damage consistent with the pollution gradient, which was further supported by metallothionein (MT) expression visualised by confocal microscopy (Figure 2). At Tanjung Priok (low exposure), secondary lamellae remained intact, epithelial cells were well organised, and no major lesions were observed. Correspondingly, fluorescence signals from Rhodamine-B–labeled MT were weak, indicating low MT expression and suggesting that detoxification mechanisms were not strongly activated due to limited metal exposure.

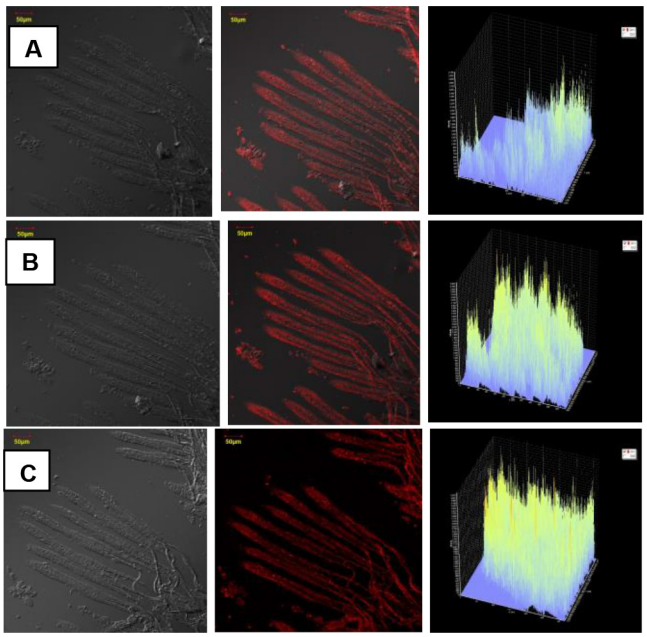



**Figure 2.** Gill morphology and metallothionein (MT) expression in *A. antiquata*. (**A**: low MT expression at Tanjung Priok; **B**: moderate MT expression at Cilincing; **C**: high MT expression at Muara Angke).

In Cilincing (moderate exposure), epithelial hypertrophy, hyperplasia, and lamellar thickening were evident, along with narrowing of interfilament spaces. These adaptive histological changes coincided with a moderate increase in MT fluorescence intensity, reflecting an induced protective response against higher pollutant stress.

By contrast, Muara Angke (high exposure) exhibited severe histopathological alterations, including lamellar oedema, fusion of secondary lamellae, epithelial desquamation, and cytoplasmic vacuolization. Fluorescence signals were markedly stronger in this location, consistent with MT overexpression as a compensatory detoxification mechanism. However, despite elevated MT levels, the extensive structural damage reduced the effective respiratory surface area, impaired oxygen diffusion, and likely compromised the metabolic capacity of the clams.

Taken together, these results demonstrate that gill tissues of *A. antiquata* not only accumulate metals but also manifest pollutant-induced stress through distinct histopathological markers and MT expression patterns, in line with previous findings in other bivalves and freshwater mussels[3,5,13,18].

In Cilincing (moderate exposure), gill tissues exhibited intermediate fluorescence intensity, reflecting an increase in MT expression. This upregulation represents an adaptive response to higher levels of heavy metal exposure compared to Tanjung Priok. Histological changes included epithelial hypertrophy and hyperplasia, thickening of the lamellae, and narrowing of interfilament spaces. The distribution of MT in these regions suggests a protective role against oxidative damage induced by transition metal ions, consistent with the established biological function of MT as a molecular buffer against heavy-metal toxicity.

