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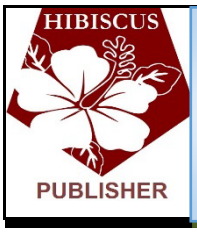

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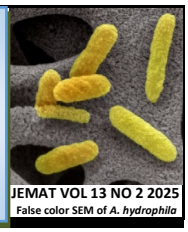

Toxicity and Bioaccumulation of Lead (Pb) in the Marine Bivalve *Geloina expansa*

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Abstract

Heavy metal pollution is a major environmental concern due to its persistence, toxicity, and ability to bioaccumulate in aquatic ecosystems. In Malaysia, increasing attention has been given to monitoring heavy metals in local marine organisms, as their accumulation in sediments and biota threatens ecosystem health and food safety. Bivalves serve as effective bioindicators, and LC_{50} provides essential insight into the acute toxicity of heavy metals across species. This study investigated the effects of lead (Pb) exposure on the bivalve *Geloina expansa* through a 96-hour acute toxicity assay. Mortality increased with rising Pb concentrations, accompanied by higher Pb accumulation in soft tissues. The 96-hour LC_{50} for *G. expansa* was calculated as 218.23 mg/L. Exposure to 250 mg/L Pb resulted in substantial Pb accumulation (1653 ± 177.51 mg/kg) compared to controls (13.00 ± 1.04 mg/kg), confirming effective uptake. These findings highlight *Geloina expansa* sensitivity to Pb and its suitability for pollution monitoring.

INTRODUCTION

Heavy metal pollution is a challenge due to its toxicity, accumulative nature, and long residence time in the environment [1]. With advancing technology and the global population, man-made contaminants in the atmosphere have gradually increased [2]. A disturbed aquatic environment with habitat loss and resource depletion can lead to pollutants being deposited in the food chain of animals and humans. Studies on environmental pollution using local organisms in relation to heavy metals are gaining interest in Malaysia [3]. One of the extremely important environmental difficulties related to pollution, toxicology, and food safety is heavy metals in organisms. Due to the impact of heavy metals on the environment and the health of consumers, studies on heavy metal concentrations in marine organisms are being investigated in detail [4]. The accumulation of heavy metal pollutants in sediments and marine organisms has either direct or indirect negative effects on the organisms and later harms humans when they consume them [5].

Heavy metal in the coastal areas may originate from various sources, including fossil fuel extraction, combustion, agriculture, refining, chemical production, and intentional and unintentional discharges. Metallic elements also enter coastal waters through natural processes such as rock weathering and river discharges [6]. The heavy metals can attach to suspended particles in the water and form complexes that can sink to the bottom and accumulate in sediments. The persistence of heavy metals in the environment makes them a long-term problem. The increase of heavy metals will pose a threat to the health of aquatic ecosystems and organisms [7,8].

According to [9], heavy metals are categorized into three groups based on their toxicity: possibly hazardous [Arsenic (As), Cadmium (Cd), Lead (Pb)], probably essential [Nickel (Ni)], and essential [Copper (Cu), Zinc (Zn), Manganese (Mn)]. Heavy metals such as Cd and Pb are referred to as non-essential metals. Even in small quantities, they are toxic and dangerous to human health. Pb pollution is caused by shipping activities such as oil effluent and fishermen's ships [5], batteries, and paints. Learning

disabilities, mental retardation, kidney damage, birth defects, muscle weakness, and degeneration of motor neurons have been recognized as adverse effects [10.11]. Pb is often used as an example of elements that are referred to as heavy metals and are considered serious pollutants due to their toxicity and tendency to accumulate [12].

The bioaccumulation potential of different bivalve species has critical implications for food safety, particularly in populations that rely on seafood as a dietary staple. Species with high bioaccumulation potential require stringent monitoring and regulatory oversight to mitigate human exposure risks. Consequently, continuous surveillance of heavy metal concentrations in bivalves, particularly macro-concentrators, alongside robust public health advisories and pollution control measures, is imperative to minimize the risks associated with contaminated seafood consumption and ensure the sustainability of safe aquatic food sources [13]. Lead (Pb) permissible limit for bivalve molluscs set in the Malaysian Food Regulations 1985 [14] and European Commission 2006 [15] is listed as 1.5 mg/kg.

The LC_{50} value is an important tool for assessing the toxicity of heavy metals in bivalves. It provides crucial information on the toxicity of heavy metals in bivalves and indicates the lethal dose at which 50% of the exposed organisms die. The LC_{50} value, a quantitative indicator of the toxicity of heavy metals in bivalves that allows comparison between different metals and bivalve species, has highlighted the importance of the LC_{50} value in the assessment of heavy metal toxicity in bivalves [16].

