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Original Article

Evaluation of lead and cadmium bioaccumulations related to oxidative responses in the tissues of two wild mud crabs, *Scylla olivacea* and *Scylla paramamosain*, from Pattani Bay, Thailand

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Abstract

Exposure to heavy metals and bioaccumulation in the tissues can directly impact animal health and well-being. Mud crabs exhibit varying capacities for accumulating heavy metals and oxidative stress in their tissues due to their unique physiology and oxidative defense mechanisms. The present study investigated tissue oxidation in response to lead (Pb) and cadmium (Cd) accumulation in two types of wild mud crabs, *Scylla olivacea* and *Scylla paramamosain*. Samples were collected from the ecologically vulnerable, anthropogenically impacted region of Pattani Bay, Thailand. Muscles, gills, hepatopancreases, and gonads were dissected to evaluate metal content and oxidative status, including lipid peroxidation, reduced glutathione, superoxide dismutase, and catalase activities. Metal content in soil and water collected from the sampling area was analyzed. Results revealed that the hepatopancreas had the highest capacity for accumulating Cd and Pb in the two mud crab species. Hepatopancreases also exhibited the greatest sensitivity to oxidative stress, as evidenced by significantly elevated lipid peroxidation levels. *S. paramamosain* was more vulnerable to bioaccumulation than *S. olivacea*. Positive correlation has been indicated between soil Pb contamination and bioaccumulation in the muscles and gills of the two mud crabs. We concluded that Pb and Cd accumulate in a tissue-specific manner; the hepatopancreas was the most accumulative. Species-specific oxidative responses to heavy metal accumulation may be considered. *S. paramamosain* exhibited more sensitivity than *S. olivacea*. Moreover, *S. paramamosain*'s oxidative response indicated some weakness characterized by a negative relationship between lipid peroxidation and reduced glutathione.

Keywords: Cadmium, Heavy metal accumulation, Hepatopancreas, Lead, Oxidative response

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Introduction

Pattani Bay, an ecologically vulnerable and anthropogenically impacted region, is located in Pattani Province in Southern Thailand. It is a semi-enclosed bay that leads into the Gulf of Thailand. Pattani Bay is one of the most important aquaculture bays in Thailand. The International Union for the Conservation of Nature endorses Pattani Bay as a substantial coastal wetland worth conserving in Southeast Asia due to its wealth of natural resources and advantageous geographic location. Pattani Bay has spurred numerous economic activities, such as industrial zone expansion, coastal aquaculture development, seafood bank establishment, and seaport enhancement. All these activities require substantial natural resources and result in environmental pollution (Tanhan et al., 2023). As a significant aquaculture bay, Pattani Bay was an ideal location for studying the ecological impact of pollution on marine life. This is because it faces major environmental challenges, primarily from various sources of pollution, including wastewater draining from industrial factories, agricultural runoff, urban sewage, and soil from land development. These pollution sources increase heavy metal contamination in the water and soil around the bay, directly impacting marine life, increasing bioaccumulation and biomagnification, and potentially affecting human health through the trophic food chain. These problems can be further exacerbated according to the rate of regional population growth (Kaewtubtim et al., 2018).

Metal-contaminated soil and water can directly accumulate in animal tissue according to their feeding habits and habitats. Humans have an advantage in using various organisms for biomonitoring purposes. Different animals have varying capacities for accumulation due to their unique physiology and oxidative defense mechanisms, and many of these organisms are as favorable as seafood. Therefore, research on health risk assessment in Pattani Bay is focused on limiting metal accumulation (Tanhan et al., 2023). Recently, research on animal health has reported results concerning the impact of metal contamination in the environment and its accumulation in the tissues of aquatic animals, such as bivalves and coastal fish, but not in crabs from Pattani Bay (Tanhan et al., 2023; Tanhan et al., 2024).

Scylla olivacea (black mud crab) and *Scylla paramamosain* (white mud crab) are used as food in Thailand. These crustaceans are economically essential, prized for their taste and size, and used in many dishes. Mud crabs are raised in coastal aquaculture systems such as ponds. They are also fattened in cages or traps in mangrove areas. However, the decline of wild mud crab populations, mainly due to environmental risks, has been worsened by the destruction of mangrove habitats in several regions. Both mud crab species inhabit estuaries, mangrove forests, and subtidal flats. *S. olivacea* is typically found in dense mangrove forests, where it resides in burrows in the inner sections of these forests, which experience fluctuating water levels and salinity. *S. paramamosain* is found in subtidal zones, inhabiting the fringes of mangrove ecosystems in its early stages (Fazhan et al., 2022). In Pattani Bay, *S. olivacea* and *S. paramamosain* are important species, serving as significant food sources and playing a role in the local ecosystem and economy. These crabs are targeted for aquaculture and contribute to the region's fisheries and aquaculture industries. Exposure to and accumulation of metals can directly impact an animal's health. In crabs, the harmful effects of metals involve metabolic dysfunction, altered growth and reproduction, and even death (Bastami and Esmailian, 2012). Highly toxic metals, such as cadmium (Cd) and lead (Pb), are considered aquatic pollutants closely linked to oxidative stress and impairment of oxidative defense mechanisms (Sevcikova et al., 2011). Research indicates that when crabs accumulate metals, especially Cd and Pb, their oxidative responses lead to a notable increase in the production of antioxidants and metabolic enzymes. (Yogeshwaran et al., 2020). A prior study has validated the link between metal exposure and accumulation and the oxidative response in crabs (Dghim et al., 2023). Furthermore, research shows that both Cd and Pb could potentially influence oxidative reactions in marine fish (Tanhan et al., 2023) and marine bivalves (Tanhan et al., 2024). Recently, it has been implicated in blue swimming crabs (Imsilp et al., 2024). Many studies have investigated mud crab bioaccumulation; however, extant studies involve limitations related to oxidative stress issues. An analysis of crabs' oxidative response revealed that metals induce or inhibit antioxidative mechanisms, depending on the species and varying among decapod crustaceans (Frías-Espericueta et al., 2022). Therefore, this study was intended to explore the variation in the organ accumulation of metals and the oxidative status of organs by comparing two wild mud crab species, *S. olivacea* and *S. paramamosain*, living in Pattani Bay. The goal was to gain new insights into the oxidative status of mud crabs in a high-risk environment and ecosystem while obtaining animal biochemical and physiological information to empower sustainable resource management and conservation.