By contrast, gills from Muara Angke (high exposure) displayed the strongest red fluorescence, indicating MT overexpression as a response to chronic and severe contamination. Despite elevated MT levels, pronounced morphological alterations were evident, including lamellar oedema, fusion of secondary lamellae, epithelial desquamation, and cytoplasmic vacuolisation. These structural disruptions reduce the effective surface area for oxygen diffusion, impairing respiratory efficiency and overall metabolic capacity. Notably, variations in fluorescence intensity among cells suggested heterogeneous cellular responses to heavy-metal stress,

underscoring the complexity of biological adaptation under toxic environmental conditions.

Overall, these findings demonstrate a strong relationship between heavy-metal contamination in Jakarta Bay and MT expression in *A. antiquata* gills. The progressive increase in MT fluorescence intensity—from weak at Tanjung Priok to moderate at Cilincing and strong at Muara Angke—mirrors the gradient of anthropogenic pollution across sites. This supports the view that MT is a cytosolic protein strongly induced by heavy metals such as Pb, Cd, and Hg, functioning in metal binding, storage, and detoxification[10,15]. Previous studies have similarly reported significant MT induction in aquatic organisms chronically exposed to metals, confirming its reliability as a sensitive biomarker for aquatic biomonitoring[22,23].

Comparable patterns have been observed in other bivalves. For example, MT expression in the oyster *Crassostrea virginica* increased in response to heavy-metal exposure in southeastern Mexico[24], while studies in the St. Lawrence Estuary (Quebec, Canada) demonstrated positive correlations between total metal concentrations and MT levels in the digestive glands and gills of *Mytilus edulis* and *Mya* arenaria[17,25]. These cross-species findings highlight the conserved role of MT in bivalves, strengthening the position of *A. antiquata* as a credible bioindicator of heavy-metal pollution in tropical coastal waters.

Biologically, the increased MT expression observed here indicates an adaptive strategy by clams to cope with environmental stress. Beyond its role in heavy-metal detoxification, MT also contributes to reducing oxidative stress by neutralising free radicals[10]. Recent work has further shown that MT distribution in *Crassostrea cuculata* and *Crassostrea glomerata* collected from East Java reflected chronic heavy-metal exposure, ultimately

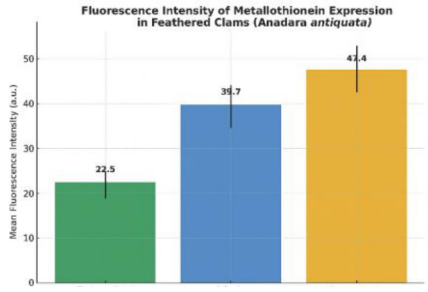



**Figure 3.** Quantitative analysis of metallothionein (MT) expression in gills of *A. antiquata* from three sites in Jakarta Bay.

affecting energy metabolism and organismal health[12]. The present findings from Jakarta Bay therefore confirm that MT expression in *A. antiquata* not only reflects environmental contamination but also represents a physiological mechanism to maintain metal homeostasis and protect against long-term toxic effects.

Cell-wise, when cells are exposed to heavy metals, this activates MTF-1, causing a drastic increase in MT gene transcription to produce cysteine-rich proteins capable of binding toxic metal ions through thiol groups (-SH)[10]. In addition to binding metals, MT also neutralises reactive free radicals produced during oxidative stress caused by metals[8]. Thus, MT induction via the MTF-1 pathway is a major cellular defence mechanism that maintains metal homeostasis and reduces the toxicity of Cd, Pb, and Hg in bivalve gill tissue.

## 3.3 Quantitative Analysis of Fluorescence Intensity

Quantitative analysis of mean fluorescence intensity (MFI) revealed variation in metallothionein (MT) expression in the gills of *A. antiquata* collected from three stations in Jakarta Bay (Figure 3). The lowest MFI was recorded at Tanjung Priok (22.5 ± 3.3 a.u.), nearly doubled at Cilincing (39.7 ± 4.9 a.u.), and reached the highest level at Muara Angke (47.4 ± 5.1 a.u.). This pattern is consistent with findings from studies of heavy-metal toxicity in bivalves from the Adyar estuary, Tamil Nadu, India[26]. The results clearly reflect a spatial gradient of heavy-metal exposure among sites, particularly in estuarine areas, and demonstrate a consistent relationship between environmental contamination and MT expression in the gills of *A. antiquata*.

