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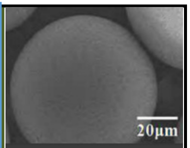

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SEM ZnO nanoparticle

Range Findings of Lethal Concentration of Zinc Sulfate Heptahydrate ($ZnSO_4.7H_2O$) to Juvenile Red Tilapia

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ABSTRACT

An acute toxicity study was conducted to determine the range of lethal concentration of zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) 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 $ZnSO_4.7H_2O$ that caused 50% mortality (LC_{50}) 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 (LC_{99}) 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 $ZnSO_4.7H_2O$ for 24, 48, 72, and 96 hours of toxicity study. The result shows that the lethal concentration of $ZnSO_4.7H_2O$ 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.

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 ($ZnSO_4.7H_2O$) 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 LC_{50} [7–9].

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]. Red tilapia (*Oreochromis* sp.) is one of the hybrid tilapias and is known to have a significant improvement in survival, growth rates, and productivity [18–21]. 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 $ZnSO_4.7H_2O$ on juvenile red tilapia. The lethal concentration that estimates to cause 50% and 99% mortality in an animal population (LC_{50} and LC_{99}) 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.

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MATERIALS AND METHODS

The stock solution was prepared as 1000 mg/L by dissolving $ZnSO_4.7H_2O$ salts in deionized water. The prepared 1000 mg/L $ZnSO_4.7H_2O$ 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] compare to seven fish as stated by OECD [8]. Each of the tanks received aeration to provide oxygen for the fish during the LC_{50} study. Fish were exposed to an initial concentration of $ZnSO_4.7H_2O$ (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_{50} and LC_{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].

By 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 $ZnSO_4.7H_2O$, 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_{50} and LC_{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 $ZnSO_4.7H_2O$ 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 $ZnSO_4.7H_2O$ 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_{50} and LC_{99} are not overlapping with each other, both estimation values are considered significant to each other [26]. **Table 2** shows the LC_{50} obtained in the previous Zn toxicity studies using tilapia as a test organism.

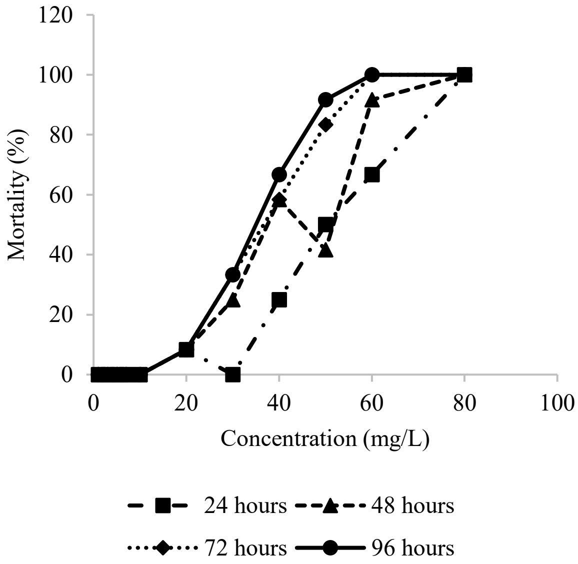

Fig 1. Mortality of red Tilapia at different concentrations of Zn. The numbers of mortality were recorded after 24, 48, 72, and 96 hours for each concentration.