Material and Methods

The Animal Ethics Committee at the Faculty of Veterinary Science, Kasetsart University, approved this study (ID#ACKU61-VET-071).

Sample collection

S. olivacea (n = 36) and *S. paramamosain* (n = 34) were collected, as shown in Fig. 1. Both males and females were collected in July 2020 and February 2021 from fishing boats in Pattani Bay, Gulf of Thailand (geographic coordinates: 6°54’51.0"N, 101°17’12.0" E). We analyzed the sample after retrieving it from a fishing boat. We ensured that it was at the same site during repetitive sampling and selected only adult crabs to ensure uniform age. In alignment with the Ethics Committee’s recommendation of 3Rs (replacement, reduction, and refinement), the sample size was analyzed to the extent possible. All were adult crabs in the fully developed reproductive stage, with carapace widths ranging from 10 to 15 cm and weights of 100–250 grams. They were kept in a cooler with ice and transported to the Faculty of Science, Kasetsart University, Bangkok, Thailand. The crabs were handled with the utmost care and respect for their well-being. All crabs were freeze-shocked before being dissected for muscle, gill, hepatopancreas, and gonad (testis and ovary) tissue, as shown in Fig. 2. Additionally, soil and seawater samples were collected from the sampling sites and used for metal analysis.

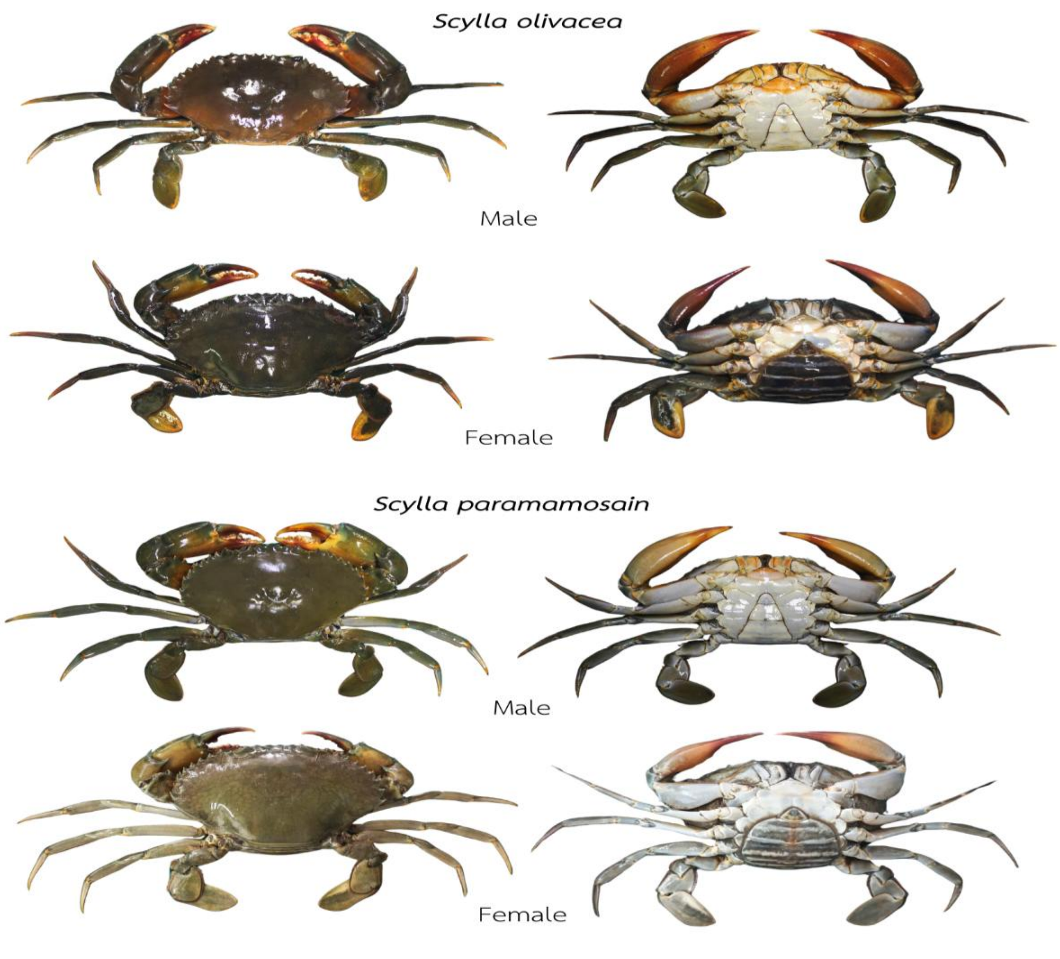

Fig-1. Photographs of *S. olivacea* and *S. paramamosain* represent the external morphology of males and females in dorsal and ventral views. The bar indicates 5 cm.

