

Acute toxicity of mercury to freshwater cultured milkfish *Chanos chanos*: Clinical symptoms and lethal concentration assessment

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Abstract

The increasing use of mercury in the industrial sector poses significant risks to freshwater ecosystems and aquatic organisms. Milkfish (*Chanos chanos*), a widely consumed species, is now being reared in freshwater environments, raising concerns about mercury bioaccumulation and food safety. This study aimed to determine the median lethal mercury concentration for milkfish at 96 hours (LC₅₀) in freshwater using an experimental approach and probit analysis. Five mercury concentrations were tested: 0 (control), 0.110 mg Hg/L, 0.195 mg Hg/L, 0.347 mg Hg/L, and 0.618 mg Hg/L. The results indicated that mercury is highly toxic to milkfish, with a 96-hour LC₅₀ of 0.147 mg Hg/L. Clinical symptoms observed included loss of reflex movements, irregular swimming, frequent surfacing with widened mouth and operculum openings, convulsions, and ventilation of the ram before mortality. These findings highlight the acute toxicity of mercury in freshwater milkfish and underscore the potential health risks associated with mercury contamination in aquaculture systems.

Keywords: Heavy metal, aquaculture, LC₅₀, bioaccumulation



Citation:

Ezraneti, R., Khalil, M., Affandi, R., & Nirmala, K. (2024). Acute toxicity of mercury to freshwater cultured milkfish *Chanos chanos*: Clinical symptoms and lethal concentration assessment. *Journal of Marine Studies*, 1(2), 1205. <https://doi.org/10.29103/joms.v1i2.17880>.

Received: July 27, 2024

Revised: July 30, 2024

Accepted: July 30, 2024

Published: July 31, 2024

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Introduction

Mercury is classified as the most dangerous pollutant among the various heavy metals. Mercury is one of the heavy metals found in nature, although only in small amounts. Mercury levels in freshwater naturally range from 1 to 3 ng/L (0.001 to 0.003 µg/L), while in marine waters range from 0.5 to 3 ng/L, equivalent to 0.0005 to 0.003 µg/L (Gworek et al., 2016). However, these levels can vary depending on specific environmental conditions and proximity to pollution sources. Furthermore, Connell and Miller (1984) stated that metal concentrations will increase as salinity decreases. However, with time, mercury levels in nature continue to increase because of its extensive use in various fields. Mercury has various industrial and commercial applications, including use in the manufacture of electrical equipment, particularly batteries (Gworek et al., 2016), chloralkali plants producing chlorine (Cl₂) and caustic soda (Garcia-Herrero et al., 2017), agricultural fungicides (Hylander & Goodsite, 2006), paint additives (primarily in the past as preservatives) (Mielke & Gonzales, 2008), and artisanal and small-scale gold mining (Esdaile & Chalker, 2018; Keane et al., 2023), which remains a significant source of mercury pollution globally. When released into the environment, mercury can contaminate freshwater ecosystems such as rivers, lakes, and reservoirs, leading to decreased water quality and potential ecological impacts (UNEP, 2019; Wang et al., 2004). The toxicity and persistence of mercury in aquatic environments make it a pollutant of particular concern (Gworek et al., 2016; Zupo et al., 2019). Further-

more, mercury will be carried by the river flow to the estuary, where the water is brackish, and finally mercury will enter marine waters. The mercury will be carried by the river flow to the estuary, where the water is brackish, and eventually the mercury will enter marine waters, potentially causing harmful effects on marine ecosystems and posing risks to human health through seafood contamination (Balshaw et al., 2007; Jinadasa et al., 2021).