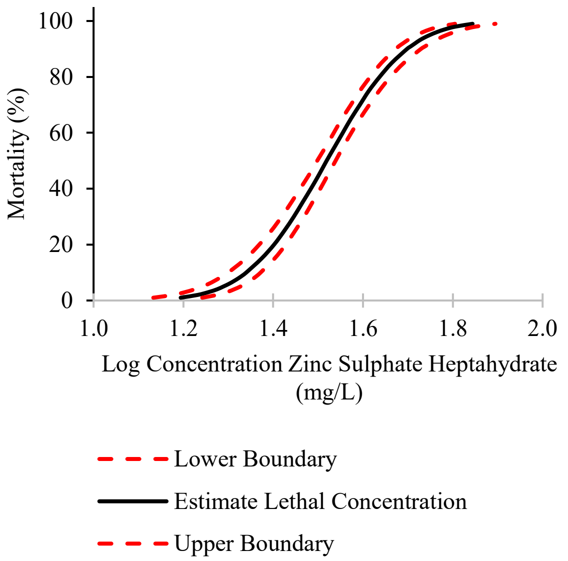

Fig 2. 95% confidence interval curves probability plot for $ZnSO_4.7H_2O$ for 96-hours predicted mortality concentration-response curve for juvenile red tilapia based on parameter estimate from the Probit analysis.

According to the previous study, the LC_{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_{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 compares 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.

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Table 1. Probit Analysis for LC_{50} and LC_{99} of $ZnSO_4.7H_2O$ to Juvenile Red Tilapia.

Time (Hours)		95% Confidence Limit for Concentration (mg/L)		
		Estimate LC	Lower Boundary	Upper Boundary
LC_{50}	24	48.710	40.583	59.865
	48	39.989	33.910	46.483
	72	34.580	32.935	36.202
	96	33.057	31.488	34.593
LC_{99}	24	116.704	83.733	294.471
	48	108.689	80.907	205.073
	72	76.158	69.245	86.023
	96	69.868	63.782	78.530

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 compare 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 $ZnSO_4.7H_2O$, zinc chloride ($ZnCl_2$), Zn bulk particle, and Zn with nanoparticle in sizes. Similar to $ZnSO_4.7H_2O$, $ZnCl_2$ is also used as a feed additive for Zn supplementation in fish feeds. However, there is more Zn content in the $ZnCl_2$ (48% of Zn content) compared to the $ZnSO_4.7H_2O$ (22.7% of Zn content) [6]. Therefore, the concentration based on the $ZnCl_2$ toxicant used in the toxicity studies [3,20,34] is much lower compared to the present and previous studies that used $ZnSO_4.7H_2O$ 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 LC_{50} is lesser than the values in the other studies that used a compound of Zn as a toxicant. The nanosized of pure Zn was 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.