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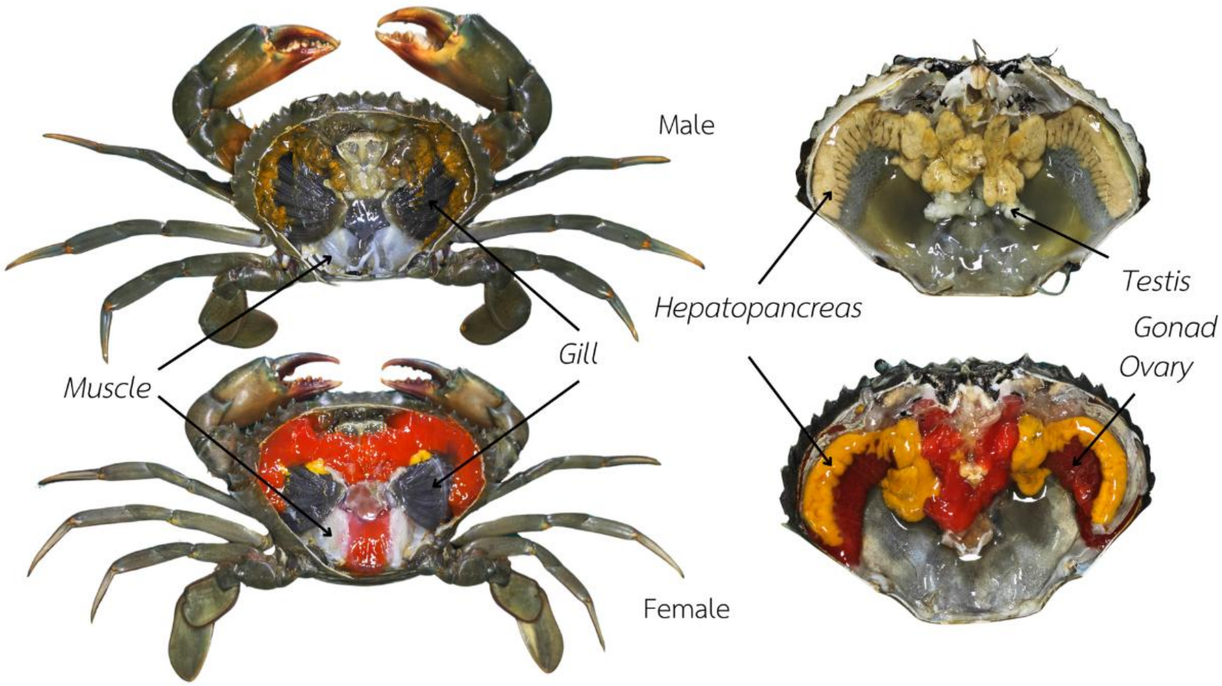

Fig-2. Photographs show internal tissue sampling from the crabs, including a gill, muscle, hepatopancreas, and gonad (testis and ovary).

Heavy metal evaluation

Fifty milliliters of seawater were adjusted with 69% nitric acid and then filtered for metal analysis. Soils were oven dried at 60 °C, weighed at 0.5 g, and digested using 5 mL of a mixture of highly purified concentrated nitric acid (69%) and hydrochloric acid in a 1:3 ratio. Crab tissues were dried at 65 °C, ground, and treated with 5 ml of concentrated nitric acid (70%) for overnight digestion at 25 °C. Further digestion occurred in water baths at 150–180 °C for 3 hours. Samples were then diluted to 25 mL with deionized water, followed by analysis of Cd and Pb using flame atomic absorption spectrometry (FAAS; 240B, Agilent Technologies). The standard heavy metal solution was 1,000 mg/L. Deionized water was used to prepare the working solution, with concentrations between 0.05 and 2.00 mg/L for Cd and from 0.50 to 20.00 mg/L for Pb. Metal concentrations were determined using calibration curves. The FAAS methods for Cd and Pb measurement were validated, showing detection limits of 0.002 mg/L for Cd and 0.020 mg/L for Pb as well as quantification limits of 0.006 mg/L for Cd and 0.060 mg/L for Pb. The recovery percentages indicate precision levels of 97.50% for Pb and 96.50% for Cd. Furthermore, the average accuracy of the Pb and Cd measurements exceeded 99.95%. Metal concentration in tissue was interpreted as mg/kg tissue wet weight (Tanhan et al., 2023).

Oxidative status evaluation

Crab tissues were rinsed with cold normal saline and then homogenized in a 10% w/v phosphate-buffered saline (50 mM, pH 7.4). Half of the homogenate was set aside for malondialdehyde (MDA) and reduced glutathione (GSH) assays. Meanwhile, the remainder was centrifuged at 10,000 g for 10 minutes at 4 °C to assess the supernatant for total protein, superoxide dismutase (SOD), and catalase (CAT) activities.