Biologically, these differences in MT expression indicate an adaptive gill response to heavy-metal exposure. MT is a cysteine-rich protein with a key role in binding metal ions (Cd, Pb, Hg, Cu, Zn) and reducing oxidative stress by neutralising reactive oxygen species (ROS)[5,7]. At Tanjung Priok, low MT expression suggests relatively light exposure, consistent with intact gill histology characterised by well-organised secondary lamellae. In Cilincing, elevated MT expression coincided with epithelial hypertrophy, hyperplasia, and lamellar thickening, representing morphological adaptations to higher metal loads[1].

The highest MT expression at Muara Angke confirmed chronic and intense heavy-metal exposure, consistent

with elevated concentrations of Cd, Pb, and Hg reported at this location[1,18,27]. Although MT upregulation functions as a protective mechanism, excessive exposure resulted in severe histological lesions, including lamellar oedema, fusion of secondary lamellae, epithelial desquamation, and cytoplasmic vacuolization. These changes reduce the effective surface area for oxygen diffusion and impair respiratory capacity, as similarly reported in the freshwater mussel *Anodonta cygnea* from the Kabul River ecosystem, Khyber Pakhtunkhwa, Pakistan[28].

These findings support recent literature affirming MT expression as a sensitive biomarker for detecting heavy-metal exposure in aquatic organisms, both at the molecular level and through histopathological changes in target organs[10,17]. Accordingly, the variation in MFI observed in the gills of *A. antiquata* across Jakarta Bay not only reflects the gradient of metal pollution but also elucidates the biological responses and histological damage experienced by filter-feeding organisms in urban estuarine environments.

### 3.4 Linking Metals, Pathology, and MT as Biomarkers

The parallel trends observed higher metal concentrations, more severe histopathology, and stronger MT expression provide strong evidence that MT in *A. antiquata* gills is a reliable biomarker of heavy-metal pollution in Jakarta Bay. These findings are consistent with studies in other bivalves (*Crassostrea virginica*, *Mytilus edulis*, *Cerastoderma edule*) showing positive correlations between environmental metal exposure and MT induction[12,16,24,25].

From a biomonitoring perspective, MT offers advantages over chemical measurements alone, as it integrates both exposure and biological effect. Moreover, since *A. antiquata* is consumed locally, its MT expression and associated tissue lesions provide not only ecological insights but also early-warning signals for food safety risk. This aligns with the One Health framework, linking environmental quality to human health in coastal communities. Given that *A. antiquata* is part of the local seafood market, chronic accumulation of high levels of Pb and Cd in the tissue poses two risks, i.e., ecosystem degradation and dietary exposure for human consumers.

This study has limitations despite providing important information regarding the relationship between heavy metal exposure and MT expression in *A. antiquata*. There are several limitations in this study, i.e., this study has not measured the activity of antioxidant enzymes involved in the defence mechanism under oxidative stress conditions due to other heavy metals, such as glutathione S-transferase (GST), Catalase (CAT), and Superoxide Dismutase (SOD), which could strengthen the biochemical evidence for histopathological and immunofluorescence findings.

This study design only used negative controls, but did not include positive controls in the form of tissue from organisms originating from reference locations with very low levels of contamination. The absence of positive controls limits the ability to make quantitative comparisons between metallothionein expression in contaminated and uncontaminated environments. Despite these limitations, this study remains an important foundation for the development of molecular biomonitoring in tropical bivalves and opens up opportunities for further research that integrates biochemical, molecular, and ecological indicators.

