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Comparative analysis of immunomodulatory biomarker responses in clams *Meretrix meretrix* and *Macra veneriformis* to waterborne cadmium exposure

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Bivalves are important indicator species for cadmium (Cd) pollution, however, the differential detoxification and physiological responses to Cd exposure among co-occurring bivalve species have not been thoroughly studied. This study investigated alterations in metallothionein (MT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels in the digestive gland and gills of clam species *Meretrix meretrix* and *Macra veneriformis* following exposure to cadmium (Cd) at concentrations of 0.05, 0.10, and 0.15 mg/L over a period of 7 days. The results indicated that gill tissue, due to its direct contact with the environment and high metabolic activity, exhibited significantly greater variations in SOD, MT, and MDA contents compared to the digestive gland, rendering it more sensitive to Cd stress and suitable for acute toxicity monitoring. *M. veneriformis* demonstrated a faster and more pronounced response, with significant alterations in biomarker levels observed in the gills at early stages even under low Cd exposure. However, its detoxification system, specifically MT synthesis reached saturation more readily; under high Cd concentrations, biomarker levels peaked and subsequently declined rapidly, indicating the potential for the early onset of irreversible damage. In contrast, *M. meretrix* exhibited a gradual adaptive response, maintaining homeostasis via sustained MT synthesis, which reflects a higher tolerance to Cd stress. MDA contents showed significant early increases in both species' gills, especially under low Cd concentrations, highlighting its value as both an early-warning indicator and a preferred biomarker for Cd pollution monitoring. The study provides candidate indicator species and identifies sensitive biomarkers for Cd biomonitoring in regions such as the Yellow River Delta and a theoretical basis for improving ecological risk assessment.

KEYWORDS

cadmium, *Meretrix meretrix*, *Macra veneriformis*, metallothionein, superoxide dismutase, malondialdehyde

1 Introduction

In environmental monitoring programs, biomarkers are important referees to evaluate the pollution of heavy metals/organic compounds in sediments, water columns, and biota as well as associated biological effects in aquatic ecosystem (González et al., 2015; da Luz et al., 2025). Varieties of biomarkers, serving as integrative measures of pollutant exposure, could provide valuable insights into the toxicological effects on organismal growth and development (Lam and Gray, 2003; Pandiyan et al., 2021). Thus a comprehensive set of biomarkers tailored to specific xenobiotics, along with chemical analyses, might be efficient tools in monitoring the marine pollution.

Benthic aquatic species, especially shellfish, are typical indicator species in marine environment some of whose physiological and/or biochemical parameters are usually directly influence the pollution degree of certain pollutants (Cai and Wang, 2024). For example, bivalves, which accumulate high concentrations of metals through filter-feeding, could reflect the spatiotemporal patterns of contaminant accumulation through different biomarkers (Ren et al., 2022). Among these biomarkers, metallothionein (MT) induction is well-established for indicating sub-lethal exposure to metals such as cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb) (McComb et al., 2014; Zhou et al., 2022; Yang et al., 2024; Wang et al., 2025). MTs are multifunctional proteins involved in regulating essential metal homeostasis, participating in immune responses, combating oxidative stress, and mediating toxic metal detoxification (Zhang et al., 2024a; Yunko et al., 2025). Elevated MT levels are generally indicative of metal exposure. Research on MT in marine invertebrates has largely focused on bivalve mollusks—such as *Ruditapes philippinarum*, *Mytilus edulis*, *Argopecten irradians*, *Meretrix meretrix*, and *Crassostrea gigas*—due to their sessile nature, low metabolic activity, and tendency to accumulate heavy metals (Wang et al., 2009; Perić et al., 2020; Cai et al., 2025; Yang et al., 2023; Zhang et al., 2023). However, MT responses vary across species, metal types, and environmental conditions such as seasonal fluctuations (Pellerin and Amiard, 2009; Perrigault and Allam, 2012), highlighting the need for comparative studies to verify its specificity and accuracy.

Parallel to MT, key components of the antioxidant system—including superoxide dismutase (SOD), glutathione peroxidase (GPx), and the lipid peroxidation product malondialdehyde (MDA)—are widely used biomarkers for pollutant-induced oxidative stress (Wang et al., 2021; Jiang et al., 2024a; Jing et al., 2025a). Metabolism of organic xenobiotics often generates reactive oxygen species (ROS), which can damage cellular components, alter gene expression, and lead to oxidative stress (Yang et al., 2022; Su et al., 2023; Tong et al., 2025; Zheng et al., 2025). MDA content, reflecting lipid peroxidation levels, is commonly used in toxicity assessments in bivalves and fish (Jiang et al., 2024b; Zhang et al., 2024b; Qiang et al., 2025; Sun et al., 2025). So the antioxidant system primarily functions to eliminate superoxide anion (O_2^-) free radicals, while lipid peroxidation (LPO) levels are directly indicated by MDA content. Therefore, combining antioxidant enzyme indicators with MDA for investigation can better reflect the organism's antioxidant response mechanism.

Cadmium represents a major environmental concern due to its toxicity and persistence in aquatic ecosystems (Hu and Wang, 2024; Lu et al., 2024). It bioaccumulates in tissues, stimulates ROS production, induces oxidative biomolecular damage, and promotes MT synthesis (Liu et al., 2024a; Lompré et al., 2024; Sun et al., 2024). Cd can also inhibit enzyme activity, disrupt cellular signaling, and dysregulate calcium homeostasis (Stohs and Bagchi, 1995; Moncaleano-Niño et al., 2017). In China, Cd contamination is a significant issue in certain aquaculture zones. For example, in the Yellow River Delta—a region rich in shellfish resources—Cd concentrations sometimes exceed water quality standards, threatening economically important species (Cai et al., 2024; Li et al., 2024a; Ren et al., 2024).

In the Yellow River Delta, *Meretrix meretrix* and *Macrta veneriformis* are two native, sympatric clams with ecological and economic value (Liu et al., 2022). Both have shown detectable biochemical responses to pollutants and are considered potential indicator species (Wu et al., 2012; Li et al., 2024b, Li et al., 2025; Zhang et al., 2021). However, comparative analyses of their responses to Cd-induced stress—particularly in terms of MT induction, antioxidant activity, and oxidative damage—remain limited.

Therefore, this study aims to systematically compare the differential responses of *M. meretrix* and *M. veneriformis* to Cd stress by quantifying tissue-specific Cd accumulation and a suite of biomarkers—MT, SOD, and MDA—in digestive gland and gill tissues. The relationships between Cd accumulation and biomarker expression will be examined to elucidate species-specific defense mechanisms and enhance the bioindicator utility of these clams.