Total protein

Total protein was measured using Lowry's assay (Lowry et al., 1951) by mixing 0.2 mL of supernatant with 2 mL of solution D (2% Na2CO3 in 0.1 N NaOH, 0.5% CuSO4 · 5H2O, and 1% C4H4KNaO6 · 4H2O) and incubating for 10 minutes. Then, 0.2 mL of 1 N Folin-Ciocalteu reagent was added, and the mixture was incubated for 30 minutes. The absorbance was read at 600 nm. Protein concentration was derived from a standard curve using bovine serum albumin at 0, 0.0045, 0.018, 0.009, 0.035, and 0.0181 mg/ml ($y = 0.0424x - 0.0029$; $R^2 = 0.9917$).

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Lipid peroxidation

MDA levels were measured in organ tissue to assess lipid peroxidation (LPO). Tissue homogenate at 0.2 mL was mixed with 4% sodium dodecyl sulfate, 20% acetic acid for 1.5 mL, and 0.5% thiobarbituric acid for 1.5 mL. Then, the mixture was heated for 1 hour at 95 °C. After cooling down, the mixture was centrifuged at 3,500 rpm for 10 minutes. We measured the supernatant at 532 nm, determining MDA concentrations using a standard curve (0, 3.61, 6.02, and 12.04 µM; $y = 14.1x - 0.6558$; $R^2 = 0.9810$). The concentration of MDA was reported as µM per mg of protein (Sakamula and Thong-Asa, 2018).

Reduced glutathione

The GSH content in the tissue was determined using a homogenate of 0.1 mL mixed with 10% trichloroacetic acid and centrifuged for 10 minutes. The collected 0.5 mL of the supernatant was mixed with 5,5'-dithiobis (2-nitrobenzoic acid), and the solution volume was adjusted to 3 mL with PBS. The absorbance at 412 nm was used for colorimetric reading. GSH standard concentrations were prepared at 0, 0.02, 0.04, and 0.06 µM, providing a linear relationship of $y = 0.669x - 0.0102$, with $R^2 = 0.9955$. The GSH concentration was expressed as mg/mg of protein (Thong-Asa et al., 2020).

Superoxide dismutase

The SOD enzyme activity was determined using 0.1 mL of supernatant mixed with 0.1 mL of 0.0001 M EDTA, 0.5 mL of carbonate buffer (pH 7.9), and 1 mL of 0.0003 M epinephrine. Absorbance readings at 480 nm were taken over a total of 180 seconds; results were obtained every 30 seconds. SOD activity was reported as U/mg of protein, referenced to the standard curve linear relationship of $y = 0.0015x + 0.0001$, with $R^2 = 0.998$, which was prepared from standard SOD activity of 6,150 U/mg (Imsilp et al., 2024).

Catalase

The CAT assay was performed by adding 50 µl of supernatant and increasing the volume to 3 ml with 0.05 M PBS (pH 7.4) that contained 0.01 M H2O2. Absorbance was measured every 30 seconds at 240 nm over 3 minutes. Subsequently, CAT activity was calculated using the extinction coefficient of H_2O_2 and the enzyme activity expressed as U/mg of protein (Dolrahman and Thong-Asa, 2024).

Statistical analysis

Data are presented as mean ± standard deviation. We assessed normality and variance utilizing the Shapiro-Wilk and Levene's tests. A t-test for group comparison and Pearson's correlation were conducted using GraphPad Prism 8.0.1. A p-value of under 0.05 is deemed statistically significant.

Results and Discussion

Heavy metals such as Cd and Pb can accumulate in crabs and other aquatic organisms, becoming toxic if they exceed certain levels. The current research evaluated the accumulation of Pb and Cd in four tissue types of mud crabs, including muscle, hepatopancreas, gill, and gonad. Results indicated that Cd in the hepatopancreas showed the highest accumulation, followed by the gonad, gill, and muscle. No significant difference was found between the two mud crab species for Cd accumulation ($p > 0.05$, Figs. 3a–3d). However, slightly higher Cd accumulation was observed in *S. paramamosain* than in *S. olivacea*. For Pb, the accumulation was higher in the hepatopancreas than in the gill, muscle, and gonad. Results also revealed that *S. paramamosain* accumulated more metals in all tissues than *S. olivacea*. However, there was no significant difference in Pb accumulation between crab species (Figs. 3e–3h). We have demonstrated that the main site for metal accumulation in mud crabs is the hepatopancreas, which is crucial in filtering and processing ingested materials. When the environment is contaminated with heavy metals, crabs can easily absorb and accumulate them through feeding and respiration, potentially contaminating their tissues. In addition, our results align with the previous report that Cd and Pb accumulate primarily in the hepatopancreases of crabs (Imsilp et al., 2024).
The Thai FDA has established maximum limits for Pb and Cd in food, including crabs. The maximum permissible limit for Pb in crustaceans is 0.5 mg/kg, according to documents related to the Codex Alimentarius Commission (WHO). The accumulation of Cd in the edible parts of these mud crabs, such as muscle (*S. olivacea* = 0.81 ± 0.16 mg/kg; *S. paramamosain* = 1.03 ± 0.26 mg/kg), was a few times higher than the recommended level (0.5 mg/kg). Pb accumulation was far higher (*S. olivacea* = 22.76 ± 10.76 mg/kg; *S. paramamosain* = 36.45 ± 36.27 mg/kg) than the recommended level (Genç and Yılmaz, 2015). In Thailand, the edible part of crab

tissue also includes the hepatopancreas and gonad (especially the female ovary). Therefore, the risk of heavy metal exposure to human health may be increased; Cd (*S. olivacea* = 2.13 ± 1.26 mg/kg; *S. paramamosain* = 6.55 ± 4.25 mg/kg) and Pb (*S. olivacea* = 16.17 ± 1.49 mg/kg; *S. paramamosain* = 26.93 ± 0.50 mg/kg) accumulation in the hepatopancreas and the gonad was relatively high, exceeding the recommended level. In addition, our results align with a previous report on human risk assessment regarding seafood consumption from Pattani Bay (Tanhan et al., 2023).

