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Zinc Sulfate Monohydrate ($ZnSO_4 \cdot H_2O$) Toxicity in Medaka Embryos: Impacts of Water Type on Lethal Concentration

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ABSTRACT

Zinc sulfate ($ZnSO_4$) is widely used in industrial and agricultural applications; however, its release into the environment raises concerns about its potential toxicity to the aquatic ecosystem. This study assesses the acute toxicity of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) on Javanese medaka (*Oryzias javanicus*) embryos by examining mortality across three water types: pure water, deionized water, and dechlorinated tap water. Embryos were exposed to $ZnSO_4$ concentrations ranging from 0.1 to 10 mg/L for 96 hours, with mortality recorded at 24, 48, 72, and 96 hours. Mortality increased in a dose- and time-dependent manner, with no deaths observed in control groups. Toxicity was highest in pure water (LC_{50} = 0.6676 mg/L), followed by dechlorinated tap water (LC_{50} = 0.9583 mg/L), and lowest in deionized water (LC_{50} = 1.021 mg/L). Water chemistry significantly influences $ZnSO_4$ toxicity, as ionic composition affects zinc uptake and its toxic effects. These results underscore the importance of site-specific water quality assessments in aquatic risk assessments. Further studies on long-term sublethal effects and metal bioaccumulation are needed to improve ecotoxicological risk assessments.

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INTRODUCTION

Zinc sulfate monohydrate plays a significant role in both agricultural and industrial applications, particularly in enhancing crop productivity and addressing micronutrient deficiencies. In agriculture, $ZnSO_4$ is utilized for biofortification, enhancing the nutritional quality of crops such as rice and wheat, particularly in zinc-deficient soils, which can result in increased yields and improved plant health (Rehman et al., 2023; Dwivedi & Srivasta. For instance, its application has been shown to enhance germination rates and oxidative stress tolerance in rice, with optimal results observed at specific concentrations (Rehman et al., 2023). However, environmental concerns arise from potential runoff and accumulation in water systems, necessitating careful management to mitigate ecological risks associated with zinc application in both agriculture and animal feed (FEEDAP, 2012). Zinc sulfate is discharged from industrial sources into aquatic ecosystems primarily through effluents from industries such as galvanizing, electroplating, and textiles, which significantly contribute to water pollution (Kavitha & Gopika, 2023). In coastal environments, anthropogenic sources, including urban runoff and historical metallurgical waste, exacerbate zinc contamination, as observed in the Valao fluvio-estuarine system in Brazil, where both modern and legacy sources influence water quality (Garnier et al., 2024). The Mediterranean Sea also experiences substantial zinc discharge from mine drainage, with estimates indicating around 330 tons/year entering the marine environment, highlighting the persistent nature of such pollution (Frau et al., 2015). Furthermore, environmental factors such as salinity and temperature are crucial in assessing zinc toxicity in marine ecosystems, necessitating tailored management strategies to mitigate the ecological risks associated with elevated zinc levels (Xu et al., 2024).

The environmental fate of zinc compounds, particularly $ZnSO_4{\cdot}H_2O$, in surface waters and sediments is influenced by various anthropogenic activities and natural processes. In mining-impacted areas, such as the Rookhope Burn catchment, zinc concentrations often exceed environmental quality standards, with significant contributions from Zn-rich groundwater and sediment interactions leading to metal attenuation through complexation with manganese oxides (Palumbo-Roe et al., 2010). In coastal regions such as southern Kaohsiung Harbor and the mouth of the Salt River in Taiwan, industrial and municipal discharges have led to high sediment zinc concentrations, indicating severe enrichment and potential ecological risks (Chang et al., 2014). Furthermore, studies using X-ray absorption spectroscopy reveal that zinc in contaminated sediments can exist in various chemical forms, with its speciation varying by location and depth, which affects its bioavailability (Webb & Gaillard, 2015). Additionally, isotopic analyses in environments like Sepetiba Bay demonstrate that anthropogenic zinc dispersal is influenced by sedimentary processes and water currents, highlighting the complex dynamics of zinc contamination in aquatic systems (Cunha et al., 2022).