2 Materials and methods

2.1 Chemicals

Chemicals with a purity $\geq 99\%$ included 5,5-dithiobis-2-nitrobenzoic acid (DTNB), phenylmethylsulphonyl fluoride (PMSF), and leupeptin were purchased from Sigma-Aldrich (Shanghai, China). The remainder of the reagents with a purity $\geq 98\%$ were purchased from Sangon Biotech (Shanghai, China).

2.2 Clam treatment

Clam individuals of *M. meretrix* and *M. veneriformis* (mean shell length: 3.89 ± 0.21 cm) used in this study were obtained from an aquaculture base in Wudi county, located along the Bohai Sea coast near Binzhou City, China. The aquaculture farm is certified for Good Agricultural Practices (GAP). Seafood from this farm is regularly screened for heavy metals, ensuring that cadmium levels in clams remain below 2.0 mg/kg wet weight, thereby avoiding any potential interference with the experimental results. In the laboratory, clams of uniform size were selected and maintained in natural seawater sourced directly from the Bohai Bay. Seawater conditions were maintained at a salinity of 28‰, a pH of 8.0, and a temperature range of 8–10°C. One-third of the seawater volume was

renewed daily. Continuous aeration was provided. The present experiment utilized a diet of food-grade powdered *Spirulina platensis*, with a cadmium content of ≤ 0.2 mg/kg. Prior to feeding, 20 mg of the algal powder was fully resuspended in 200 mL of culture water. A fixed volume of 10 mL of this algal suspension was evenly distributed into each rearing tank at scheduled intervals using a pipette, ensuring consistent individual food intake. The cadmium content in this feeding quantity was extremely low (≤ 0.01 $\mu\text{g/L}$) and thus considered negligible, with no anticipated impact on the experimental outcomes. Prior to experimentation, clams were acclimatized under these conditions for one week.

2.3 Experimental designs

Following acclimatization, clams were transferred to plastic tanks ($50 \times 40 \times 30$ cm) containing seawater. Salinity (28‰), pH (8.0), temperature (8–10°C), and feeding regimen (powdered *Spirulina platensis*) were maintained consistent with the acclimatization conditions. The average concentration of Cd in seawater was 0.219 ± 0.011 ng/L. For exposure groups, CdCl_2 was dissolved and diluted in seawater to achieve nominal concentrations of 0.05, 0.1, and 0.15 mg/L, corresponding to $10 \times$, $20 \times$, and $30 \times$ the aquaculture water quality standard for Cd (≤ 0.005 mg/L), respectively. Each treatment, including the control, comprised triplicate tanks stocked with 40 clams. One-third of the seawater volume, containing corresponding Cd concentrations, was replenished daily. The Cd concentrations in the exposure tanks were verified every 24 hours. No mortality was observed across all concentrations during the 7-day exposure period. Samples of digestive gland and gill tissues were collected from both species on days 0, 1, 3, 5, and 7 following exposure initiation.

2.4 Sample preparation

Following the exposure period, clams were dissected on ice. Digestive glands and gills from three individuals in each replicate tank were pooled. Half of each tissue sample was immediately frozen at -80°C in 1.5 mL centrifuge tubes for MT analysis. The remaining tissue was rinsed in distilled water and homogenized (1:10 w/v) in cold phosphate buffer (100 mM, pH 7.4) using a glass homogenizer in an ice bath. The homogenate was maintained at 4°C and centrifuged at $3,500 \times g$ for 10 min at 4°C . The resulting supernatant was aliquoted for analysis of SOD activity and MDA content.

2.5 SOD activity

SOD activity was measured using a Total Superoxide Dismutase Assay Kit (WST-1 method, Nanjing Jiancheng Bioengineering Institute). One unit of SOD activity (U) was defined as the amount of enzyme causing 50% inhibition of the formazan dye reduction reaction per milligram of protein in a 1 mL reaction volume. The activity is expressed as U/mg/min.

2.6 MDA content

MDA content was quantified using a thiobarbituric acid (TBA) assay adapted from Wills (1987). Briefly, 50 μL of tissue homogenate was diluted to 1 mL with distilled water. Subsequently, 500 μL of ice-cold 20% trichloroacetic acid (containing 1 mM FeSO_4) and 1 mL of 0.67% (w/v) TBA solution were added. Samples were vortexed and incubated at 90°C for 10 min to form the MDA-TBA adduct. After cooling on ice, precipitates were removed by centrifugation ($3,000 \times g$, 5 min, 4°C). A 1 mL aliquot of supernatant was diluted 1:3 with distilled water, and the absorbance was measured at 532 nm using a spectrophotometer. MDA concentration was calculated from a standard curve generated with 1,1,3,3-tetramethoxypropane and normalized to protein content, expressed as nmol MDA/mg protein.

2.7 MT quantification

MT content was determined using a modified Viarengo et al. (1997) protocol. Frozen tissues were homogenized (1:3 w/w) in ice-cold buffer [100 mM phosphate buffer (pH 8.0), 0.5 M sucrose, 20 mM Tris-HCl (pH 8.6), 0.006 mM leupeptin, 0.5 mM PMSF, 0.01% (v/v) β -mercaptoethanol]. The homogenate was centrifuged ($30,000 \times g$, 20 min, 4°C). High-molecular-weight proteins were precipitated from the supernatant using ethanol-chloroform (1.005:0.08 v/v) and pelleted by centrifugation ($7,000 \times g$, 10 min, 4°C). The MT-containing supernatant was incubated with 1 mg RNA, 40 μL 37% HCl, and 6 mL ethanol at -20°C for 1 h. MTs were pelleted by centrifugation ($2,000 \times g$, 15 min, 4°C), washed twice with sucrose-Tris/ethanol/chloroform (13:87:1), and air-dried. The pellet was dissolved in 150 μL 0.25 M NaCl and 150 μL 4 mM HCl-EDTA. MT concentration was determined spectrophotometrically using Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid), DTNB] at 412 nm (BioTEK ELx800). Reduced glutathione standards were used for calibration. MT content was calculated based on cysteine reactivity (21 residues per molecule, MW ~ 8.6 kDa) and expressed as μg MT-like protein/mg total protein.

2.8 Protein determination

Soluble protein concentration in enzyme extracts was determined using the Bradford (1976) method (Bio-Rad Protein Assay Kit) with bovine serum albumin standards. Absorbance of the Coomassie Brilliant Blue G-250 dye-protein complex was measured at 595 nm.