Evaluation of soil and water contamination indicated that the accumulation of Pb in soil was significantly higher than that of Cd, whereas water contamination remained low. A significant difference was found between Pb and Cd in soil, with $p < 0.05$ as shown in Table 1. We conducted a correlation test and found a strongly positive relationship between the levels of Pb in soil and water and tissue accumulation, specifically in the muscle and gill. In *S. olivacea*, the positive correlation between water and muscle was high at $r = 0.803$ ($p = 0.005$, Fig. 3i). The correlation between water and gill was also high at $r = 0.707$ ($p = 0.022$, Fig. 3l). The positive correlation between soil and muscle was substantial, with a correlation coefficient of $r = 0.769$ ($p = 0.009$, Fig. 3j). Similarly, the correlation between soil and gill was significant, with a correlation coefficient of $r = 0.655$ ($p = 0.040$, Fig. 3m). Additionally, *S. paramamosain* exhibited positive correlations only between soil and muscle and gill; specifically, the correlation between soil and muscle was $r = 0.661$ ($p = 0.037$, Fig. 3k), and the correlation between soil and gill was $r = 0.672$ ($p = 0.033$, Fig. 3n). The correlation test results may not reflect causal and relative results but may help depict some of the risks associated with exposure to both mud crab habitats. Several environmental factors contribute to differences in heavy metal accumulation between mud crab species. These factors encompass heavy metal concentrations in aquatic environments, temperature, pH levels, salinity, dissolved oxygen, and the crabs' physiological and metabolic activities. Additionally, the size and age of the crabs can influence their ability to accumulate heavy metals, with larger crabs generally exhibiting higher accumulation rates (Hannan et al., 2024). Both crab species inhabit estuaries, mangrove forests, and subtidal flats. *S. olivacea* lives in burrows in the inner sections of dense mangrove forests. Therefore, this crab species experiences fluctuating water levels and salinity, including oscillating contaminated metals. *S. paramamosain* is more likely to live in subtidal zones, especially in its early stages, inhabiting the fringes of mangrove ecosystems (Fazhan et al., 2022). Heavy metal contamination in mud crab tissue is directly derived from their surrounding soil and water. Among the two heavy metals, Pb exhibited the highest accumulation in tissue, whereas Cd exhibited the lowest concentrations represented in mud crabs (Hannan et al., 2024). The highest heavy metal accumulation was found in the hepatopancreas, and no correlation was observed with metal contamination in the soil and water. Therefore, our results imply a variation of organ capacity for metal accumulation. Factors such as environments, species-specific characteristics, and the form of the heavy metal itself also influence metal accumulation (Barath et al., 2019). Gills of aquatic animals were the first organs to encounter metal contamination from the environment through filtration and respiration in water. The hepatopancreas plays a crucial role in food digestion and body waste excretion. This organ has a higher capacity for metal detoxification and accumulation compared to other organs (i.e., gills and muscles). Concerning the least amount of metal accumulated, the muscles exhibited the lowest oxidative capacity in response to metal accumulation. In fact, muscles had lower metal concentrations than the hepatopancreas, and their accumulation of metals in muscle relies on the contaminant level in the environment (Razali et al., 2024). This is confirmed by our results regarding the positive correlation of metal contamination in soil and water with muscle accumulation (Figs. 3i–3n). The study indicated that metal accumulation in crabs' tissue can be linked to pollution from aquaculture, sewage, and industrial discharge in the bay (Tanhan et al., 2023). This finding is consistent with other studies conducted in various regions where mud crabs are exposed to sources of pollution regarding specific metal concentrations, the relative contribution of sources varying between regions, and their local industrial activities, agricultural practices, and waste management (Barath et al., 2019; de Jesus et al., 2021; Sherly et al., 2022).

Table-1. Accumulation of Pb and Cd in soil and water presented as the mean ± standard deviation (SD).

Metal accumulation (mg/kg)	Soil	Water
Pb	100.2 ± 33.01*	0.2232 ± 0.10
Cd	0.6327 ± 0.11	0.01545 ± 0.01

* indicates $p < 0.05$.