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REFERENCES

1. Authman M, Zaki M, Khallaf E, Abbas H. Use of Fish as Bio-Indicator of The Effects of Heavy Metals Pollution. J Aquac Res Dev. 2015 Apr 1;J Aquaculture Research and Development:328.
2. Hogstrand C. Zinc. In: Fish Physiology: Homeostasis and Toxicology of Essential Metal. Academic Press; P. 135–200.
3. Siddiki Akmna, Khair Mu, Naser Mn, Salam Ma. Biophysicochemical Changes In Nile Tilapia, Oreochromis Niloticus Exposed To Znso4.7h2o and Zncl2 Metal Toxicant. J Innov Pharm Biol Sci. 2018 Apr 5;113–8.
4. Sires J. A Review of Potential Zinc and Copper Pollution Sources In Kenai River Watershed. Alaska Department of Environmental Conservation; 2017.
5. European Commision. Opinion of The Scientific Committee for Animal Nutrition on The Use of Zinc In Feeding Stuffs. European Commision (Ec); 2003.
6. European Food Safety Authority. Scientific Opinion on The Safety and Efficacy of Zinc Compounds (E6) as Feed Additives for All Animal Species (Zinc Acetate, Dihydrate; Zinc Chloride, Anhydrous; Zinc Oxide; Zinc Sulphate, Heptahydrate; Zinc Sulphate, Monohydrate; Zinc Chelate of Amino Acids, Hydrate; Zinc Chelate of Glycine, Hydrate), Based on A Dossier Submitted By Fefana asbl. 2015. Available From: Https://Www.Efsa.Europa.Eu/En/Efsajournal/Pub/4058
7. Finney Dj. Probit Analysis: A Statistical Treatment of The Sigmoid Response Curve. University Press; 1952. 346 P.
8. Oecd. Test No. 203: Fish, Acute Toxicity Test. Paris: Organisation for Economic Co-Operation and Development; 2019. Available From: Https://Www.Oecd-Ilibrary.Org/Environment/Test-No-203-Fish-Acute-Toxicity-Test_9789264069961-En
9. American Society for Testing and Materials International (ASTM International). Standard Guide for Conducting Acute Toxicity Tests on Test Materials With Fishes, Macroinvertebrates, and Amphibians. Available From: Https://Www.astm.Org/E0729-96r14.Html
10. Almeida Ja, Diniz Ys, Marques Sfg, Faine La, Ribas Bo, Burneiko Rc, Et Al. The Use of The Oxidative Stress Responses as Biomarkers In Nile Tilapia (Oreochromis Niloticus) Exposed To In Vivo Cadmium Contamination. Environ Int. 2002 Mar;27(8):673–9.
11. Garcia-Santos S, Fontaínhas-Fernandes A, Wilson Jm. Cadmium Tolerance In The Nile Tilapia (Oreochromis Niloticus) Following Acute Exposure: assessment of Some Ionoregulatory Parameters. Environ Toxicol. 2006 Feb;21(1):33–46.
12. Ayotunde Eo, ofem Bo. Acute and Chronic Toxicity of Pawpaw (Carica Papaya) Seed Powder To Adult Nile Tilapia (Oreochromis Niloticus Linne 1757). Afr J Biotechnol. 2008:7(13).
13. Firat O, Kargin F. Individual and Combined Effects of Heavy Metals on Serum Biochemistry of Nile Tilapia Oreochromis Niloticus. Arch Environ Contam Toxicol. 2010 Jan;58(1):151–7.
14. Taweel A, Shuhaimi-Othman M, Ahmad Ak. In Vivo Acute Toxicity Tests of Some Heavy Metals To Tilapia Fish (Oreochromis Niloticus). J Biol Sci. 2013;13(5):365–71.
15. Abdel-Khalek A Adel, Kadry M, Hamed A, Marie Ma. Ecotoxicological Impacts of Zinc Metal In Comparison To Its Nanoparticles In Nile Tilapia; Oreochromis Niloticus. J Basic Appl Zool. 2015 Oct 1;72:113–25.
16. Abdel-Tawwab M, El-Sayed Go, Shady Sh. Effects of Dietary Protein Levels and Environmental Zinc Exposure on The Growth, Feed Utilization, and Biochemical Variables of Nile Tilapia, Oreochromis Niloticus (L.). Toxicol Environ Chem. 2012 Aug 1;94(7):1368–82.