## 4. Conclusion

This study confirms that the feathered clam (*A. antiquata*) is highly sensitive to heavy-metal pollution in Jakarta Bay, as demonstrated by three key indicators: bioaccumulation of Cd, Pb, and Hg in gill tissues, progressive histopathological alterations, and differential expression of MT. The highest contamination and tissue damage were found at Muara Angke, followed by Cilincing and Tanjung Priok, reflecting a clear spatial gradient of pollution. Increasing metal concentrations were consistently associated with structural damage in the gills and elevated MT expression, supporting the role of MT as a sensitive biomarker of heavy-metal stress.

Overall, *A. antiquata* not only reflects environmental contamination through physiological and molecular responses but also holds strong potential as a reliable bioindicator for coastal biomonitoring programs. Furthermore, as a widely consumed species, its contamination highlights an urgent need to assess and manage food-safety risks associated with heavy-metal bioaccumulation in urban coastal ecosystems. Future monitoring strategies should integrate biomarker-based approaches to strengthen environmental management and public health protection. Future studies should integrate metallothionein expression with transcriptomic and enzymatic biomarkers to build a multi-level biomonitoring framework for tropical estuaries.

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## 6. References

1. Puspitasari R, Takarina ND, Soesilo TE, Agustina H. Potential risks of heavy metals in green mussels (*Perna viridis*) harvested from Cilincing and Kamal Muara, Jakarta Bay, Indonesia to human health. Mar Pollut Bull. 2023; 189:114754. https://doi.org/10.1016/j.marpolbul.2023.114754 PMid:36913801.
2. Phaenark C, Phankamolsil Y, Sawangproh W. Ecological and health implications of heavy metal bioaccumulation in Thai Fauna: A systematic review. Ecotox and Environ Saf. 2024; 285:117086. https://doi.org/10.1016/j.ecoenv.2024.117086 PMid:39353378.
3. Vernanda JA, Isswa Zatri Z, Vita Hidayati N, Fitrani M, Binti Abd Manaf L, Andriyono S. Study of heavy metals (Pb, Cd, As, Hg) content in feathered clam (*Anadara antiquata*) and undulate venus clam (*Paratapes undulata*) collected from Ujungpangkah Gresik, Indonesia. Transact of the Chin Soc of Agricul Machin. 2024; 55(1):95-107. https://doi.org/10.62321/issn.1000-1298.2024.01.08
4. Kunda RM, Rumanta M, Hutasoit LR. Bioaccumulation of Pb in the gills of *Perna viridis* (Bivalvia: Mytilidae) in Jakarta Bay, Indonesia and its histological alteration. J of Res in Sci Edu. 2024; 10(5):2488-2494. https://doi.org/10.29303/jppipa.v10i5.7109
5. Riani E, Cordova MR, Arifin Z. Heavy metal pollution and its relation to the malformation of green mussels cultured in Cilincing waters, Jakarta Bay, Indonesia. Mar Pollut Bull. 2018; 133:664-670. https://doi.org/10.1016/j.marpolbul.2018.06.029 PMid:30041363