Zinc sulfate exhibits significant toxicity in both freshwater and marine fish species, with varying lethal concentrations reported across different studies. For instance, juvenile red tilapia (*Oreochromis* sp.) demonstrated an LC_{50} of 33.1 mg/L after 96 hours of exposure, indicating a time-dependent increase in lethality with prolonged exposure (Rohaidi et al., 2022). Similarly, the freshwater fish *Clarias batrachus* showed hematological disturbances when exposed to excessive zinc, suggesting chronic toxicity effects (Verma et al., 2023). In another study, fingerlings of *Percocypris pingi* exhibited an LC_{50} of 2.852 mg/L at 96 hours, highlighting zinc's acute toxicity (Zeng et al., 2018).

The type of water significantly influences metal toxicity in fish, with variations observed across freshwater, dechlorinated, and seawater environments. Freshwater fish, such as *Oreochromis niloticus* and *Labeo rohita*, exhibit notable bioaccumulation of heavy metals like mercury, lead, and cadmium, primarily due to industrial pollution and agricultural runoff, leading to adverse health effects including organ damage and impaired reproductive capabilities (Nandi et al., 2012; Suhendrayatna et al., 2019; Thirumala et al., 2024). The bioavailability of metals is also influenced by water chemistry; for instance, biotic ligand models (BLMs) suggest that factors such as pH and dissolved organic carbon can alter metal toxicity levels in freshwater ecosystems (Mebane, 2022). In contrast, seawater's ionic composition may mitigate some of the toxic effects, although specific studies on the impact of seawater on metal toxicity remain limited (Nandi et al., 2012). These findings underscore the ecological risks associated with zinc exposure in aquatic environments, underscoring the need for further research to understand the implications for fish populations and ecosystem health.

Javanese medaka embryos (*Oryzias javanicus*) offer several advantages in ecotoxicology studies compared to other model organisms. Their small size, rapid development, and transparent embryos facilitate observation of toxic effects and developmental changes, making them ideal for acute and chronic toxicity assessments (Amin et al., 2025, 2021; Ibrahim et al., 2020a). The species exhibits high sensitivity to various pollutants, such as zinc oxide nanoparticles and herbicides like diuron, allowing for the precise determination of lethal and sublethal concentrations (Amin et al., 2021; Ibrahim et al., 2020a, 2020 b. Furthermore, the availability of a sequenced genome enhances genetic studies and the understanding of physiological responses to environmental stressors (Takehana et al., 2020). Additionally, Javanese medaka embryos serve as a valuable model in ecotoxicology studies due to their sensitivity to various environmental contaminants. Research has shown that exposure to metals such as nickel, copper, and selenium can lead to significant developmental abnormalities and mortality, with gene expression analysis revealing extensive impacts on embryonic development (Addai-Arhin et al., 2025).

Additionally, zinc oxide nanoparticles (ZnO NPs) have been shown to induce increased heart rates and various deformities, including spinal and pigmentation issues, at concentrations as low as 10 µg/L (Amin et al., 2024). Furthermore, herbicides like diuron exhibit biphasic effects, causing both stimulatory and inhibitory responses in heart rates and hatchability, indicating potential endocrine disruption (Ibrahim et al., 2020a). Lastly, exposure to 3,4-dichloroaniline has been linked to severe acute and sublethal toxicity, highlighting the long-term risks associated with chemical exposure (Ibrahim et al., 2020b). Collectively, these findings underscore the importance of Javanese medaka embryos in assessing the ecological risks posed by pollutants.

The objective of this study was to assess and compare the acute toxicity of $ZnSO_4 \cdot H_2O$ on Javanese medaka embryos across three distinct water types: pure water, deionized water, and dechlorinated tap water. By determining concentration–response relationships and calculating LC_{50} values over a 96-hour exposure period, this study aims to elucidate the role of water chemistry in modulating zinc toxicity.

To achieve this objective, we tested the hypothesis that the acute toxicity of $ZnSO_4 \cdot H_2O$, as measured by the 96-h LC_{50}, varies across different water types for Javanese medaka embryos.