Metal mercury enters aquatic ecosystems in both organic and inorganic forms, poses significant toxicity risks and exhibits a strong tendency to bioaccumulate in aquatic organisms (de Almeida Rodrigues et al., 2019; La Colla et al., 2019). The toxicity and behavior of mercury in water bodies vary depending on salinity, with distinct differences observed between freshwater, brackish and marine environments (Boeing, 2000). One of the aquatic organisms that are known to accumulate mercury is the milkfish (*Chanos chanos*), a species of fish commonly found in tropical and subtropical waters. Milkfish is classified as a highly euryhaline species with notable salinity tolerance. Milkfish is an exceptionally euryhaline fish species that can thrive across an extensive salinity range, from freshwater (0 ppt) to hypersaline conditions exceeding 50 ppt (Swanson, 1998), demonstrating their extraordinary osmoregulatory capabilities. This adaptability allows milkfish to inhabit various aquatic environments, including freshwater, brackish water, and oceanic waters. Traditionally, milkfish have been cultured in coastal ponds and floating net cages in marine and brackish water environments. However, its cultivation has expanded to include freshwater systems such as reservoirs, showcasing its versatility (Lin et al., 2023). This adaptability makes milkfish an ideal candidate for aquaculture in various water bodies. Furthermore, milkfish rearing has recently been extended to freshwater environments, including reservoirs, to improve aquaculture production and diversify farming practices. The salinity of the rearing environment significantly affects osmoregulatory processes in milkfish. In response to different salinities, milkfish adjust their physiological mechanisms to maintain internal osmotic balance (Kultz, 2015).

The presence of mercury compounds in aquatic ecosystems poses a significant threat to milkfish cultivation, especially in freshwater environments where this species has recently been introduced for aquaculture. Mercury exposure can induce physiological stress in milkfish, disrupting endocrine function, altering osmoregulation, and causing oxidative stress, thus compromising their health and growth (Mwakalapa et al., 2019). Despite the importance of this issue, mercury toxicity levels that specifically affect milkfish in freshwater conditions have not been widely studied or documented in the published scientific literature. Therefore, this study was carried out to determine the acute level of mercury toxicity in milkfish in a freshwater environment. The outcome of this experiment aims to provide valuable and important information to aquaculture stakeholders about the potential hazards posed by mercury contamination in the aquatic environment. These studies will be instrumental in raising awareness of the need for improved management practices to reduce the risk of mercury toxicity, which will ensure the sustainability and health of milkfish farming in freshwater systems.

Methods

This study comprises two stages: the range value test and the acute toxicity test of mercury on milkfish (*Chanos chanos*) in freshwater media. The experimental setup utilized 15 aquariums, each with dimensions of 30 x 30 x 30 cm³, and each filled with 20 liters of freshwater. The test subjects were milkfish, each measuring 7-8 cm in length and weighing 3-5 grams. A total of 150 fish were used, with a stocking density of 10 fish per aquarium. The pollutant tested was mercury nitrate (Hg(NO₃)₂). In the first phase of the study, mercury concentrations were determined using a base-10 logarithmic method. The treatments included a control (A), and concentrations of 0.006 mg/L (B), 0.06 mg/L (C), 0.6 mg/L (D), and 6 mg/L (E) of Hg, each replicated three times. Throughout the experiment, aeration was provided for each aquarium, but no water changes or feedings were performed. The primary parameter measured was fish mortality, recorded at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours. Subsequent measurements were taken every 6 hours until the 48-hour mark.

Phase 2 of the study involved conducting an acute toxicity test to determine the LC₅₀ value of mercury, which represents the concentration lethal to 50% of the fish within 48 and 96 hours. The range-finding test established that the upper threshold concentration (N) was 0.6 mg Hg/L and the lower threshold concentration was 0.06 mg Hg/L. These threshold values were then used to calculate the appropriate concentrations for the acute toxicity test using the formula outlined by Wardoyo (1977). The formula is as follows:

$$\text{Log } N/n = k (\log a - \log n)$$

$$a/n = b/a = c/b = d/c = N/d$$

Description:

N : Upper threshold concentration

n : Lower threshold concentration

k : Number of concentrations tested

a,b,c,d : Concentrations tested with the value of a as the smallest concentration.

Throughout the study, no water changes were made, and each treatment was consistently aerated to ensure that fish mortality was not attributable to oxygen deprivation. Fish mortality was recorded at 0, 6, 12, 18, and 24 hours, with subsequent measurements taken every 12 hours until the 96-hour mark. Observational indicators included behavioral symptoms such as ramjet ventilation (characterized by continuous mouth opening and operculum abduction), respiratory frequency (measured by counting the operculum and mouth movements per minute starting 30 minutes after the introduction of the test substance and compared with the control group), and swimming and reflex patterns (noting behaviors such as normal swimming, remaining at the bottom, moving towards the surface, imbalance, or loss of reflexes). Additionally, water quality was monitored daily. The LC₅₀ concentration value was determined using probit analysis with SPSS 17 software.

