Range Findings of Lethal Concentration of Zinc Sulfate Heptahydrate (ZnSO₄.7H₂O) to Juvenile Red Tilapia

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INTRODUCTION

Zinc (Zn) is one of the most commonly abundant heavy metals and the second required element in living organisms after iron (Fe) [1,2]. Common anthropogenic sources of Zn are the products from the galvanization process and the usage of pesticides, fertilizers, and also fungicides [1,3,4]. According to European Commission (EC) [5] and European Food Safety Authority (EFSA) (2015), zinc sulfate heptahydrate (ZnSO₄.7H₂O) is also used as one of the supplementations for Zn requirement in animal feeds including fish feeds. The nutritional requirement of Zn in fish feeds for tilapia fish is considered to be 30 mg per kg and permitted up to a maximum of 250 mg of feedstuff per kg [2,5,6]. Despite Zn has been used as a nutrient requirement in fish, it can induce toxicity effects to the fish at a certain level of concentration. The lethal concentration level, however, differs based on certain factors which are discussed in the present study. Estimation of the lethal range of concentration of the toxicant can be done by obtaining the LC₅₀ [7–9].

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ABSTRACT

An acute toxicity study was conducted to determine the range of lethal concentration of zinc sulfate heptahydrate (ZnSO₄.7H₂O) to juvenile red tilapia (Oreochromis sp.). The mortality of the fish was recorded at 24, 48, 72, and 96 hours of study. The lethal concentration of ZnSO₄.7H₂O that caused 50% mortality (LC₅₀) was 48.7, 40.0, 34.6, and 33.1 mg/L for 24, 48, 72, and 96 hours, respectively. Meanwhile, the lethal concentration that caused 99% mortality (LC₉₉) was 116.7, 108.7, 76.2, and 69.9 mg/L for 24, 48, 72, and 96 hours, respectively. The Probit analysis with a 95% confidence interval is used to determine the range of lethal concentration of ZnSO₄.7H₂O for 24, 48, 72, and 96 hours of toxicity study. The result shows that the lethal concentration of ZnSO₄.7H₂O decreased over time indicating that the low concentration could become lethal to juvenile red tilapia when exposed for longer periods and act as a preliminary study for further study.

Tilapia species is one of the aquatic organisms used as test organisms in toxicity studies. Apart from easy handling, culture, and maintenance in the laboratory, this species responds promptly to different environmental alterations and have the adaptability to a broad range of environmental stresses [10–17]. Unfortunately, the toxicity of Zn to the hybrid red tilapia is less reported as it is not commonly used in the toxicity study of Zn [20].

In this study, juvenile red tilapia was selected as a test organism and a preliminary study was conducted to determine the range of lethal concentration of ZnSO₄.7H₂O on juvenile red tilapia. The lethal concentration that estimates to cause 50% and 99% mortality in an animal population (LC₅₀ and LC₉₉) was reported, and aspects that differentiate the obtained result from some previous studies were discussed. The findings of this study will act as a baseline for future studies such as the effect of Zn toxicity on juvenile red tilapia and also the study to prevent the toxicity effect of Zn on juvenile red tilapia.
MATERIALS AND METHODS

The stock solution was prepared as 1000 mg/L by dissolving ZnSO\textsubscript{4}.7H\textsubscript{2}O salts in deionized water. The prepared 1000 mg/L ZnSO\textsubscript{4}.7H\textsubscript{2}O stock solution was then diluted to the desired concentration (0, 1, 3, 5, 7, 10, 20, 30, 40, 50, 60, 80 mg/L) in 40 L water. Juvenile red tilapia were acclimatized for two weeks in an aerated 1500 L poly tank. The fish were fed twice a day during the period and the water in the poly tank was replaced once a week. Fish were then transferred to a tank with 100 L water before the toxicity study and fasted for 24 hours to remove waste from the fish. The waste produced by the fish was siphoned out from the tank after 12 and 24 hours of the fasting period.

The fish (length = 11.0±0.26 cm; weight = 26.8±2.3 g) were then transferred to the tank filled with 40 L of dechlorinated tap water. For each tank with different concentrations, six fish were used [22,23] compared to seven fish as stated by OECD [8]. Each of the tanks received aeration to provide oxygen for the fish during the LC\textsubscript{50} study. Fish were exposed to an initial concentration of ZnSO\textsubscript{4}.7H\textsubscript{2}O (0, 1, 3, 5, 7, 10, 20, 30, 40, 50, 60, 80 mg/L) for 96 hours. Static non-renewable test solution design is used according to the OECD [8] and ASTM International [9]. The mortality is recorded at 24, 48, 72, and 96 hours and all the dead fish were removed from the tank. The water in the aquarium was discarded following the hazardous waste disposal procedure and fish were burned and buried following the animal disposal procedure.

Probit analysis, a specialized regression model of binomial response variables, was used to determine the range of LC\textsubscript{50} and LC\textsubscript{99} by transforming the sigmoid dose-response curve based on the mortality (in %) of the fish for every 24 hours into a straight line before further analyzed by running a regression on the relationship [24,25].

Using SPSS Statistics (IBM, version 25) software to conduct the analysis, the output values (expressed as the lower and upper boundary of the output range) were able to furnish including the 95% confidence interval for a more accurate maximum likelihood method [24].

RESULTS AND DISCUSSION

The mortality (in %) of the fish is shown in Fig 1. The results show that the higher the exposure of ZnSO\textsubscript{4}.7H\textsubscript{2}O, the higher the mortality of the fish, indicating that at a higher concentration, the test chemical is lethal to the fish. Figure 2 shows the 95% confidence interval curve (concentration expressed in the log) from the result of Probit modeling for 96 hours of toxicity study.