METHODS AND MATERIALS

Stock Preparation and Embryo Collection

Zinc sulfate monohydrate was obtained from Sigma Aldrich and dissolved in pure water, deionized water, and dechlorinated tap water to prepare the stock solution. Exposure concentrations of 0.100, 0.250, 0.500, 1.0, 5.0, and 10.0 mg/L were then prepared by diluting the stock solution.

Javanese medaka were previously collected from the estuary area in Sepang, Selangor, Malaysia, and housed in overflow containers, where they were fed fresh *Artemia nauplii* (brine shrimp) larvae three times a day. Prior to the experiment, newly fertilized cluster eggs were carefully collected from the female body and washed multiple times to remove any residual substances on the egg surface. Healthy embryos, no older than 8 hours post-fertilization (hpf), were then selected for the subsequent experimental procedures.

Toxicity Test

This study employed an empirical and experimental approach to assess the acute toxicity of $ZnSO4 \cdot 2H_2O$. The experimental design involved the direct manipulation of two key variables, $ZnSO_4 \cdot H_2O$ concentration and water type, to observe and quantify the measurable outcome of embryo mortality over a 96-hour exposure period.

The exposure method for Javanese medaka embryos was adapted from the Organization for Economic Co-operation and Development (OECD) guidelines for testing chemicals through the fish: early life stage toxicity test (Organization for Economic Co-operation and Development, 2013). For the 96-hour exposure, 10 embryos were placed in each well of a 6-well multiplate, with each well containing 10 mL of the zinc sulfate test solution. A total of 210 embryos were used in the exposure groups, with 30 embryos in the control group. Each concentration was tested in triplicate. The embryos were maintained under a 14-hour light and 10-hour dark cycle at 26°C ± 1°C. Static toxicity tests were conducted, and after 24 hours, the embryos were examined under a stereomicroscope (Olympus CX31 2D, Tokyo, Japan). Any dead embryos were removed and recorded. At this point, the exposure solutions were replaced with fresh stock and diluted solutions.

Statistical Analysis

Probit analysis was performed to calculate the LC50 using log-transformed concentrations in GraphPad Prism version 8.0.2 for Windows (GraphPad Software, La Jolla, CA, USA). All the data are expressed as mean ± standard deviation (SD).

FINDINGS

The acute toxicity of $ZnSO_4 \cdot H_2O$ in the early life stages of Javanese medaka fish varied across different water types, with mortality increasing in a concentration- and time-dependent manner. No mortality was observed in the control groups across all water types, confirming the natural survival of the test organisms under normal conditions. In pure water, mortality increased progressively with concentration and exposure duration (Figure 1). At the highest concentration (10 mg/L), all replicates exhibited complete mortality by 96 hours. Even at 1 mg/L, a notable increase in mortality was observed at 96 hours, reaching up to 73.33%. In deionized water, a delayed toxic response was evident compared to pure water, with minimal mortality at lower concentrations during the initial 72 hours, but a sharp increase at 96 hours, particularly at concentrations of 1 mg/L and higher (Figure 2). In dechlorinated tap water, mortality followed a more gradual pattern, with significant effects observed at 5 and 10 mg/L by 96 hours (Figure 3). Across all water types, time-dependent toxicity was evident, with the most significant mortality occurring after 72 hours, particularly at higher concentrations. These findings highlight the importance of considering water chemistry in assessing Zn toxicity in aquatic organisms.

