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RESEARCH ARTICLE

ESTABLISHING WATER QUALITY THRESHOLDS FOR SODIUM HYPOCHLORITE: ACUTE TOXICITY ASSESSMENT IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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ABSTRACT

Sodium hypochlorite (NaOCl) is widely applied as a disinfectant, yet its discharge into freshwater systems poses a growing water quality concern. This study assessed the acute toxicity of NaOCl to juvenile Nile tilapia *Oreochromis niloticus* to establish water quality thresholds for chlorine management in tropical freshwater aquatic environments. Fish were exposed to graded NaOCl concentrations (1.40–1.60 ppm) for 96 hours under controlled experimental conditions. Probit analysis resulted a steep concentration–response relationship, with a 96-hour LC_{50} of 1.55 ppm (95% CI: 1.52–1.58 ppm), LC_{10} of 1.42 ppm, and LC_{90} of 1.62 ppm. The narrow margin between these thresholds suggests that small increases in chlorine concentration can rapidly increase ecological risk. These findings provide critical baseline data for establishing chlorine discharge limits and suggest the need for improved monitoring of chlorinated effluents to protect freshwater resources in tropical environments.

KEYWORDS

Acute Toxicity, Aquaculture, Aquatic Pollution, Chlorine Disinfectants, Median Lethal Concentration, Probit Analysis.

1. INTRODUCTION

Human activities have exerted increasing pressure on freshwater ecosystems, with chemical pollution becoming a very important concern for water quality and aquatic resource management. This pressure has increased in recent years following the increased use of disinfectants in domestic, agricultural, healthcare and industrial activities. During and after the global health crisis in the early 2020s, the use of household and industrial disinfectants increased markedly worldwide, with reported increases of approximately 30–40% (Zheng, et al., 2020; Guo et al., 2021). A wide range of disinfecting agents is currently applied in industrial and domestic purposes, such as sodium hypochlorite NaOCl, ethanol, hydrogen peroxide, isopropanol, quaternary ammonium compounds, glutaraldehyde and phenolic derivatives (Artasensi et al., 2021). Among these, NaOCl is one of the widely used chlorine-based disinfectants due to its strong antimicrobial effects, low cost, and broad application in water and wastewater treatment, dairy production, food processing, agriculture, glass industries, paint manufacturing and dental practices (Affrald, 2022; Cai et al., 2023).

Although chlorine-based disinfectants are useful for controlling microbial contamination, their widespread application also has implications for freshwater resource and water quality management. Chlorine-containing compounds may enter aquatic ecosystems through multiplel pathways, including treated and inadequately treated wastewater, industrial effluent, surface runoff, urban drainage and accidental or inappropriate discharge of disinfectant-containing wastes. These pathways are very important in developing tropical regions, where industrial development and rapid urbanization may occur alongside inadequate wastewater collection and treatment infrastructure. In many developing countries, wastewater treatment systems are either insufficient or operating beyond capacity, which allows chlorinated effluents to be discharged directly into freshwater bodies including rivers with minimal treatment (Owhonka et al., 2021; Oladimeji et al., 2024). Urban runoff can provide an additional pathway by transporting chemical compounds from households, commercial facilities, and other urban surfaces into freshwater environments, particularly during intense rainfall events. Consequently, chlorine contamination needs to be considered not only a toxicological issue but also a water-quality management challenge (Li et al., 2022; Valentukeviciene et al., 2024).

Once entered water, NaOCL undergoes rapid breakdown and forms hypochlorous acid (HOCl) and hypochlorite ions (OCl^-), collectively called as free available chlorine. The relative concentration of these species strongly depends on aquatic environment, particularly pH and temperature. These reactive chlorine species are highly oxidative and may

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interact readily with many biological macromolecules (Tang et al., 2022). Although they tend to breakdown relatively quickly under natural conditions such as sunlight exposure, their short-term persistence is sufficient to cause acute toxic effects in aquatic organisms immediately following discharge events. Evidence from previous research indicates that reactive chlorine species may induce oxidative stress in many fish by impairing antioxidant defense systems, including key enzymes particularly superoxide dismutase (SOD) and catalase (CAT) (Ma et al., 2025). Furthermore, exposure can lead to structural damage in gill tissues, characterized by excessive mucus production, epithelial disruption and compromised respiratory efficiency. Such biological consequences provide important evidence for evaluating the potential ecological effects of chlorine contamination and may complement conventional chemical monitoring of residual chlorine in receiving aquatic environments.