2.9 Statistical analysis

Data analysis was performed using SPSS 19.0, with results expressed as mean $\pm$ standard deviation (SD). After verifying normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test), one-way ANOVA was first used to determine overall significance among groups. When ANOVA indicated significance, *post-hoc* tests were applied as follows: Dunnett's test

was used specifically for comparisons between each exposure group and the control group, while pairwise comparisons among different exposure concentrations were conducted using Duncan's test (under equal variances) or the Games–Howell test (under unequal variances). A unified significance threshold of $P < 0.05$ was adopted for all analyses.

3 Results

3.1 Responses of SOD activities to Cd exposure in *M. meretrix* and *M. veneriformis* tissues

Figures 1 illustrates SOD activity in *M. meretrix*. In the digestive gland (Figure 1A), SOD activities of 0.05 mg/L Cd exposure showed no significant difference compared to the controls. Conversely, significantly increased SOD activity in the 0.10 mg/L group was observed at Day 3 onward ($P < 0.05$), and peaked on Day 7. While that in the 0.15 mg/L group peaked over 110 U/mg prot on Day 5.

As shown in Figure 1B, in the gills, SOD activity in the 0.05 mg/L Cd group was significantly higher than that in the control at Day 3 onward, and maintained an upward trend. While those in the 0.1 mg/L Cd group and 0.15 mg/L Cd group were became significantly higher than the control at Day 1 and reached their peak values on Day 5 and Day 3, respectively. significantly lower SOD activity was found in the 0.15 mg/L Cd group ($P < 0.05$).

As for *M. veneriformis*, SOD activities in the digestive gland (Figure 2A) exposed to 0.05 mg/L of Cd exceeded those in controls from Day 3 and continued to rise. While those in the 0.10 mg/L group showed early activation (Day 1), peaked on Day 3, and declined to near-control levels on Day 7. In the high-concentration group (0.15 mg/L), SOD activity peaked on Day 3, then declined, and below that in the control group on Day 7. Gill SOD activity (Figure 2B) revealed distinct responses: that in the 0.05 mg/L group increased steadily from Day 1; SOD activity in the 0.10 mg/L group peaked sharply (46.95 U/mg) on Day 1 and kept on declining, and began to be lower than the control on Day 5 onwards; as well as that in the 0.15 mg/L group exhibited persistent suppression from Day 1 onward.

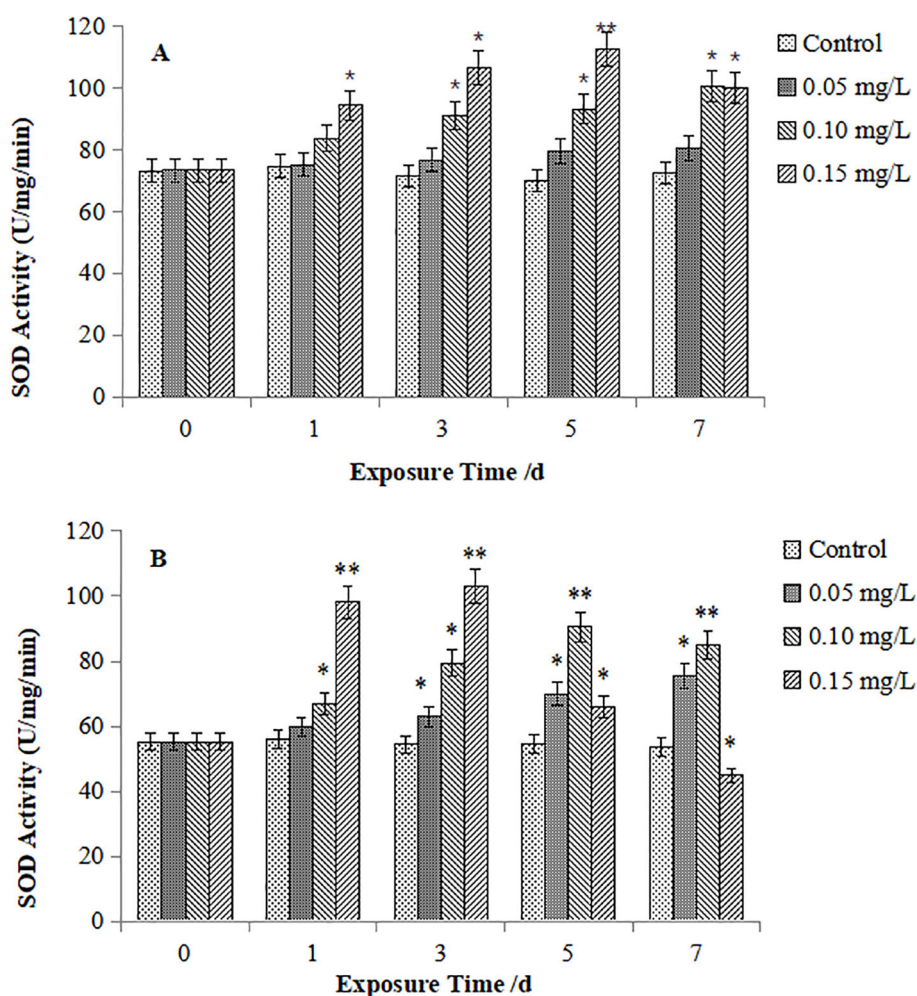


FIGURE 1

Effect of Cd on SOD activity in the digestive gland (A) and gills (B) of *M. meretrix*. The asterisk (*) indicates a significant difference compared to the control group ($P < 0.05$), and the double asterisk (**) indicates a highly significant difference compared to the control group ($P < 0.01$).

Comparative analysis indicated that gill SOD activity in both species underwent more pronounced and sensitive fluctuations than that in the digestive gland. *M. veneriformis* exhibited greater SOD variability in homologous tissues than *M. meretrix*. Mechanistically, low Cd (0.05 mg/L) exposure universally enhanced SOD activity, while, higher Cd concentrations (≥ 0.10 mg/L) induced suppression.

3.2 Effects of Cd exposure on MT contents in *M. meretrix* and *M. veneriformis* tissues

Figures 3 depicts MT contents in *M. meretrix*. As for the 0.05 mg/L Cd exposure, no significant variation MT contents was observed in the digestive gland (Figure 3A), whereas significant increase of MT contents was observed from Day 3 onward

($P < 0.05$), sustaining an upward trend continuously (Figure 3B). While under the 0.10 mg/L Cd exposure, MT contents in both tissues exhibited significant elevation from Day 1 ($P < 0.05$), reaching extreme values at Day 7 and Day 5 in digestive gland and gills, respectively ($P < 0.05$). And under exposure to 0.15 mg/L Cd, MT contents rose extremely significantly from Day 3 (digestive gland) and Day 1 (gills) ($P < 0.05$). Overall, MT accumulation in *M. meretrix* displayed dose- and time-dependence, with those in gills showing greater responsiveness and steeper increases.