Geloina expansa is a typical mangrove bivalve that lives almost all its life partially submerged in the sediment. It feeds by filtering much of the suspended material in the water around it. Lots of elements are trapped and accumulate in their bodies through this feeding habit, including toxins such as heavy metals [17]. This edible species has been taken directly by humans as their regular diet. Due to its ability to filter water, *G. expansa* has been used to track heavy metal levels in aquatic environments at significant sites, such as industrial areas. [18] reported this clam is highly tolerant, able to survive in different levels of salinities, living in mud, brackish, and freshwater areas of mangrove forests.

Previous studies show that the biochemical biomarker has a strong correlation with the lead, Pb concentration in the soft tissues of *G. expansa* [19]. Therefore, a laboratory study is needed to determine the degree of Pb toxicity to obtain a response to the effects of Pb pollution. While biomarker and field accumulation studies exist for *G. expansa*, acute laboratory toxicity thresholds (LC_{50}) have not been reported. The data obtained may contribute to the data of toxicity studies on this species. They can show the survivability of this species to Pb pollution in its environment and can serve as a reference model for other bivalve species. The aim of this study was to assess the impact of Pb toxicity by determining its LC_{50} of *G. expansa*. These findings will provide valuable insights into the physiological responses of *G. expansa* to Pb exposure and its potential as a bioindicator of metal contamination.

MATERIALS AND METHODOLOGY

Geloina expansa collection and acclimatization

Samples of *G. expansa* were collected at low tide in a mangrove area along the Sepang Besar River, Sepang, Selangor, Peninsular Malaysia (2°38'48.9" N, 101°43'53.6" E). *In situ* environmental parameters were measured at the collection site to characterise baseline conditions influencing lead (Pb) bioavailability. Salinity, water temperature, and pH were recorded during sampling and were 20.00 ± 0.03 ppt, 29.10 ± 0.09 °C, and 7.32 ± 0.01, respectively. These parameters provide essential context for interpreting background Pb levels at the sampling location. For this experiment, *G. expansa* with a length of 7.18 ± 1.51 cm were collected and transported alive to the laboratory. The samples were acclimatized at room temperature in an aquarium glass tank used [40 cm (length) x 25 cm (width) x 25 cm (height)]. Three replicates were set up to assess the effects of Pb exposure on *G. expansa*. Each replicate consisted of five separate tanks, each representing a different concentration of Pb. Each tank was stocked with 10 individual *G. expansa* and filled with 10 litres of water, ensuring a standardized ratio of 1 litre per individual. This setup allowed for a controlled evaluation of Pb accumulation and its physiological impact on *G. expansa* across varying exposure levels. The clams were acclimatized for 7 days in a room with ambient conditions. During the acclimatization period, the clams were given chlorella powder as food, and the water was slightly aerated.

Preparation of lead (II) nitrate solution

Lead (II) nitrate [$Pb(NO_3)_2$] was used to perform the heavy metal toxicity studies. All laboratory equipment was previously cleaned in a 10% HNO_3 bath and rinsed well with distilled water. $Pb(NO_3)_2$ solutions were prepared at concentrations of 0, 200, 400, 600, and 800 mg/L. For range finding tests, the test concentration of Pb was made on a logarithmic scale, i.e., 1000, 100, 10. 1, 0.01, and 0.001 mg/L [20].

Determination of toxicity parameters

The number of dead *G. expansa* was calculated, and the LC_{50} of the treatment with Pb of each sample was subjected to Probit analysis. The concentration of Pb that caused the LC_{50} of the clam population tested was calculated and used for acute toxicity for future studies. Pb exposure occurred over 96 hours, and observations were recorded daily. The test solutions to which the clams were exposed were changed daily to maintain the test concentration and simulate the natural environment of the clams. The static renewal toxicity tests were conducted as described by [21] and [22].

The clams were not fed, and the medium was not aerated during the bioassay test. The main water parameters were recorded and maintained: Salinity 22.3 ± 0.13 ppt, dissolved pH 6.63 ± 0.05, temperature maintained at 30.6 ± 0.1 ºC, and ambient light with a 12:12 light: dark regime was used. For the results to be acceptable, the survival rate of the control must be ≥90 % at the end of each test. The tests are discarded if the mortality of the control is more than 10%. During observation, dead clams were immediately removed from the test chamber.

Acute toxicity of lead (Pb)

G. expansa were exposed to a predetermined LC_{50} concentration of metal for 96 hours. The acute Pb toxicity test was performed in three replicates. *G. expansa* that survived after 96 h of acute Pb toxicity were stored at -20 °C until they were used to determine heavy metal concentrations in the soft tissues of treated *G. expansa*.

Determination of lead (Pb) concentrations in soft tissue of *G. expansa*

The acid digestion for soft tissue of *G. expansa* was carried out according to the standard method USEPA 3050B [23]. The Pb content was measured according to the standard method US EPA 6010B [24] using the inductively coupled plasma optical emission spectrometer 5100 (ICP-OES).