Table 1. Milkfish mortality rate in the range value test.

| Concentration (mg Hg/l) | Fish number (species) | Mortality at hour - (%) | | | | | | |
|----------------------------|--------------------------|-------------------------|-----|-----|-----|-----|-----|-----|
| | | 0 | 6 | 12 | 18 | 24 | 36 | 48 |
| A (0) | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B (0.006) | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C (0.06) | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| D (0.6) | 30 | 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| E (6) | 30 | 0 | 100 | 100 | 100 | 100 | 100 | 100 |

Results

Range value test

The results of the range value test for mercury on milkfish revealed that the lowest threshold concentration was 0.06 mg Hg/L. This concentration represents the highest level of mercury that did not result in fish mortality within 48 hours of exposure. Conversely, the upper threshold concentration was 0.6 mg Hg/L, which was the lowest concentration causing 100% mortality within 24 hours of exposure. The following table presents the mortality data for milkfish observed during the range value test. In the control group, no mortality was recorded after 48 hours, indicating that the water quality was well-maintained throughout the exposure period.

Acute toxicity test

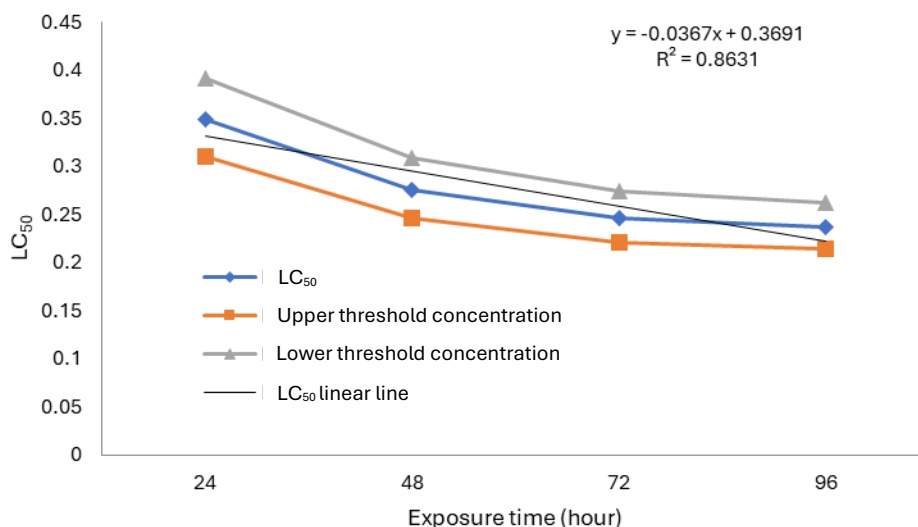
The acute toxicity test, conducted over 96 hours, utilized mercury concentrations lower than those used in the range value test. The concentrations employed were derived from the range test results and included: Treatment A (no mercury), Treatment B (0.110 mg Hg/L), Treatment C (0.195 mg Hg/L), Treatment D (0.346 mg Hg/L), and Treatment E (0.618 mg Hg/L). Clinical observations and fish survival were recorded at 6, 12, 18, 24, 36, 48, 60, 72, 84, and 96 hours following mercury exposure. At the 6-hour mark, Treatment E (0.618 mg Hg/L) exhibited 100% mortality. Treatment D (0.346 mg Hg/L) also reached 100% mortality by the 36th hour. In contrast, Treatment C (0.195 mg Hg/L) resulted in a 90% mortality rate

by the study's end. Treatment B (0.110 mg Hg/L) demonstrated a 90% survival rate at 96 hours. The control group showed no mortality or stress-related clinical symptoms throughout the 96-hour exposure, indicating that the water quality and fish conditions were well-maintained during the acute toxicity test.

Mortality data were analyzed using probit analysis to determine the LC_{50} values at 24, 48, 72, and 96 hours. The resulting LC_{50} values were 0.3497 mg/L (0.216 mg Hg/L) at 24 hours, 0.2758 mg/L (0.171 mg Hg/L) at 48 hours, 0.2467 mg/L (0.152 mg Hg/L) at 72 hours, and 0.2371 mg/L (0.147 mg Hg/L) at 96 hours. A graph depicting these LC_{50} values from the acute toxicity test is presented in Figure 1.