Figures 4 presents MT contents in *M. veneriformis*. MT contents in the digestive gland (Figure 4A) increased significantly from Day 3 under 0.05 mg/L Cd exposure ($P < 0.05$). For the 0.10 mg/L and 0.15 mg/L groups, MT contents began to be significantly higher than that in the control from Day 1 ($P < 0.05$), and being extremely significant greater at Day 5 and Day 1, respectively

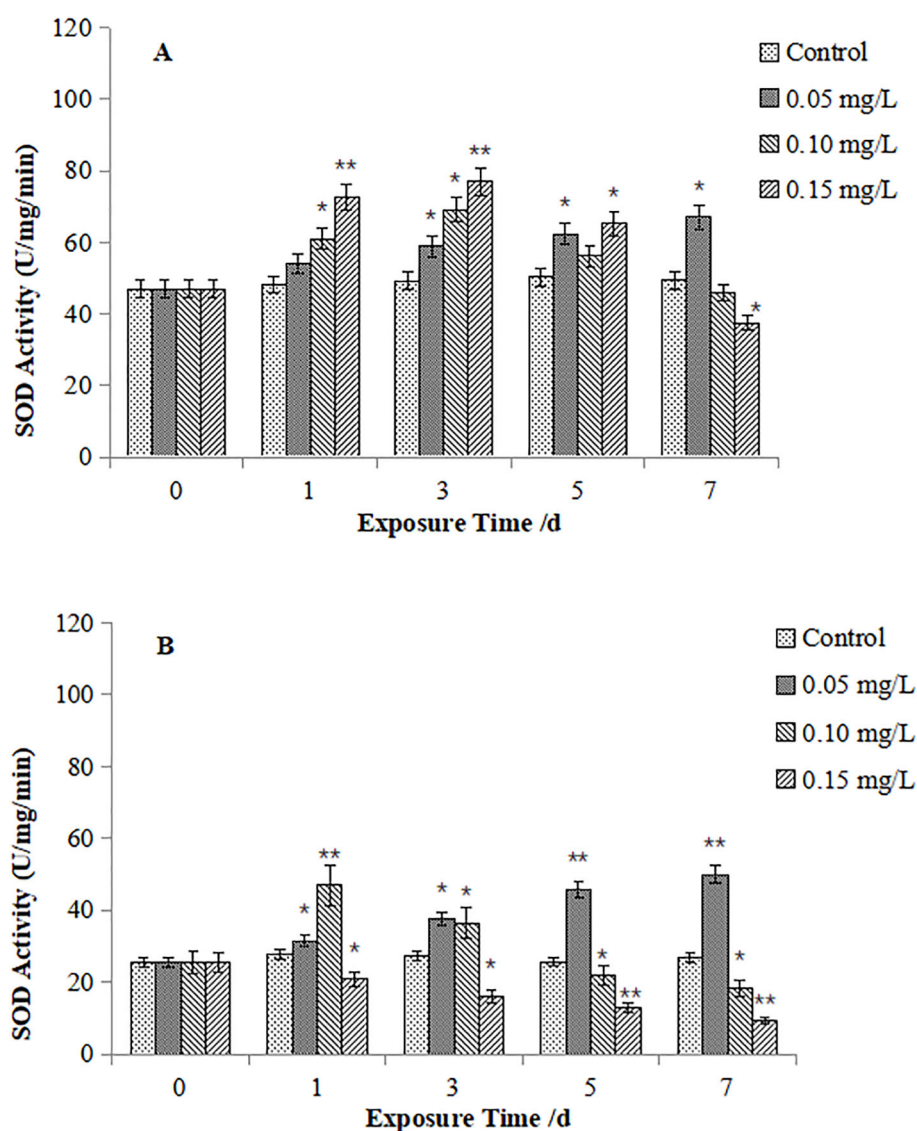


FIGURE 2

Effect of Cd on SOD activity in the digestive gland (A) and gills (B) of *M. veneriformis*. The asterisk (*) indicates a significant difference compared to the control group ($P < 0.05$), and the double asterisk (**) indicates a highly significant difference compared to the control group ($P < 0.01$).