Statistical analysis
Data were arcsine-transformed prior to one-way ANOVA, followed by Tukey's post hoc test. LC_{50} values were estimated using Probit regression (95% confidence limit).

RESULTS

LC_{50} determination using probit analysis
The determination of LC_{50} for treated *G. expansa* with Pb was performed by plotting the mortality percentage against the Pb concentration using Probit analysis. **Table** 1 shows the mortality of *G. expansa* exposed to different concentrations of Pb over a period of 96 hours. The results showed that the mortality of *G. expansa* increases with the Pb concentration. At a concentration of 0 mg/L, the mortality rate is 0 %; at 200 mg/L, it is 50 %. The mortality of clams increased to 66.7 % at 400 mg/L and to 80 % at a concentration of 600 mg/L. At the maximum dose of 800 mg/L, the mortality rate of *G. expansa* was 93.3 %.

Table 1. Percentage of mortality of *G. expansa* exposed to Pb after 96 hours of exposure (mean ± standard error)

Concentration (mg/L)	0	200	400	600	800
Mortality (%)	0	50.0 ± 5.8	66.7 ± 8.8	80.0 ± 5.8	93.3 ± 3.3

Percentage mortality between treated and control clam was determined using Probit analysis. Probit-transformed responses show that the log concentration estimate for 50% of mortality is 2.339. The LC_{50} value was estimated to be 218.23 mg/L as antilog for 2.339 (**Fig. 1**). It indicates the Pb concentration at which 50% of the clams should die after 96 hours of exposure. The value on the x-axis (concentration) at which the response (probability of death) is 50 % when the concentration is zero is referred to as the intercept with the value -5.079. It approximates the concentration at which 50 % of the population of *G. expansa* will die. The probit analysis shows that the standard error of the intercept is 1.485, indicating the variability or ambiguity of the intercept estimate. A more precise estimate has a smaller standard error.

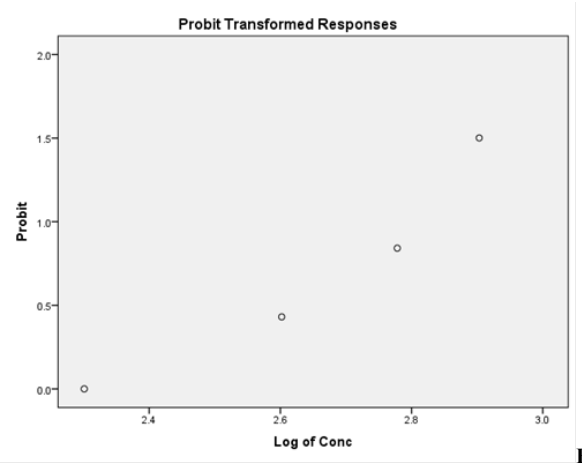

Fig. 1. Probit transformed response for the estimation of the LC_{50} of *G. expansa* at 96 hours of exposure to Pb.

Table 2 shows the estimated concentration-response curves of the mortality of *G. expansa* after 96-hour exposure to Pb. For the LC_{50}, the 95% confidence interval is (104.62, 296.41). This interval indicates the range in which the actual LC_{50} falls with 95% confidence. It is a measure of the precision of the LC_{50} estimate. A more precise estimate is indicated by a narrower interval. A limitation in the present study is that the LC_{50} estimation was derived from a small number of exposure concentrations. According to OECD [25] aquatic toxicity guidelines, accurate determination of LC_{50} values and their confidence intervals requires at least 5–7 graded concentrations arranged in a geometric series and spanning the full response range from 0% to 100% mortality. Insufficient concentration gradation or incomplete mortality coverage may reduce the precision of LC_{50} and CI estimates. Therefore, the LC_{50} value reported here should be interpreted with caution, and future studies should incorporate a wider range of graded concentrations to fully comply with OECD recommendations.

Table 2. Estimated concentration-response curves of mortality of *G. expansa* after 96 hours of exposure to Pb.

Intercept	Standard error	Estimate	LC_{50}	95% Confidence Interval
-5.079	1.485	2.3339	218.23	(104.62, 296.41)

Acute toxicity of lead (Pb)
The acute toxicity of Pb in *G. expansa* was investigated after determining the LC_{50} of Pb in this clam. The LC_{50} of Pb after 96 hours was found to be 218.23 mg/L. In the present study, *G. expansa* was exposed to 250 mg/L Pb for 96 hours after acclimatization. **Table 3** shows the concentrations of Pb in *G. expansa* tissue after 96 hours of treatment. Comparison of Pb between the exposed clam and control was determined using an independent sample t-test ($p<0.05$).

Table 3. Concentrations of Pb in *G. expansa* tissue, presented as mean ± standard error.