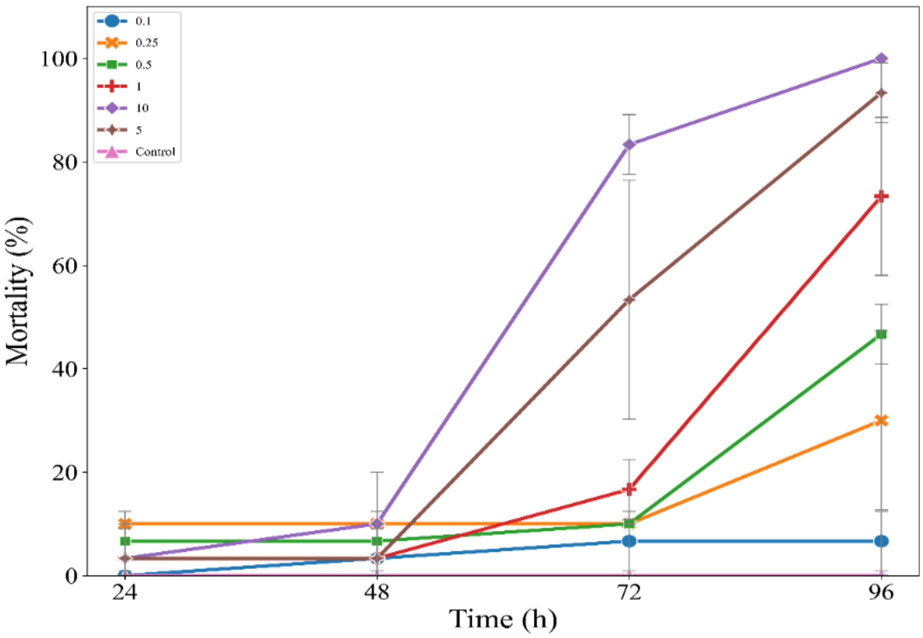

Figure 1. *Mortality of Javanese medaka embryos at various time intervals after exposure to $ZnSO_4$ in pure water*

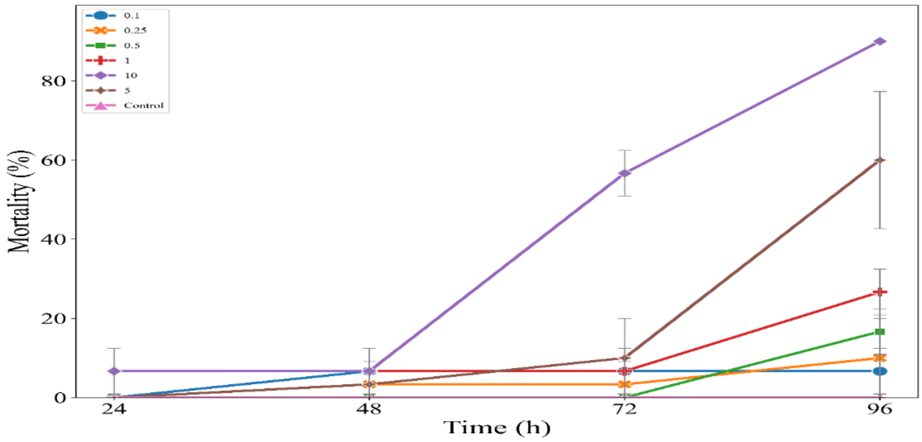

Figure 2. *Mortality of Javanese medaka embryos at various time intervals after exposure to $ZnSO_4$ in deionized water*

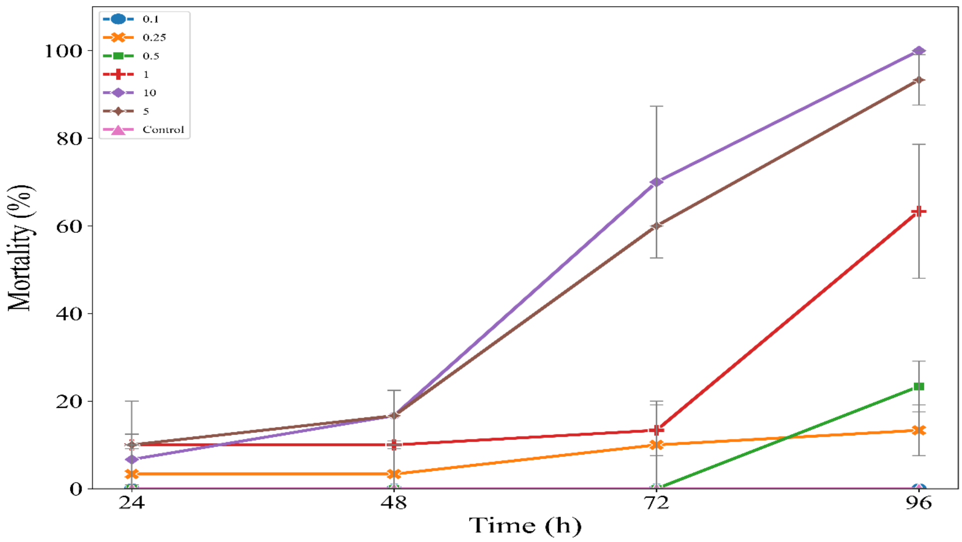

Figure 3. *Mortality of Javanese medaka embryos at various time intervals after exposure to $ZnSO_4$ in dechlorinated tap water*