During the acute toxicity test, the frequency of operculum movement (the rate at which the milkfish opened and closed their operculum) increased with rising mercury concentrations. In the control group (Treatment A, 0 ppm), the average operculum opening frequency was 68.13 times per minute. This frequency increased in the subsequent treatments: Treatment B (0.110 mg Hg/L) showed an average of 124.93 times per minute, Treatment C (0.195 mg Hg/L) had an average of 130.5 times per minute, Treatment D (0.347 mg Hg/L) recorded 139.62 times per minute, and Treatment E (0.618 mg Hg/L) exhibited a frequency of 173.17 times per minute. This trend is illustrated in Figure 2.

Exposure to mercury results in significant damage to the respiratory system of milkfish, leading them to increase their mouth and operculum movements in an attempt to obtain

**Figure 1.** LC_{50} values of mercury in milkfish during the acute toxicity test.

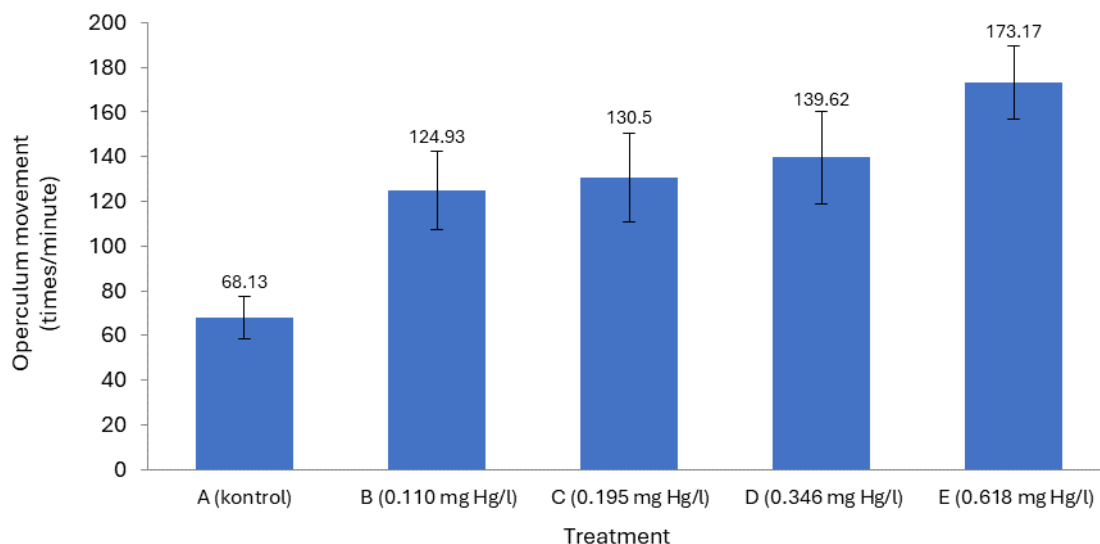


Figure 2. Frequency of operculum movement in milkfish during the acute toxicity test.

more oxygen. This heightened respiratory effort represents a physiological adaptation aimed at prolonging survival or delaying mortality.

Behavioral responses observed in milkfish following mercury exposure indicate that higher mercury concentrations accelerate the onset of behavioral changes. These changes include a loss of reflexes, irregular swimming patterns, and frequent surface visits accompanied by rapid and wide mouth and operculum movements. Fish also exhibit a tendency to return to the bottom of the aquarium in an upright position, often landing with their ventral side down. Prior to death, the fish may experience convulsions at the bottom of the tank.

In Treatment C (0.195 mg Hg/L), fish displayed symptoms of ramjet ventilation before dying. This is attributed to the severe damage to their respiratory system caused by prolonged exposure to high mercury concentrations.

Discussion

Mercury (Hg) is a unique chemical element with atomic number 80 and atomic weight 200.59 g/mol; it is the only metallic element that is liquid at standard temperature and pressure, with a melting point of -38.83°C and a boiling point of 356.73°C (Wu et al., 2024). In aquatic ecosystems, mercury can exist in various forms, including elemental, inorganic, and organic compounds. Mercury is considered one of the most hazardous heavy metal pollutants due to its high toxicity, volatility, and ability to bioaccumulate and biomagnify through food webs (Ali et al., 2019; Raj & Maiti, 2019). It readily binds to tissues in aquatic organisms, posing significant risks to ecosystem and human health (de Almeida Rodrigues et al., 2019).