6. Fitri SR, Patria MP. Microplastic contamination on *Anadara antiquata* Linnaeus 1758 in Pangkal Babu mangrove forest area, Tanjung Jabung Barat district, Jambi. J of Physics: Conferen Series. 2019; 1282:012109. https://doi.org/10.1088/1742-6596/1282/1/012109
7. Bensellam M, Laybutt DR, Jonas J. Emerging roles of metallothioneins in beta cell pathophysiology: Beyond and above metal homeostasis and antioxidant response. Biology. 2021; 10(3):176. https://doi.org/10.3390/biology10030176 PMid:33652748 PMCid: PMC7996892
8. Yang R, Roshani D, Gao B, Li P, Shang N. Metallothionein: A comprehensive review of its classification, structure, biological functions, and applications. Antioxidants (Basel). 2024; 13(7):825. https://doi.org/10.3390/antiox13070825 PMid:39061894 PMCid:PMC11273490
9. Liu Y, Wu Z, Guo K, Zhou Y, Xing K, Zheng J, Sun Y, Zhang J. Metallothionein-1 gene from *Exopalaemon carinicauda* and its response to heavy metal ions challenge. Mar Pollut Bull. 2022; 175:113324. https://doi.org/10.1016/j.marpolbul.2022.113324 PMid:35051848
10. Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquat Toxicol. 2006; 76(2):160-202. https://doi.org/10.1016/j.aquatox.2005.08.015 PMid:16289342
11. Wang H, Yu H, Bai X, Wang X, Shao H. Materials Today Bio Recent Advances in Hydrogel-Based Delivery Systems for the Treatment of Pancreatic Cancer. Mater Today Bio. 2025; 35:102487. Doi: 10.1016/j.mtbio.2025.102487.
12. Hertika AM, Kusriani K, Indrayani E, Yona D, Putra RB. Metallothionein expression on oysters (*Crassostrea cuculata* and *Crassostrea glomerata*) from the Southern coastal region of East Java. F1000Res. 2019; 8:56. https://doi.org/10.12688/f1000research.17381.2 PMid:32477493 PMCid: PMC7217233.
13. Kalaronis D, Ainali NM, Evgenidou E, Kyzas GZ, Yang X, Bikiaris DN, Lambropoulou DA. Microscopic techniques as tools for the determination of microplastics and nanoplastics in the aquatic environment: A short review. Green Anal Chem. 2022; 3:100036. https://doi.org/10.1016/j.greeac.2022.100036
14. Saad AA, El-Sikaily A, Kassem H. Metallothionein and glutathione content as biomarkers of metal pollution in mussels and local fishermen in Abu Qir Bay, Egypt. J Health Pollut. 2017; 6(12):50-60. https://doi.org/10.5696/2156-9614-6-12.50 PMid:30524805 PMCid: PMC6221500.
15. Nam YK, Kim EJ. Diversification and domain evolution of molluskan metallothioneins: A mini review. Fish Aquatic Sci. 2017; 20:8. https://doi.org/10.1186/s41240-017-0054-z
16. 16. Aly W, Williams ID, Hudson MD. Limitations of metallothioneins in common cockles (*Cerastoderma edule*) and sponges (*Haliclona oculata*) as biomarkers of metal contamination in a semi-enclosed coastal area. Sci Total Environ. 2014; 473-474:391-397. https://doi.org/10.1016/j.scitotenv.2013.11.136 PMid:24378929.