Treatment	Concentrations of Pb (mg/kg)
250 mg/L of Pb	1653.33 ± 177.51^{b}
Control	13.00 ± 1.04^{a}

Note: Values with different superscript alphabet (a, b) within the same column are significantly different

The accumulation of Pb in the soft tissue of *G. expansa* was determined by ICP-OES, and the result showed that a high Pb concentration was detected in the treated sample with a value of 1653.33 ± 177.51 mg/kg, compared to the control group that was not treated with Pb and whose Pb concentration was 13 ± 1.04 mg/kg.

DISCUSSION

The mortality of *G. expansa* increases with the Pb concentration. It is assumed that higher Pb concentrations are associated with a higher mortality rate. The toxicity of Pb increases in water and has a toxic effect on *G. expansa*. The predicted LC_{50} value indicates a concentration at which 50% mortality can be expected after 96 hours of exposure, and the probit analysis showed that the concentration of Pb has a significant effect on the mortality of *G. expansa*. The LC_{50} is essential for determining the relative toxicity of various heavy metals to bivalves and for indicating the most hazardous metals and their potential effects on the bivalve population [26]. As an endpoint in acute toxicity testing, the LC_{50} value provides crucial information on the lethal consequences of heavy metal exposure for bivalves.

The LC_{50} can also be used to identify regions of heavy metal pollution that may be hazardous to bivalve populations and provides information for environmental management and water quality requirements [16]. In this study, a selected Pb exposure range (0, 200, 400, 600, and 800 mg/L) was employed to comprehensively evaluate toxicity responses in bivalves. Previously, to determine the acute toxicity of Pb, *Corbicula*

fluminea was exposed to concentrations between 100 and 5000 mg/L of Pb [27]. Although acute LC_{50} values for *Perna viridis* and similar mussels are often reported in the low mg/L range (e.g., ~2.62 mg/L under standard acute toxicity tests), toxicity thresholds vary widely across taxa and environmental conditions, and some species and experimental systems exhibit markedly higher tolerance or require elevated concentrations to elicit sublethal endpoints [20,28,29]. This range allows us to capture not only traditional lethal responses but also sublethal, physiological, and mechanistic effects that may emerge at higher exposures and to account for variation in water chemistry, and potential environmental contamination scenarios. The unusually high LC_{50} value of 218.2 mg/L for *G. expansa* reflects its low sensitivity to Pb when compared with species summarized in **Table 4**.

Table 4. The LC_{50} values for bivalves subjected to a 96-hour bioassay with lead (Pb).

Species	LC_{50} (mg/L)	Reference
Perna viridis	2.62	[28]
Modiolus philippinarum	2.876	[20]
Parreysia corrugata	3.22	[30]
Lamellidens jenkinsianus obesa	192.14	[30]
Corbicula fluminea	1023.3	[27]
Geloina expansa	218.2	Current

The accumulation of heavy metals in bivalve tissue following acute toxic exposure is an important aspect of environmental toxicology studies. Heavy metals can be harmful to aquatic organisms, including bivalves, and understanding the accumulation patterns helps to assess the potential environmental impact. Bivalves are filter feeders and are exposed to contaminants in the water, which can lead to an accumulation of heavy metals in their tissue. Heavy metals can accumulate in the kidneys, stomach, and gills of bivalves exposed to acute toxic stress [31]. Bivalves are also known for their low susceptibility to heavy metal toxicity [32]. The accumulation of heavy metals in bivalves can be used as a biomarker for environmental pollution. Heavy metals can also affect the health of bivalves as they can trigger oxidative stress and cause other physiological disorders [31]. The acute toxicity of Pb in freshwater bivalves (*Lamellidens jenkinsianus obesa*) was studied by [30].

The LC_{50} values were much higher than the usual Pb concentrations in natural waters, indicating the tolerance of these bivalves to acute, short-term Pb exposure. The acute toxicity of Pb was also investigated in the freshwater bivalve *Lamellidens marginalis,* and the study provided information on the sublethal effects of Pb on acetylcholinesterase and catalase activity, lipid peroxidation, and behaviour of the bivalves [33]. Future studies should focus on the mechanistic effects of Pb accumulation in *G. expansa*, particularly its impact on the enzyme system. Following the findings of [19], which demonstrated a positive correlation between Pb exposure and GST activity, subsequent research could explore the induction of oxidative stress as a result of acute Pb toxicity.

CONCLUSION

The LC_{50} value was estimated to be 218.23 mg/L for *G. expansa* after exposure to various concentrations of Pb. The mortality of *G. expansa* increases with Pb concentration. *G. expansa* exposed to 250 mg/L Pb in an acute toxicity challenge showed an accumulation of Pb concentration (1653 ± 177.51 mg/kg) in the soft tissue compared to the control group (13.00 ± 1.04), indicating the success of Pb exposure in this bivalve.

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