Using the mortality results, LC\textsubscript{50} and LC\textsubscript{99} were obtained using Probit analysis as shown in Table 1. The results show that both LC values decreased with acute 96-hour exposure time, allowing the possibility of the low level of ZnSO\textsubscript{4}.7H\textsubscript{2}O could bring toxicity effect to the fish in long-term exposure from the initial exposure concentration. The 95% confidence interval (expressed as a lower and upper boundary) obtained during the Probit analysis shows that at any concentration of ZnSO\textsubscript{4}.7H\textsubscript{2}O within the interval, there is a 95% probability that the mortality of the fish could happen. Based on Fig 2 and Table 1, since the interval between the lower and upper boundaries of estimated values LC\textsubscript{50} and LC\textsubscript{99} are not overlapping with each other, both estimation values are considered significant to each other [26]. Table 2 shows the LC values obtained in the previous Zn toxicity studies using tilapia as a test organism.

According to the previous study, the LC\textsubscript{50} values for the tested toxicant also decreased with the increase in time of exposure or it could be said that the relationship between LC\textsubscript{50} and exposure time showed an opposite relationship [3,14]. This would also indicate the potential of the low level of tested toxicants to cause toxicity and the possibility to be a threat to the freshwater ecosystem and aquatic animals if exposed longer [3]. However, there are fewer studies reported about red tilapia as a test organism in the Zn toxicity study compared to most of the previous studies that used Nile tilapia (O. niloticus) species. Both red tilapia and O. niloticus might have different physiological conditions on the adaptation to Zn exposure. Therefore, the selected tilapia species can be one of the factors in assessing the toxicity of Zn.

Fig 1. Mortality of red Tilapia at different concentrations of Zn. The numbers of mortality were recorded after 24, 48, 72, and 96 hours for each concentration.

Fig 2. 95% confidence interval curves probability plot for ZnSO\textsubscript{4}.7H\textsubscript{2}O for 96-hours predicted mortality concentration-response curve for juvenile red tilapia based on parameter estimate from the Probit analysis.

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Other than that, the age and size of the fish are also factors in assessing the toxicity of Zn. Smaller-sized or younger species are more sensitive to acute toxicity of heavy metals compared to larger or mature fish [14,20,27–29]. The youngest stage used in the previous study is the fingerlings stage compared to the present study that used the juvenile stage of the fish. However, the size of the fish used in the Abdel-Tawwab et al. [30] study was much smaller (4.6 ± 0.2 g) compared to the 2016 study (25 ± 0.5 g). According to US EPA [31], all organisms should be approximately the same age. It is also noted that the fish should be taken from the same source to minimize the diversity of response to the experimental material [14,31]. Even if the smaller size and younger fish are used, the type of toxicant used in the toxicity study should be taken into consideration as Zn content in any toxicants differ.

The degree of heavy metal toxicity in natural water depends on the type, dosage, and exposure duration of the chemical [32,33]. According to the previous study, the toxicants used to assess the Zn toxicity to the fish are ZnSO₄·7H₂O, zinc chloride (ZnCl₂), Zn bulk particle, and Zn with nanoparticle in sizes. Similar to ZnSO₄·7H₂O, ZnCl₂ is also used as a feed additive for Zn supplementation in fish feeds. However, there is more Zn content in the ZnCl₂ (48% of Zn content) compared to the ZnSO₄·7H₂O (22.7% of Zn content) [6]. Therefore, the concentration based on the ZnCl₂ toxicant used in the toxicity studies [3,20,34] is much lower compared to the present and previous studies that used ZnSO₄·7H₂O as a toxicant [14,17,35]. Plus, using pure Zn as a stock solution as in a study by Abdel-Khalek et al. [15], the LC₅₀ is lesser than the values in the other studies that used a compound of Zn as a toxicant. The nanosized of pure Zn were proven to be more toxic than the bulk particle of pure Zn. This indicates that the particle sizes of Zn also play an important role in assessing its toxicity effects on fish [15]. Otherwise, the duration of the experiment is usually kept at 96 hours or less due to the amount of oxygen decrease and metabolic waste increase that could become problematic in long-term experiments, according to the standardized method of bioassay systems [8,35].

CONCLUSION

Despite being one of the required nutrients in fish, Zn is still able to induce toxicity effects in the fish. However, some of the aspects need to be taken into account such as species of fish used as test organisms as well as the toxicant or chemical used in the toxicity study. The smaller and younger species of fish tend to be more sensitive to the toxicity effect of tested toxicants. Zn toxicity is not only concentration-dependent but also depends on the exposure time. This study will act as a preliminary study for future studies and also will be added to the toxicity study of Zn to the red tilapia species as there are fewer studies reported for these species.

Table 1. Probit Analysis for LC₉₀ and LC₅₀ of ZnSO₄·7H₂O to Juvenile Red Tilapia.

<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>LC₉₀ Estimate (mg/L)</th>
<th>LC₉₀ Lower Boundary</th>
<th>LC₉₀ Upper Boundary</th>
<th>LC₅₀ Estimate (mg/L)</th>
<th>LC₅₀ Lower Boundary</th>
<th>LC₅₀ Upper Boundary</th>
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<td>33.910</td>
<td>46.483</td>
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<td>32.955</td>
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<td>34.593</td>
<td>96</td>
<td>69.868</td>
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</table>

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