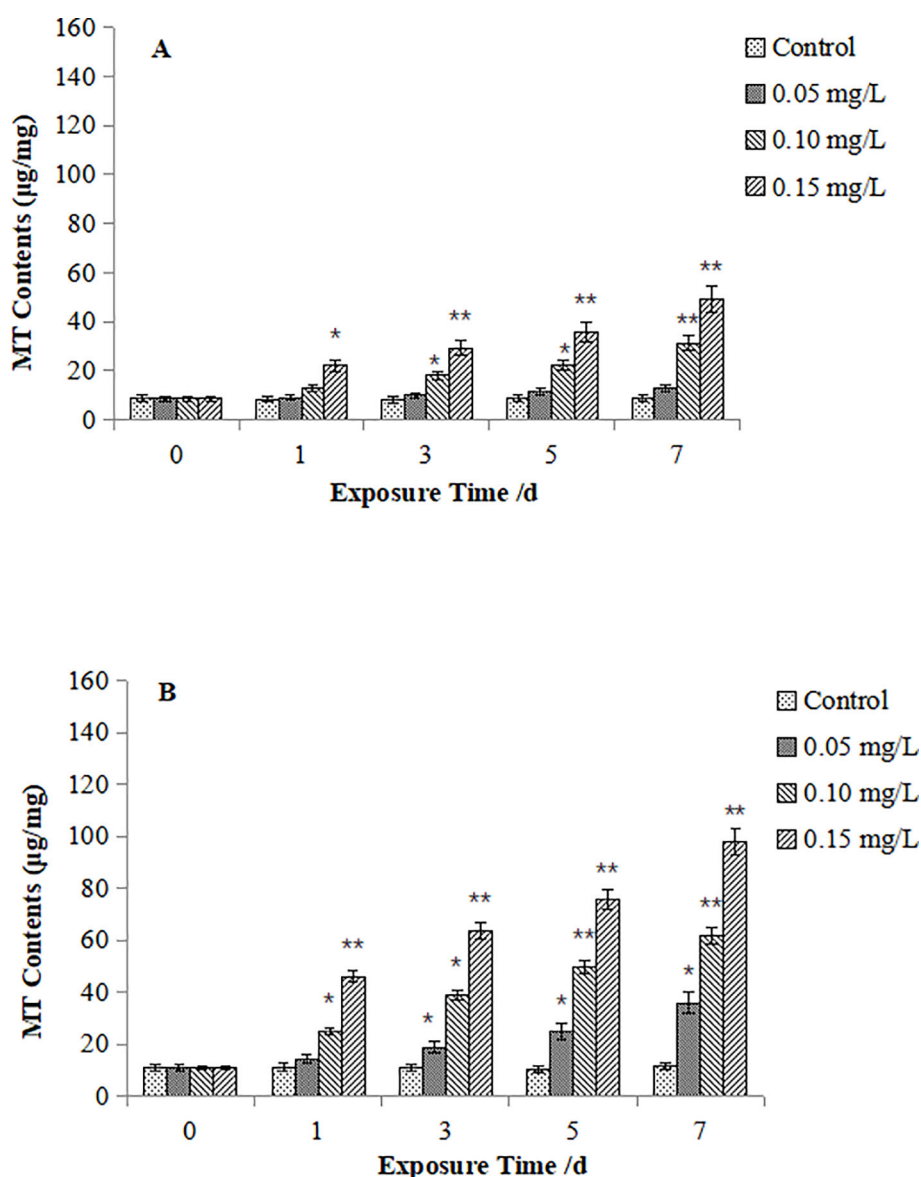


FIGURE 3

Effect of Cd on MT contents in the digestive gland (A) and gills (B) of *M. meretrix*. The asterisk (*) indicates a significant difference compared to the control group ($P < 0.05$), and the double asterisk (**) indicates a highly significant difference compared to the control group ($P < 0.01$).

($P < 0.05$). For those in the gill, (Figure 4B) significant and extremely significant increase were firstly observed at Day 1 and day 5 under the 0.05 mg/L Cd exposure ($P < 0.05$), while extremely significant increase were both occurred at Day 1 in the 0.10 mg/L and 0.15 mg/L groups ($P < 0.05$). Notably, gills exhibited earlier and more pronounced MT upregulation than digestive glands at equivalent concentrations.

Comparative analysis reveals conserved dose- and time-dependent MT accumulation in both species, aligning with classical dose-response relationships. However, *M. veneriformis* demonstrated significantly stronger MT induction in both tissues than *M. meretrix* (gill: peak 2.1-fold higher; digestive gland: 1.8-fold), indicating heightened sensitivity to Cd stress.

3.3 Effects of Cd exposure on MDA contents of clams *M. meretrix* and *M. veneriformis*

Figures 5 illustrates MDA contents in *M. meretrix*. In the digestive gland (Figure 5A), MDA content remained stable under 0.05 mg/L Cd exposure but significantly increased ($P < 0.05$) from Day 3 and Day 1 for the 0.10 mg/L and 0.15 mg/L Cd groups respectively. Gill filaments (Figure 5B) exhibited significant MDA elevation from Day 3, Day 1 and Day 1 for the 0.05, 0.10, 0.15 mg/L Cd group respectively ($P < 0.05$). Baseline MDA contents in control gills was lower than those in digestive glands ($P < 0.05$). Crucially, once exposed to Cd, gills showed significantly greater MDA

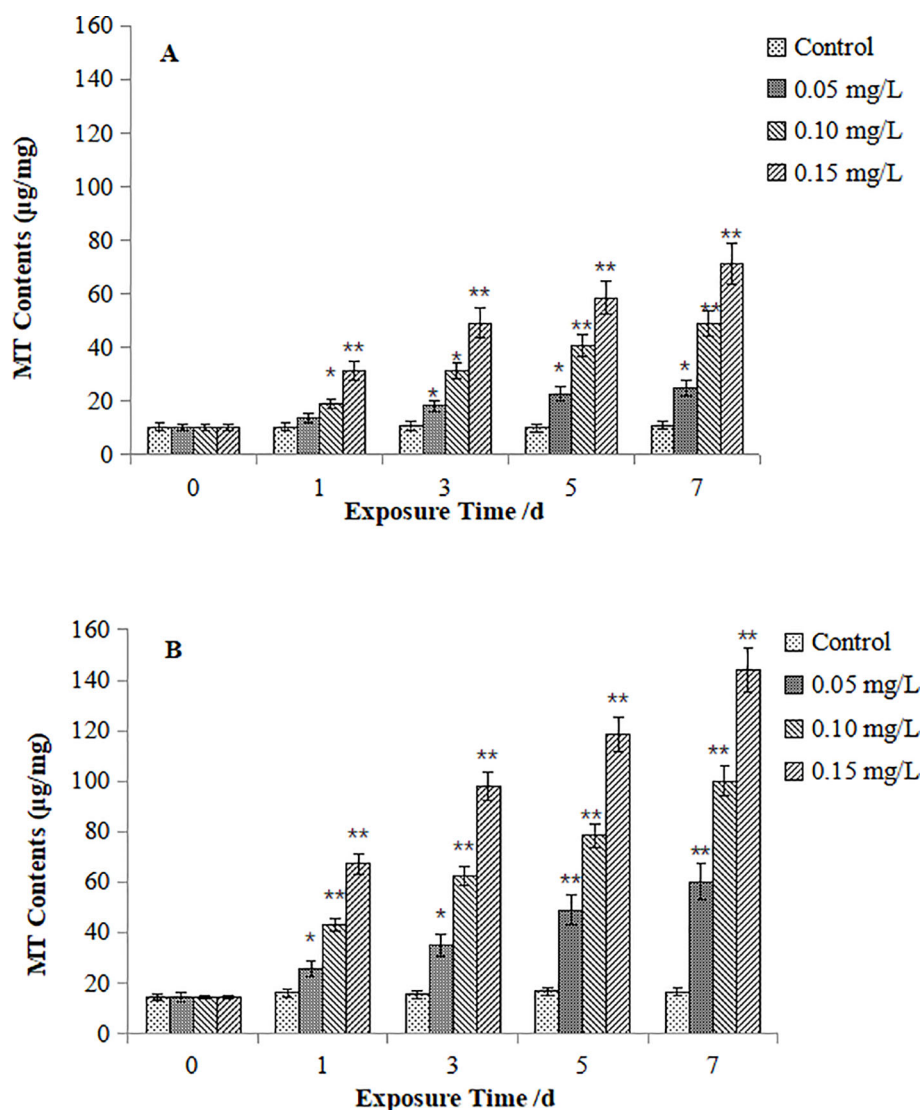


FIGURE 4

Effect of Cd on MT contents in the digestive gland (A) and gills (B) of *M. veneriformis*. The asterisk (*) indicates a significant difference compared to the control group ($P < 0.05$), and the double asterisk (**) indicates a highly significant difference compared to the control group ($P < 0.01$).

induction than digestive glands for all the treatments ($P < 0.05$), indicating heightened gill sensitivity.