The toxicity of mercury to aquatic organisms can be assessed through acute and chronic exposure studies. Acute toxicity tests typically last up to 96 hours, while chronic toxicity evaluations extend beyond this timeframe. The direct effect of pollutants, including mercury, on fish is usually expressed by acute toxicity as a result that occurs in less than 96 hours or sublethal (chronic) as a result that occurs in more than 96 hours (four days). In this study, a range value test for

48 hours, an acute toxicity test for 96 hours, and a 30-day primary study were conducted to see the effect of mercury toxicity and different salinities on the physiological conditions of milkfish.

Observations from the 96-hour acute toxicity test revealed that the LC_{50} value of mercury for milkfish reared in freshwater was 0.2371 mg/L (0.147 mg Hg/L). In comparison, Siahaan (2003) reported that the LC_{50} value of lead (Pb) for milkfish in freshwater is 62.248 mg/L, while (Biuki et al., 2010) found the LC_{50} value of cadmium (Cd) for milkfish to be 62.8 mg/L. These results highlight mercury as a highly toxic heavy metal compared to lead and cadmium, as it can cause 50% mortality in milkfish at much lower concentrations within 96 hours. This aligns with Balazs (1970), who classified toxicants with an LC_{50} value of less than 1 mg/L as having very high toxicity potential. Additionally, Darmono (1995) ranked heavy metals in terms of toxicity as follows: $\text{Hg}^{2+} > \text{Cd}^{2+} > \text{Ag}^{2+} > \text{Ni}^{2+} > \text{Pb}^{2+} > \text{As}^{2+} > \text{Cr}^{2+} > \text{Sn}^{2+} > \text{Zn}^{2+}$.

Clinical symptoms observed during the acute toxicity test included loss of reflexes, irregular swimming patterns, frequent surfacing with wider mouth and operculum openings, and increased ventilation frequency. The fish would then return to the bottom of the aquarium in an upright position, often with their ventral side facing up. The milkfish also exhibited convulsions and ram jet ventilation before succumbing at the bottom of the tank. These symptoms are consistent with mercury's known effects on the central nervous system, as mercury targets this tissue in milkfish. This observation supports the findings of (Connell & Miller, 1984), who noted that exposure to heavy metals disrupts the central nervous system in organisms.

The absorption of mercury by milkfish is significantly influenced by osmotic gradients. Higher osmotic gradients facilitate greater mercury uptake, while iso-osmotic or lower gradient conditions reduce the risk of mercury accumulation. This relationship between osmotic pressure and mercury uptake adds another layer of complexity to understanding mercury toxicity in aquatic environments. As Modassir (2000) emphasized that increased water turnover due to

osmoregulation accelerates mercury accumulation, thereby enhancing its toxicity.

Conclusions

Heavy metal, poses significant risks to aquatic environments and organisms as a result of its ability to bioaccumulate and biomagnifying. This study determined that the mercury LC₅₀ value for freshwater milkfish (*Chanos chanos*) was 0.147 mg/L, indicating its extreme toxicity compared to other heavy metals such as lead (Pb) and cadmium (Cd). The clinical symptoms of mercury exposure in milkfish included loss of reflex movements, irregular swimming, frequent surface, convulsions, and ramjet ventilation, which ultimately led to mortality. These symptoms are attributed to the impact of mercury on the central nervous system. The study also highlighted the influence of osmotic gradients on mercury absorption, suggesting that maintaining milkfish under isosmotic conditions could mitigate mercury accumulation. These findings underscore the critical need for strict monitoring and regulation of mercury levels in aquatic environments.

Acknowledgements

The authors would like to thank the Aquaculture Laboratory technicians, IPB University for their support during this experiment.

Authorship contribution

Riri Ezraneti: Conceptualization, methodology, investigation, resources, sample processing and analysis, data curation, formal analysis, visualization, writing - original draft preparation, writing - review and editing. **Munawar Khalil:** writing - review and editing. **Ridwan Affandi:** Writing - review and editing, supervision. **Kukuh Nirmala:** Writing - review and editing, supervision. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Data availability

Datasets generated during and/or analysed throughout the present study are available from the corresponding author upon reasonable request.

Conflict of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

Funding

No external funding or financial support was received while conducting this research.

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