17. Abdel-Tawwab M, El-Sayed Go, Shady Sh. Growth, Biochemical Variables, and Zinc Bioaccumulation In Nile Tilapia, Oreochromis Niloticus (L.) as Affected By Water-Born Zinc Toxicity and Exposure Period. Int Aquat Res. 2016 Sep 1;8(3):197–206.
18. Ponzoni Rw, Hamzah A, Tan S, Kamaruzzaman N. Genetic Parameters and Response To Selection for Live Weight In The Gift Strain of Nile Tilapia (Oreochromis Niloticus). Aquaculture. 2005 Jun 30;247(1):203–10.
19. Ponzoni Rw, Khaw Hl, Yee Hy. Gift: The Story Since Leaving Iclarm (Now Known as The Worldfish Center): Socioeconomic, Access and Benefit Sharing and Dissemination aspects. Fridtjof Nanesn Institute; 2010. Available From: Https://Digitalarchive.Worldfishcenter.Org/Handle/20.500.12348/1294
20. Aldoghachi M, Rahman M, Yusoff I, Azirun M. Acute Toxicity and Bioaccumulation of Heavy Metals In Red Tilapia Fish. 2016 Apr 1;26:507–13.
21. Abdul Rahman S, Zambry H, Kamarzaman S, Jalal A. The Potential Role of Red Tilapia (Oreochromis Niloticus) Scales: Allergic Reaction Test In Mice. J Appl Pharm Sci. 2013 Oct 1;3:45–50.
22. Jeram S, Sintes Jmr, Halder M, Fentanes Jb, Sokull-Klüttgen B, Hutchinson Th. A Strategy To Reduce The Use of Fish In Acute Ecotoxicity Testing of New Chemical Substances Notified In The European Union. Regul Toxicol Pharmacol Rtp. 2005 Jul;42(2):218–24.
23. Rufli H, Springer Ta. Can We Reduce The Number of Fish In The Oecd Acute Toxicity Test? Environ Toxicol Chem. 2011 Apr;30(4):1006–11.
24. Halmi Mie, Rahim Mbha, Othman Ar. Estimation of Lc50 and Its Confidence Interval for The Effect of Ferrous Sulphate on Catla Catla. J Environ Microbiol Toxicol. 2018 Jul 31;6(1):21–3.
25. Jaafar Inm, Ahmad Sa, Yasid Na. Estimation of Lc50 and Its Confidence Interval for The Effect of Nano-Zero Valent Iron on The Freshwater Zooplankton Species Daphnia Magna. J Environ Microbiol Toxicol. 2018 Dec 31;6(2):25–8.
26. Schenker N, Gentleman Jf. on Judging The Significance of Differences By Examining The Overlap Between Confidence Intervals. Am Stat. 2001 Aug 1;55(3):182–6.
27. Grosell M, Nielsen C, Bianchini A. Sodium Turnover Rate Determines Sensitivity To Acute Copper and Silver Exposure In Freshwater Animals. Comp Biochem Physiol Toxicol Pharmacol Cbp. 2002 Sep;133(1–2):287–303.
28. Dung Lq, Cuong Nm, Huyen Nt, Cu Nd. Acute Toxicity Test To Determine The Effects of Copper, Zinc and Cyanide on Cobia (Rachycentron Canadum) Resources In North Vietnam. 2005;11:4.
29. Kamaruzzaman By, Akbar B, Jalal Kca, Shahbudin S. Accumulation of Metals In The Gills of Tilapia Fingerlings (Oreochromis Niloticus) From In Vitro Toxicology Study. J Fish Aquat Sci. 2010;5(6):503–9.
30. Abdel-Tawwab M, El-Sayed Go, Shady Shhh. Acute Toxicity of Water-Born Zinc In Nile Tilapia, Oreochromis Niloticus (L.) Fingerlings. Better Sci Better Fish Better Life Proc Ninth Int Symp Tilapia Aquac Shanghai China 22-24 April 2011. 2011;39–44.
31. United States Environmental Protection Agency. Methods for Measuring The Acute Toxicity of Effluents and Receiving Waters To Freshwater and Marine Organisms. Washington, Dc: Usepa; 2002. Report No.: Epa-821-R-02-012.
32. Ezemonye Lin, Ogeleka Df, Okieimen Fe. Lethal Toxicity of Industrial Chemicals To Early Life Stages of Tilapia Guineensis. J Hazard Mater. 2008 Aug 30;157(1):64–8.
33. Ebrahimpour M, Alipour H, Rakhshah S. Influence of Water Hardness on Acute Toxicity of Copper and Zinc on Fish. Toxicol Ind Health. 2010 Jul 1;26(6):361–5.
34. Abdel-Warith, A A, Younis, M E, Al-asgah, A N, Et Al. Effect of Zinc Toxicity on Liver Histology of Nile Tilapia, Oreochromis Niloticus. Sci Res Essays. 2011 Aug 26;6(17):3760–9.
35. Ezeonyejiaku C, Obiakor M. Toxicological Study of Single Action of Zinc on Tilapia Species (Oreochromis Niloticus). online J Anim Feed Res. 2011 Apr 7;1:139–43.