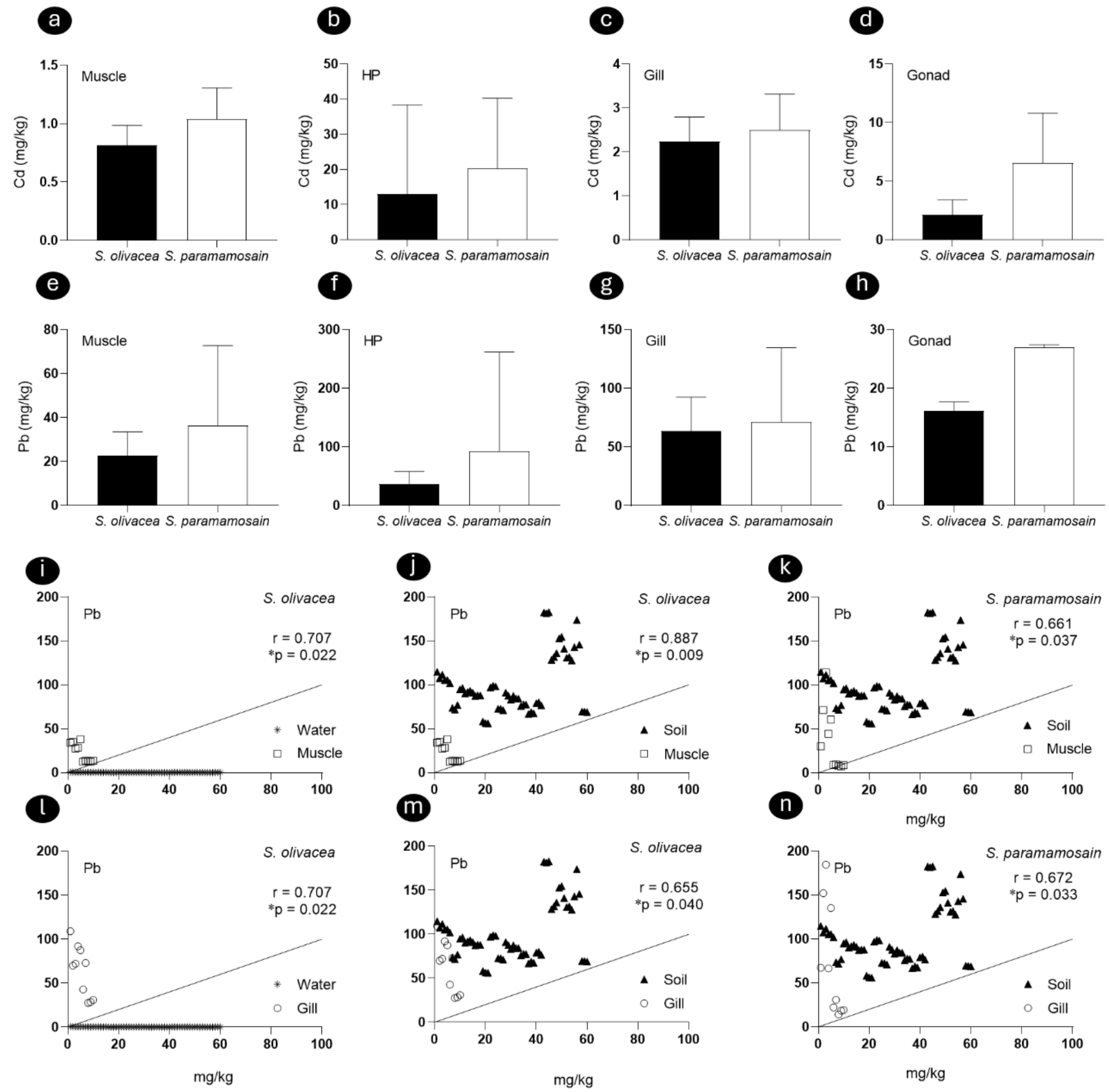

Fig-3. Heavy metal accumulation in the tissue of *S. olivacea* and *S. paramamosain* (a-h). Correlation between water, soil, and tissue accumulation (i-n). Cd = cadmium; Pb = lead; HP = hepatopancreas; * indicate $p < 0.05$.

We focus on the impact of heavy metal accumulation on oxidative status in each type of mud crab tissue, considering biological changes in the oxidative defense mechanism of organisms that live close to environmental risks and pollution exposure. Crabs are commonly used as bioindicators due to their ability to accumulate heavy metals. Therefore, they provide benefits in monitoring heavy metal pollution levels in aquatic ecosystems. However, their tissue accumulation capacity and the concentration of heavy metals in their bodies, particularly in specific organs, can lead to oxidative stress (Dghim et al., 2023). Heavy metals can disrupt the natural antioxidant defense mechanism and lead to an imbalance of ROS formation and the counteraction ability of the body. As a result, increased ROS can cause cellular damage and impair animals' physiological functions. Elevated levels of heavy metals and disruption of crab physiology can be linked with ROS formation, including an increase in oxidative stress, impaired immune responses, and potential reproductive issues (Yu et al., 2020). One of the important oxidative responses to various biological hazards is the LPO process. LPO levels can be used to determine the increase in oxidative stress (Yadav, 2015). The present study revealed increased LPO levels in crab tissues, specifically the hepatopancreas, indicating that the hepatopancreas is more susceptible to oxidative stress than other organs. We found a notable difference in LPO levels between the hepatopancreas and the muscle ($p < 0.0001$) in *S. olivacea* and *S. paramamosain.* This result indicated tissue variability in oxidative response and capacity. As a result, high levels of Cd and Pb accumulation were found in the hepatopancreas. The assessment of the relationship between metal accumulation and LPO levels indicated no significant difference ($p > 0.05$). Our results suggested that LPO level and heavy metal accumulation in tissue are independent of each other.