Figures 6 presents MDA contents in *M. veneriformis*. The digestive gland MDA (Figure 6A) increased significantly beginning on Day 3 ($P < 0.05$), Day 1 ($P < 0.05$) and Day 1 ($P < 0.05$) for the 0.05, 0.10, and 0.15 mg/L Cd groups, respectively. While those in Gill filaments (Figure 6B) began to show significant increases at Day 1 for the 0.05 mg/L group ($P < 0.05$) and extremely significant rises at Day 1 for both the 0.10 and 0.15 mg/L Cd groups ($P < 0.05$). The magnitude of MDA induction was consistently greater in the gills compared to the digestive glands.

Comparative analysis revealed significantly stronger MDA responses in *M. veneriformis* than in *M. meretrix* within homologous tissues at equivalent Cd concentrations (gill: 1.7-fold higher; digestive gland: 1.4-fold higher; $P < 0.05$). Most treatments

exhibited time-progressive MDA accumulation, aligning with dose/time-dependent oxidative damage mechanisms.

4 Discussion

4.1 Immunomodulatory biomarker responses to Cd exposure in *M. meretrix* and *M. veneriformis*

Cd exerts toxicity in aquatic organisms through multiple pathways, including disruption of antioxidant defenses, induction of lipid peroxidation, and activation of metallothionein (MT) synthesis (Liu et al., 2024b). In this study, SOD activity in the digestive gland of *M. meretrix* remained unchanged under low Cd

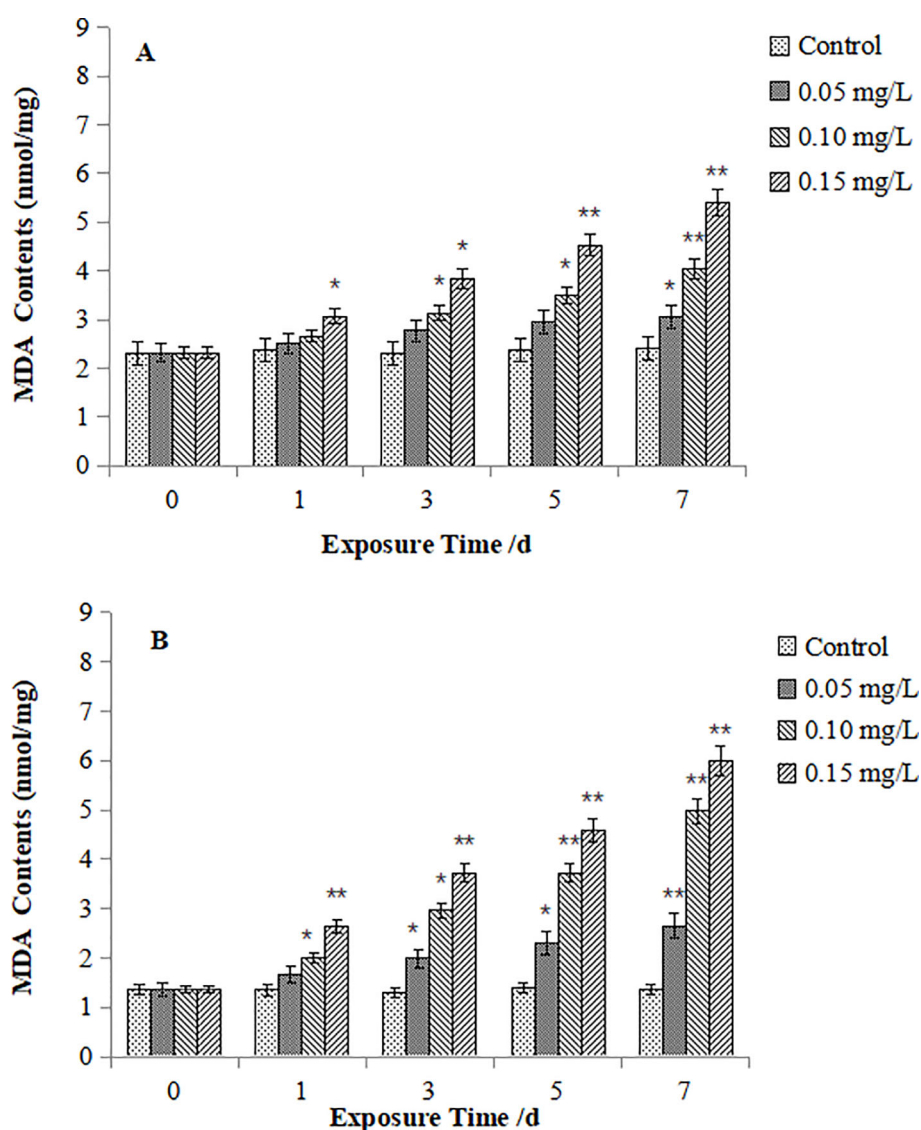


FIGURE 5

Effect of Cd on MDA contents in the digestive gland (A) and gills (B) of *M. meretrix*. The asterisk (*) indicates a significant difference compared to the control group ($P < 0.05$), and the double asterisk (**) indicates a highly significant difference compared to the control group ($P < 0.01$).

exposure (0.05 mg/L), whereas gill SOD increased significantly from Day 3 (Figures 1A, B), indicating that gills—directly exposed to the environment—are more sensitive to Cd-induced oxidative stress. Similarly, *M. veneriformis* exhibited rapid gill SOD activation at 0.05 mg/L Cd from Day 1 (Figure 2B). Under higher Cd levels (≥ 0.10 mg/L), both species showed a biphasic “induction-suppression” pattern, though the peak occurred later in *M. meretrix* (Days 3–5) than in *M. veneriformis* (Days 1–3). This earlier saturation in *M. veneriformis* may be linked to its higher metabolic rate and consequent rapid ROS accumulation in gill tissue (Li et al., 2024b).