In the acute toxicity assessment of $ZnSO_4{\cdot}H_2O$ on the early life stages of Javanese medaka, the calculated LC_{50} values varied across different water types, indicating significant differences in $ZnSO_4$ toxicity based on water chemistry. The LC_{50} value in pure water was 0.6676 mg/L, representing the highest toxicity among the three water types (Figure 4). In deionized water, the LC_{50} value was 1.021 mg/L (Figure 5), indicating reduced toxicity compared to pure water. For dechlorinated tap water, the LC_{50} was 0.9583 mg/L (Figure 6), which falls between the values for pure and deionized water.

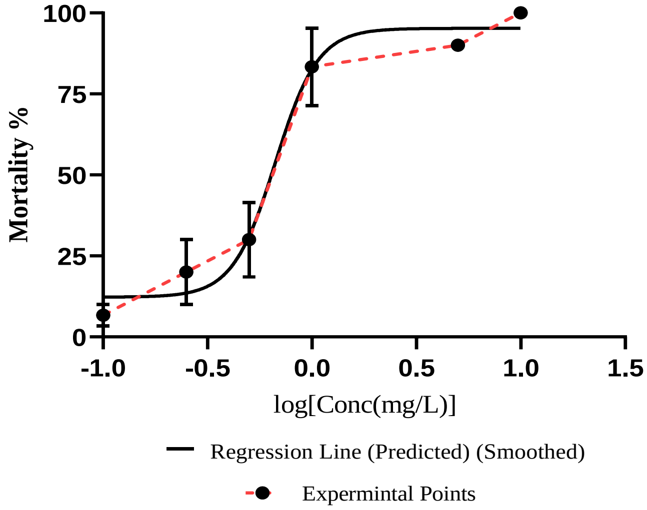

Figure 4. *96-hr LC_{50} values of $ZnSO_4$ for Javanese medaka embryos in pure water*

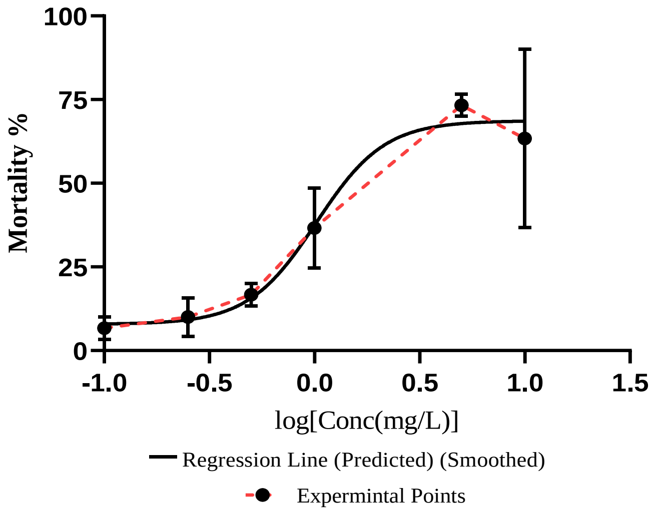

Figure 5. *96-hr LC_{50} values of $ZnSO_4$ for Javanese medaka embryos in deionized water*

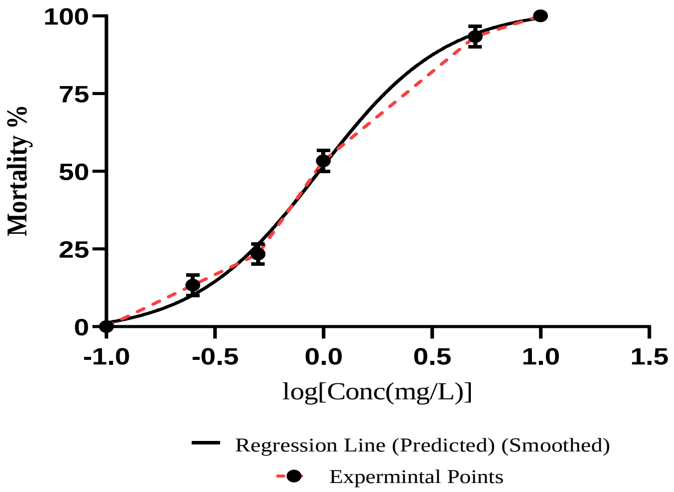

Figure 6. *96-hr LC_{50} values of $ZnSO_4$ for Javanese medaka embryos in dechlorinated tap water.*