When comparing the two species of crabs, the LPO level in muscle and gonad tissues was not different ($p > 0.05$, Fig. 4a). Interestingly, the MDA level in hepatopancreas and gill tissues depicted a significant difference between these two crab species; *S. paramamosain* showed significantly higher levels of LPO than *S. olivacea* in the hepatopancreas ($p = 0.0226$, Fig. 4b) and gill ($p = 0.0138$, Fig. 4c). Similar to other organisms, LPO in crabs involves a chain reaction in which free radicals attack the fatty acids in lipids, causing them to degrade and produce harmful compounds, such as MDA. LPO is related to the oxidative degradation of lipids within crabs' tissues, primarily occurring in the hepatopancreas, their main digestive organ. Therefore, the feeding habitat as well as dietary and storage content can significantly influence this LPO process, leading to potential cellular damage and affecting the crabs' overall health (Hector et al., 2019). Although organs such as the hepatopancreas and gills can accumulate heavy metals (e.g., Cd and Pb) with no significant difference in concentration, the two crab species showed a difference in oxidative response, as indicated by LPO. This implies an organ- and species-specific response to oxidative stress. *S. paramamosain* showed greater sensitivity in response to oxidative stress than *S. olivacea*. Our results align with a previous study on potential differences in physiological responses of various crab species to heavy metal accumulation (Imsilp et al., 2024).

Several mechanisms were used to manage oxidative stress induced by heavy metal accumulation in mud crabs. These mechanisms included antioxidant enzyme activation (i.e., SOD and CAT), upregulation of stress-response proteins such as Hsp70, and mobilization of metal-binding proteins to sequester heavy metals. Detoxification pathways, cellular responses to DNA damage, and apoptosis were also mentioned (Cheng et al., 2021). When exposed to high levels of metals, antioxidant enzyme activity, such as CAT, often increases as a defense mechanism against oxidative stress. CAT production was used to counteract the harmful effects of the metal-induced free radical production, such as against hydroxyl radical (de Jesus et al., 2021). In our study, CAT activity in the hepatopancreas was significantly higher in *S. paramamosain* than in *S. olivacea* ($p = 0.0348$, Fig. 4j). A similar result was also found in the gonad ($p = 0.0094$, Fig. 4l). Our results align with a previous report indicating that the accumulation of metals typically occurs in specific tissues of crabs, such as the hepatopancreas, where the highest levels of CAT activity are often observed in response to metal exposure (Yogeshwaran et al., 2020). However, the level of CAT activity in crab tissues can vary depending on the concentration of metals present and the length of exposure time (Anandkumar et al., 2019). Moreover, crab species may respond differently to metal accumulation and regulation of CAT activity (Cheng et al., 2021).

The present study also evaluated another antioxidative enzyme activity, SOD, and a reducing agent, GSH. No significant difference was indicated when comparing crab species or organ tissues ($p > 0.05$). Correlation analysis of oxidative parameters revealed a strong negative relationship between MDA and GSH levels. A notable disparity was identified in the hepatopancreas ($p = 0.011$, Fig. 4q) of *S. paramamosain*. This result indicated two important issues: (1) increased LPO in tissue decreasing the availability of reducing agents such as GSH and (2) the species-specific oxidative response. When free radicals damage membrane phospholipids, it increases

MDA production and activates the body to utilize GSH to neutralize free radicals, counteracting this damage and leading to depleted GSH levels. A negative correlation between MDA and GSH confirmed that higher oxidative stress is associated with lower antioxidant defenses (Ghafoor, 2023). According to the previous detail, an adaptive stage and an inhibitory stage exist in the oxidative response mechanism. The adaptive stage involves activating antioxidant enzymes upon initial exposure, effectively decreasing free radical creation and maintaining cellular homeostasis. Conversely, the latter inhibitory stage removes the enzyme's ability to scavenge free radicals, leading to considerable oxidative damage (Li et al., 2012). It is possible that the oxidative stress response of *S. olivacea* can still be controlled. At the same time, *S. paramamosain* may undergo a slight inhibitory phase of the oxidative reaction to metal buildup in its tissue, represented by some weakness of the oxidative defense mechanism.

Our present results depicted only a few parameters in which Pb and Cd can affect oxidative stress mechanisms. Considering this limitation of our study, further molecular evidence is required to confirm and address the variety and the precise alteration mechanism involved in Pb and Cd toxicological effects. Recently, a study has reported the effects of Pb and Cd related to mitochondrial dysfunction, leakage, and facilitated free radical formation. These include mitochondrial destruction, such as decreased mitochondrial mass, increased mitochondrial swelling, enhanced membrane permeability to H^+ and K^+ ions, and elevated mitochondrial membrane fluidity, as well as increased mitophagy protein expression. The significant suppressed effect of Pb and Cd on respiratory chain complex activities leads to an increase in free radical formation. As a key component of cellular respiration, the electron transport chain can also be a significant source of reactive oxygen species. They are produced when electrons prematurely leak from the chain and interact with oxygen, primarily at Complexes I and III. Therefore, respiratory chain complex damage can lead to oxidative stress and cellular damage (Elmorsy et al., 2025).

Heavy metal accumulation in mud crab populations within Pattani Bay gradually affects the oxidative defense mechanism and can lead to severe long-term health impacts, potentially impacting the entire ecosystem. At a molecular level, the oxidative defense mechanisms in response to metal accumulation in specific *S. olivacea* and *S. paramamosain* tissues still require clarification. However, further effects include reduced reproductive success, weakened immune systems, developmental abnormalities, and population declines, which should be of concern. Furthermore, the effects are amplified as heavy metals bioaccumulate in the food chain, potentially impacting other marine organisms and even humans who consume affected crabs.