MT exhibited dose- and time-dependent induction in both species, though *M. veneriformis* showed stronger upregulation—e.g., gill MT increased markedly at 0.15 mg/L Cd as early as Day 1 (Figure 4B). This may reflect species-specific traits such as larger gill surface area and higher Cd uptake rate, necessitating rapid MT

synthesis for metal sequestration (Fang et al., 2010; Moncaleano-Niño et al., 2017). Across all concentrations, gill MT responded earlier than digestive gland MT, reinforcing the role of gills as sentinel organs. Notably, MT induction was not sustained: at 0.15 mg/L Cd, gill MT in *M. veneriformis* plateaued by Day 7, suggesting saturation of metal-binding capacity or a shift in stress response pathways (Jing et al., 2025b; Lompré et al., 2024), consistent with reports of progressive DNA damage in this species under high Cd load (Zhang et al., 2023).

MDA, a terminal product of lipid peroxidation, rose significantly in gill tissue of *M. meretrix* by Day 3 at 0.05 mg/L Cd (Figure 5B), while digestive gland MDA increased only at ≥ 0.10 mg/L (Figure 5A), again highlighting gill susceptibility. *M. veneriformis* showed more amplified MDA responses: gill MDA surged from Day 1 at 0.05 mg/L, with higher increments than in *M. meretrix* at equivalent doses (Figure 6B). These results are consistent with our previous findings

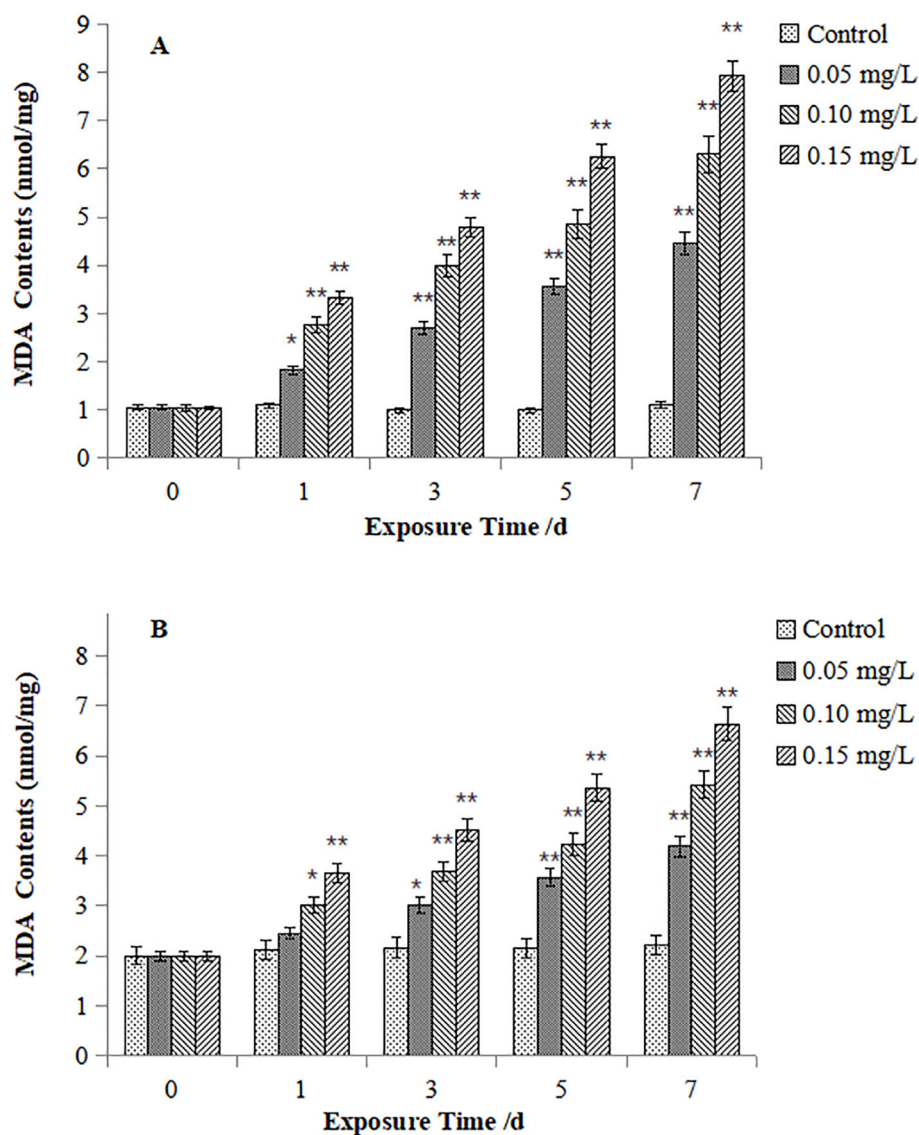


FIGURE 6

Effect of Cd on MDA contents in the digestive gland (A) and gills (B) of *M. veneriformis*. The asterisk (*) indicates a significant difference compared to the control group ($P < 0.05$), and the double asterisk (**) indicates a highly significant difference compared to the control group ($P < 0.01$).

(Ren et al., 2021). Temporal correlation analysis revealed that MDA accumulation coincided with SOD collapse—e.g., in *M. meretrix* gills at 0.15 mg/L, SOD dropped sharply on Day 7 while MDA peaked, indicating antioxidant failure. Similarly, in *M. veneriformis* gills at 0.10 mg/L, MDA exceeded controls on Day 5 as SOD fell below baseline, confirming ROS overwhelm. Integrating SOD and MDA dynamics thus clarified interspecific differences in Cd-induced oxidative damage.

4.2 Comparative analysis of Cd response mechanisms in *M. meretrix* and *M. veneriformis*

Table 1 summarizes key differential responses between the two species. Integrated analysis indicates that *M. veneriformis* is more

sensitive to Cd, as shown by: (1) earlier biomarker activation (e.g., gill SOD elevation at 0.05 mg/L on Day 1); (2) faster post-peak decline under high Cd (e.g., gill SOD below control by Day 7 at 0.15 mg/L); and (3) greater oxidative damage (higher MDA increments). These findings align with earlier reports of Cd-induced behavioral and hemocytic alterations in *M. veneriformis* (Wang, 2009; Giarratano et al., 2023), supporting its potential as a coastal Cd bioindicator.

Firstly, the differential sensitivity arises from physiological and ecological adaptations. *M. veneriformis* has a larger gill surface area that enhances Cd uptake but is coupled with lower antioxidant capacity, leading to earlier oxidative injury. In contrast, *M. meretrix* exhibits slower but sustained MT synthesis, favoring gradual detoxification. Ecologically, the intertidal habitat of *M. meretrix* may select for robust antioxidant systems, whereas the subtidal *M. veneriformis* likely has less adaptive capacity to acute pollution.

TABLE 1 Comparative analysis of key characteristics between *M. meretrix* and *M. veneriformis*.