DISCUSSION

The acute toxicity of $ZnSO_4 \cdot H_2O$ on Javanese medaka embryos was revealed to be concentration- and time-dependent, with significant mortality observed across different water types. Pure water exhibited the highest toxicity, followed by dechlorinated tap water, and deionized water showed delayed effects. These findings align with previous research indicating that water chemistry, particularly pH and hardness, influences zinc bioavailability and toxicity, as lower hardness and varying pH levels can enhance zinc's toxic effects on aquatic organisms (X. F. Li et al., 2019a). The observed rapid mortality in pure water may be attributed to the higher bioavailability of zinc ions, which can damage gill tissues and induce stress responses in fish (Skidmore, 1964). Comparatively, the results are consistent with studies indicating varying sensitivity among aquatic species to zinc, emphasizing the need for aquatic risk assessments that consider local water chemistry (Trenfield et al., 2023; Daryoush & Ismail, 2012). The overall trend indicated that pure water exhibited the highest toxicity, followed by deionized water, while dechlorinated tap water showed the lowest mortality rates. This suggests that water chemistry plays a critical role in Zn bioavailability, with ions present in dechlorinated tap water potentially mitigating Zn^{2+} toxicity. The delayed onset of mortality in deionized and dechlorinated tap water indicates that Zn bioavailability and uptake rates vary depending on the ionic composition of the exposure medium. The toxicity of zinc sulfate ($ZnSO_4$) is significantly influenced by water chemistry, particularly the presence of major cations and environmental conditions. Studies indicate that increased concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) can reduce $ZnSO_4$ toxicity in aquatic organisms, such as *Daphnia magna*, by factors of 6.3 and 2.1, respectively (Heijerick et al., 2002).

Furthermore, the presence of sediment can mitigate toxicity by reducing water column concentrations of zinc, as observed in *Hyalella azteca*, where sediment treatments decreased toxicity by a factor of ten (Poynton et al., 2019). Research indicates that as the pH increases, the toxicity of zinc also rises, with studies showing a marked increase in toxicity from a pH of 6.7 to 8.3, where growth inhibition concentrations decreased from 185 to 53 µg/L (Price et al., 2021). Additionally, lower pH levels correlate with heightened sensitivity to zinc across various species, with hazardous concentrations being significantly lower at acidic pH levels (Li et al., 2019). Hardness consistently affects zinc toxicity across species, with higher hardness levels generally reducing the toxicity of zinc. Additionally, DOC plays a variable role in modifying zinc's bioavailability and toxicity, although its influence is less consistent than that of hardness (DeForest et al., 2023). The Biotic Ligand Model (BLM) effectively incorporates these parameters to predict zinc toxicity, demonstrating improved accuracy over traditional hardness-based models (DeForest & Genderen, 2012; Santore et al., 2002). Thus, understanding these water chemistry factors is crucial for assessing the ecological risks associated with zinc exposure in aquatic environments.

Zinc sulfate exhibits significant embryotoxicity across various fish species, with both lethal and sublethal effects observed. In marine environments, studies on species like the yellowstriped goby (*Mugilogobius chulae*) and red sea bream (*Pagrus major*) reveal that $ZnSO_4$ concentrations as low as 2.78 mg/L can lead to high mortality rates and developmental deformities, including spinal malformations and reduced hatching success (Huang et al., 2010; Li et al., 2018). The Brazilian silverside (*Atherinella brasiliensis*) demonstrated sensitivity to Zn^{2+}, indicating that salinity influences toxicity, with increased mortality at elevated temperatures (Feitosa et al., 2021). Additionally, zinc oxide nanoparticles (ZnO NPs) were found to cause increased heartbeat and various deformities in Javanese medaka embryos, highlighting the broader implications of zinc compounds on aquatic life (Amin et al., 2024).