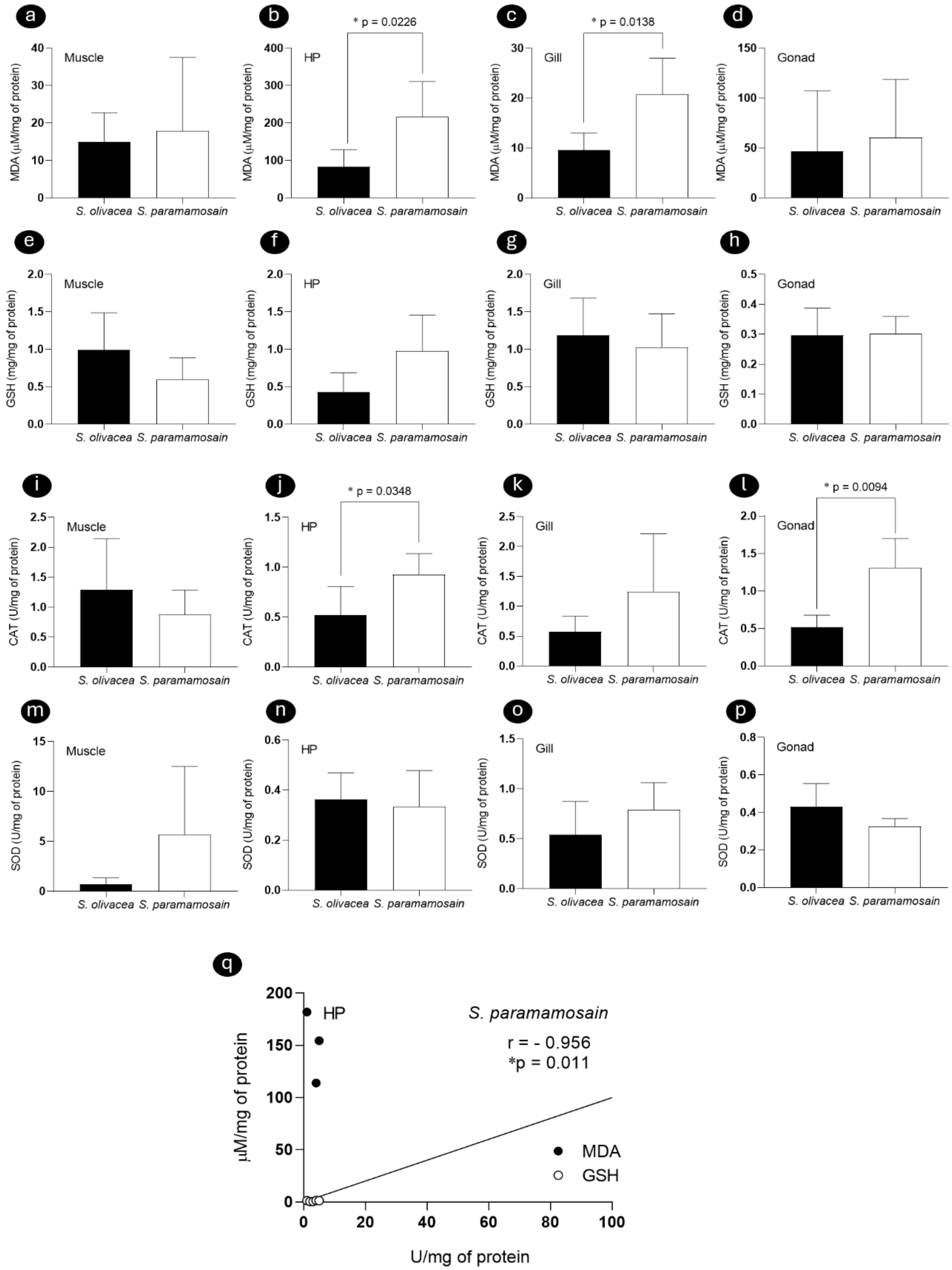

Fig-4. Oxidative response indicated by MDA, GSH, CAT, and SOD in the *S. olivacea* and *S. paramamosain* tissue (a-p). Pearson's correlation test (q) indicates a strongly negative relationship between the LPO and GSH levels in the hepatopancreas of *S. paramamosain*. LPO = lipid peroxidation; MDA = malondialdehyde; GSH = reduced glutathione; CAT = catalase; SOD = superoxide dismutase; HP = hepatopancreas, * indicates $p < 0.05$.

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Conclusion

The present study indicated that heavy metals, specifically Cd and Pb, accumulate in a tissue-specific manner, with the hepatopancreas being the most accumulative. A species-specific difference appears in oxidative responses to similar levels of heavy metal accumulation in tissue; that is, *S. paramamosain* is more sensitive than *S. olivacea*. In *S. paramamosain*, some weakness in the oxidative response was recognized, characterized by a strong negative correlation between LPO and reduced GSH levels.

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Conflict of Interest: None.
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Contribution of Authors

Thong-asa W, Tanhan P, Imsilp K & Lansubsakul N: Prepared the materials, collected the data, and performed the analysis.
Thong-asa W: Wrote the first draft of the manuscript, and all authors commented on previous versions.
All authors contributed to the conception and design of the study. All authors read and approved of the final manuscript.

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