Index	<i>M. meretrix</i>	<i>M. veneriformis</i>
SOD activity	Peak values appeared late (Day 3–5), and the decline was slow under high Cd concentration (Figure 1).	Peak values occurred early (Day 1–3), and the activity collapsed faster under high Cd concentration (Figure 2).
MT content	The increase in gill filaments was slow (Figure 3B), and the response of digestive gland was delayed (Figure 3A).	The extremely remarkable increase of MT in gills was much earlier (Day 1, Figure 4B), and the overall increase was 1.5–2 times that of <i>M. meretrix</i> under same treatment (Figure 4).
MDA content	The response was delayed under low concentration (gills on Day 3, Figure 5B), and the increase was slow under high concentration (Figure 5)	The increase of MDA in all concentrations was earlier than that in <i>M. meretrix</i> (gills on day 1, Figure 6B), and the increase was significantly higher (Figure 6).

Transcriptomic analyses have revealed developmental stage-specific gene regulation in *M. meretrix* larvae under Cd stress. The upregulation of CCO, Ndh, and pro-C3 in later stages contrasted with HPX and A2M induction in early developmental phases (Wang et al., 2010; Xu et al., 2024). Notably, these genes showed progressive downregulation during metamorphosis, suggesting Cd-mediated disruption of mitochondrial electron transport and immune modulation (Xu et al., 2024). The genetic response of *M. veneriformis* has not been extensively studied. However, hemocyte function experiments have demonstrated that Cd exposure induces a faster ROS generation rate and earlier onset of DNA damage in this species, suggesting that its molecular regulatory network may exhibit heightened susceptibility to Cd interference.

Moreover, tissue-specific regulatory mechanisms differ between the two bivalve species. In *M. meretrix*, MT expression shows concentration-dependent variation across tissues (Gao et al., 2021), suggesting distinct detoxification strategies: gills prioritize rapid Cd sequestration, while the digestive gland maintains homeostasis through balanced MT expression (Sun et al., 2024). Post-depuration analyses further indicate its recovery capacity, with reduced Cd levels and oxidative stress markers in ovarian tissue, along with downregulation of apoptosis-related genes (Zhou et al., 2024). In contrast, the repair mechanisms in *M. veneriformis* remain poorly characterized, potentially due to Cd-induced suppression of cellular recovery processes. Hemocytic responses also vary interspecifically—*M. meretrix* exhibits greater membrane stability and more effective DNA repair (Ren et al., 2021). Behaviorally, *M. meretrix* shows preferential inhibition of filtration rate over respiration rate under Cd stress (González et al., 2015), a phenomenon linked to glutathione S-transferase dynamics—initially enhanced to detoxify lipid peroxidation products, followed by rapid inhibition under high Cd, leading to suppressed feeding activity.

Correlation analysis in this study clarified the differential response mechanisms to Cd stress. At low exposure (0.05 mg/L), synergistic rises in SOD and MT suppressed MDA accumulation in gills until Day 3. Under high Cd (≥ 0.10 mg/L), SOD collapse combined with MT saturation led to a sharp MDA increase. Gills—directly exposed and metabolically active—showed acute sensitivity, while the digestive gland exhibited delayed but sustained responses, likely due to Cd redistribution and long-term detoxification demands. Quantitative evaluation revealed a sensitivity order of Gill MDA > Gill SOD > Gill MT, with gill

MDA in *M. veneriformis* increasing significantly at 0.05 mg/L Cd from Day 1 (Figure 6B). In terms of stability, MT > MDA > SOD, as MT rose consistently while SOD fluctuated markedly. These distinct patterns underscore temporal and mechanistic divergence in biomarker behaviors under Cd stress. These findings, derived from a short-term (7-day) exposure, suggest the following biomarker selection criteria for pollution monitoring: Gill MDA in *M. veneriformis* is an optimal early-warning indicator for acute stress, given its rapid response and high sensitivity. Although this study design was acute, the response pattern of digestive gland MT in *M. meretrix* suggests that it may be a promising candidate for monitoring chronic exposure; however, this requires validation through longer-term studies.

While this study elucidates the differential physiological responses to Cd in these bivalves, two limitations should be noted: (1) It is important to note that this study did not measure tissue-specific cadmium accumulation. While the biomarker responses are consistent with known mechanisms of cadmium toxicity, the lack of internal dose data means that the mechanistic interpretations remain associative. Future studies incorporating tissue burden analysis are necessary to conclusively link the biomarker responses to internal cadmium dose. (2) the synergistic roles of auxiliary antioxidant enzymes (e.g., CAT, GPx) remain unexamined. Furthermore, the chronic effects beyond seven days require additional validation. Collectively, Cd exposure impairs both species via antioxidant disruption, lipid peroxidation, and MT pathway activation. These findings advance theoretical frameworks for coastal Cd biomonitoring and underscore the need to integrate tissue-specific biomarkers with long-term exposure studies to refine ecological risk assessments.

5 Conclusions

This study demonstrated distinct toxic responses in *M. veneriformis* and *M. meretrix* under short-term (7-day) Cd exposure. Gill tissue, due to its direct contact with contaminants and high metabolic activity, exhibited greater sensitivity than the digestive gland, as reflected in the biomarkers SOD, MT, and MDA. These findings support the use of gill tissue as a suitable target for acute toxicity monitoring under similar exposure conditions. *M. veneriformis* exhibited faster and more pronounced biomarker responses to acute Cd stress; however, its detoxification capacity

(e.g., MT synthesis) appeared to saturate more readily, suggesting a lower threshold for irreversible damage under sustained exposure. In contrast, *M. meretrix* demonstrated a more gradual physiological adjustment, indicating a potentially higher tolerance within the acute exposure period. MDA, as a terminal product of lipid peroxidation, showed early and significant elevation in gill tissue, even at low Cd concentrations. This pattern supports its use as a sensitive biomarker for early warning of Cd-induced oxidative damage in short-term exposure scenarios. It should be noted that the 7-day exposure design limits extrapolation to chronic monitoring applications. While the differential responses observed here suggest potential species-specific advantages for longer-term biomonitoring, such conclusions would require validation through studies with extended exposure durations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Ethics Committee of Shandong University of Aeronautics. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JR: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. ZZ: Conceptualization, Validation, Writing – review & editing. BL: Funding acquisition, Writing – review & editing. QZ: Formal Analysis, Investigation, Methodology, Writing – review & editing. JZ: Formal Analysis, Software, Visualization, Writing – review &

editing. SS: Conceptualization, Formal Analysis, Methodology, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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