17. Pellerin J, Amiard JC. Comparison of bioaccumulation of metals and induction of metallothioneins in two marine bivalves (*Mytilus edulis* and *Mya arenaria*). Comp Biochem Physiol C Toxicol Pharmacol. 2009; 150(2):186-195. https://doi.org/10.1016/j.cbpc.2009.04.008 PMid:19409510.
18. Andriani Y, Fitri AD, Arief MC, Zahidah Z. Lead pollution in the Angke Kapuk mangrove forest of the Jakarta Bay area. Pol J Environ Stud. 2024; 34(1):13-20. https://doi.org/10.15244/pjoes/185542
19. Dey S, Rajak P, Sen K. Bioaccumulation of metals and metalloids in seafood: A comprehensive overview of mobilization, interactive effects in eutrophic environments, and implications for public health risks. J Trace Elem Miner. 2024; 8:100141. https://doi.org/10.1016/j.jtemin.2024.100141
20. 20. Yulianto B, Radjasa OK, Soegianto A. Heavy metals (Cd, Pb, Cu, Zn) in Green Mussel (*Perna viridis*) and health risk analysis on residents of Semarang coastal waters, Central Java, Indonesia. Asian J Water Environ Poll. 2020; 17(3):71-76. https://doi.org/10.3233/AJW200039
21. Nguyen Thanh Kim H, Duong V-H, Chu T-T, Pham-Thi T-X, Nguyen X-Q, Van Vu S, Van TP, Ta D-T, Duong D-T, Tursunov O, Priyadharshini M, Musthafa MS, Hegedűs M, Shahrokhi A, Kovács T. Mussels as bioindicators for the rapid detection of heavy metal fluctuations in marine coastal waters: A case study of seasonal bioaccumulation monitoring and assessment of *Perna viridis* from the Gulf of Tonkin Coastline, Hai Phong, Vietnam. Water. 2025; 17(17):2552. https://doi.org/10.3390/w17172552
22. Knapen D, Reynders H, Bervoets L, Verheyen E, Blust R. Metallothionein gene and protein expression as a biomarker for metal pollution in natural gudgeon populations. Aquat Toxicol. 2007; 82(3):163-172. https://doi.org/10.1016/j.aquatox.2007.02.008 PMid:17379326.
23. Won E, Raisuddin S, Shin K. Evaluation of induction of metallothionein-like proteins (MTLPs) in the polychaetes for biomonitoring of heavy metal pollution in marine sediments. Mar Pollut Bull. 2008; 57(6-12):544-551. https://doi.org/10.1016/j.marpolbul.2008.02.025 PMid:18395758.
24. Aguilar C, Montalvo C, Canedo Y, Ruíz A, Cerón JG, Cerón R, Abatal M, Anguebes F. Analysis of Cadmium, Mercury and Lead in oysters (*Crassostrea virginica*) in a protected area of southeastern Mexico associated with the risk to human health. J Trace Elem Miner. 2024; 9:100175. https://doi.org/10.1016/j.jtemin.2024.100175
25. Oaten JF, Hudson MD, Jensen AC, Williams ID. Effects of organism preparation in metallothionein and metal analysis in marine invertebrates for biomonitoring marine pollution. Sci Total Environ. 2015; 518-519:238-247. https://doi.org/10.1016/j.scitotenv.2015.03.003 PMid:25765376.
26. Chandrasekaran TS, Milton J, Santhanabharathi B, Pradhoshini KP, Cojandaraj L, Priyadharshini M, Ahmed MS, Musthafa MS, Balaji P, Faggio C. Heavy metals toxicity in edible bivalves and risk exposure to humans through its consumption from Adyar Estuary, Tamil Nadu, India - A baseline study. Regio Stud Mar Scie. 2024; 79(1):103854. https://doi.org/10.1016/j.rsma.2024.103854
27. Lestari L, Budiyanto F. Metal speciation in sediment from Muara Angke, Jakarta Bay using of BCR sequential extraction procedure. Indo J Mar Sci. 2019; 24(1):23-30. https://doi.org/10.14710/ik.ijms.24.1.23-30
28. Khan MI, Khisroon M, Khan A, Gulfam N, Siraj M, Zaidi F, Ahmadullah, Abidullah, Fatima SH, Noreen S, Hamidullah, Shah ZA, Qadir F. Bioaccumulation of heavy metals in water, sediments, and tissues and their histopathological effects on *Anodonta cygnea* (Linea, 1876) in Kabul River, Khyber Pakhtunkhwa, Pakistan. Biomed Res Int. 2018; 2018:1910274. https://doi.org/10.1155/2018/1910274 PMid:29693003 PMCid:PMC5859875.

## Author Contributions

Conceptualisation, M.R and R.M.K.; methodology, R.M.K, L.R.H., and A.R.P.R.; validation, A.R.P.R, P.U and L.R.H.; formal analysis, M.R and R.M.K.; investigation, R.M.K, A.R.P.R., and L.R.H.; resources, R.M.K and A.R.P.R.; data curation, M.R and R.M.K.; writing-original draft preparation, R.M.K, P.U., and A.R.P.R.; writing-review and editing, R.M.K and P.U.; visualisation, R.M.K and A.R.P.R.; All authors have read and approved the published version of the manuscript.


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