The acute toxicity of $ZnSO_4 \cdot H_2O$ to Javanese medaka embryos varied with water type, with pure water showing the highest toxicity (LC_{50} = 0.6676 mg/L), followed by dechlorinated tap water (LC_{50} = 0.9583 mg/L), and the lowest toxicity observed in deionized water (LC_{50} = 1.021 mg/L), highlighting the influence of water chemistry on zinc toxicity. The dose-response relationship of zinc sulfate toxicity in fish reveals both lethal and sublethal effects, significantly influenced by concentration and exposure duration. For juvenile red tilapia, the LC_{50} decreased over time, indicating that prolonged exposure to lower concentrations can be fatal, with values ranging from 48.7 mg/L at 24 hours to 33.1 mg/L at 96 hours (Rohaidi et al., 2022). In contrast, fingerlings of *Percocypris pingi* exhibited much lower LC_{50} values, with 3.504 mg/L at 24 hours and stabilizing at 2.852 mg/L from 72 to 96 hours (Zeng et al., 2018). For *Oreochromis niloticus*, the 96-hour LC_{50} was determined to be 72.431 mg/L (Ezeonyejiaku & Obiakor, 2011). In zebrafish embryos, exposure to zinc chloride resulted in an LC50 of 1.36 mg/L, indicating significant developmental toxicity (Küçükoğlu et al., 2013). Guppies (*Poecilia reticulata*) exhibited an LC50 of 30.826 mg/L over 96 hours (Gül et al., 2009). Additionally, tests on various Rocky Mountain fish species, including cutthroat trout, revealed LC_{50} values ranging from 166 µg/L to over 67,000 µg/L, highlighting the diverse responses to zinc toxicity among aquatic organisms (Brinkman & Johnston, 2012). These findings highlight the varying sensitivity of aquatic organisms to zinc compounds, emphasizing the need for species-specific assessments in environmental risk evaluations.

CONCLUSION

This study demonstrates that the acute toxicity of zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) to Javanese medaka embryos is significantly influenced by water chemistry, with mortality increasing in a concentration and time-dependent manner. Pure water exhibited the highest toxicity (LC_{50} = 0.6676 mg/L), followed by dechlorinated tap water (LC_{50} = 0.9583 mg/L), while deionized water showed the lowest toxicity (LC_{50} = 1.021 mg/L). These variations suggest that the ionic composition of water plays a critical role in zinc bioavailability and toxicity, with dissolved ions in dechlorinated tap water potentially mitigating Zn^{2+} toxicity. Given the observed variability in toxicity across different water types, this study highlights the importance of considering site-specific water chemistry parameters in ecotoxicological risk assessments of zinc compounds. Future research should investigate the chronic and sublethal effects of $ZnSO_4 \cdot H_2O$ on various developmental and physiological endpoints in Javanese medaka, including hatching success, growth, and behavioral changes, under environmentally relevant concentrations. Additionally, studies incorporating a broader range of water chemistries, such as variations in hardness, pH, and dissolved organic matter, are recommended to understand better the mechanisms by which water quality influences zinc toxicity and bioavailability in natural aquatic systems.

ETHICS STATEMENT

The fish used in this experiment were sampled, handled, and treated following the guidelines and regulations approved by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (AUP No.: R006/2016).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

AUTHOR CONTRIBUTIONS

Conceptualization: Naweedullah Amin, Qudratullah Oryakhil, Mohammad Navid Wais, Khalida Aziz; Methodology: Naweedullah Amin, Mohammad Navid Wais, Khalida Aziz; Formal analysis: Naweedullah Amin, Mohammad Navid Wais; Investigation: Naweedullah Amin, Qudratullah Oryakhil, Khalida Aziz; Writing-original draft: Naweedullah Amin, Mohammad Navid Wais, Qudratullah Oryakhil, Khalida Aziz; Writing-review & editing: Naweedullah Amin.

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DATA AVAILABILITY

Data are available upon request from the corresponding author.

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