Although chlorine-based disinfectants are widely applied, scientific information on their acute toxicity to freshwater biota, particularly tropical fish, remains limited. For instance, observed effects of NaOCL exposure on changes swimming behaviour in zebrafish (*Danio rerio*) (Ren et al., 2022). As a study reported the physiological changes of chloramine-T and its degradation products, including NaOCL, on acid–base regulation and ion transport in rainbow trout (*Oncorhynchus mykiss*) (Powell and Perry, 1998). Earlier, Powell and Perry (1996) observed respiratory and acid–base disturbances in rainbow trout following exposure to hypochlorite, chloramine-T and associated compounds. However, more recent research suggest that tropical fish species respond differently to chemical stressors compared to temperate species due to physiological and metabolic variations (Zhang et al., 2025). Therefore, toxicity information generated from temperate species may not represent the sensitivity of fish in tropical freshwater ecosystems.

Nile tilapia (*Oreochromis niloticus*) is particularly relevant for evaluating chemical stress in tropical freshwater environments because this fish is widely distributed and extensively cultured in subtropical and tropical regions (El-Sayed and Fitzsimmons, 2023; Sriprateep et al., 2025). The fish is also often associated with freshwater environments influenced by agricultural, urban and domestic activities. In developing countries, aquaculture ponds and other freshwater environments may depend indirectly or directly on surface-water resources, increasing the potential for exposure to contaminants transported through stormwater runoff and wastewater discharge. During heavy rainfall, agricultural and urban runoff may carry accumulated chemical residues into rivers, lakes, ponds and other receiving aquatic environments (Kaown et al., 2024; Jose, 2026). Therefore, understanding the sensitivity of Nile tilapia to chlorine exposure my provide important information for assessing the potential ecological changes of chlorine contamination in tropical freshwater environments.

From a water-resource management point of view, the widespread application of chlorine-based disinfectants has important implications for water quality management, particularly in regions where wastewater treatment facility is insufficient (Parveen et al., 2022; Yi et al., 2024). Understanding the toxicity of aquatic biota is crucial for developing evidence-based discharge limits, water quality management and environmental monitoring activities. Biological toxicity thresholds may provide complementary information to chemical measurements by suggesting concentrations at which residual chlorine can pose a direct risk to aquatic biota (Li, 2021; Vannoni et al., 2024). Such information is particularly very important for environmental risk analysis and for establishing appropriate controls on industrial discharges and wastewater into freshwater systems.

Therefore, the present study evaluated the acute toxicity of NaOCL to juvenile Nile tilapia. In particular, the 96-hour median lethal concentration (LC_{50}) was observed as an indicator of the sensitivity of this tropical freshwater fish to chlorine exposure. The LC_{50} is widely applied in ecotoxicology to characterize the acute toxicity of chemical stressors and to generate toxicity thresholds that may contribute to environmental risk assessment (Sharmin et al., 2021; Hossain et al., 2022). By establishing an acute toxicity threshold for NaOCl in juvenile tilapia, this study provides important baseline information that can support pollution-risk assessment, water-quality monitoring and the development of evidence-based strategies for managing chlorine-containing discharges in tropical freshwater ecosystems. In subtropical and tropical regions, many aquaculture activities and natural water bodies receive intermittent inputs of chlorinated water from industrial, municipal or domestic sources. Concentrations used in this study (1.40–1.60 ppm) are within the range that can occur in poorly treated effluents or following shock chlorination events. The rapid breakdown of chlorine under sunlight and organic matter load may lead to fluctuating exposure patterns, making acute toxicity information crucial for risk assessment.

2. Materials and Methods

2.1 Collection of Test Organisms and Their Acclimatization

The experiment was conducted using juvenile Nile tilapia at the Wet Laboratory of the Department of Marine Science, Kulliyyah (Faculty) of Science, IIUM (International Islamic University Malaysia). A total of 200 juveniles were collected from a commercial hatchery in Pahang, Malaysia, with an average individual body weight of 22.5 g and an average individual total length of 3.8 cm. Upon arrival, the fish were acclimated for 14 days in 200-L fiberglass tanks containing dechlorinated tap water. The tank water was aerated continuously. The water temperature was maintained between 26 and 30 °C and photoperiod was controlled as 12 h light and 12 h dark. Dissolved oxygen (DO) concentration were consistently kept above 6.0 mg L^{-1}.

2.2 Experimental Design and Exposure Procedure

The experiment was carried out using 18 glass aquaria (each with a capacity of 40 L), containing 30 L of test solution. Six experimental treatments were executed in triplicate, comprising five exposure concentrations (1.40, 1.45, 1.50, 1.55, and 1.60 ppm NaOCl) and a control group with dechlorinated water free of NaOCl. Commercial NaOCL was applied as a test substance. A stock solution was first prepared in distilled water and then diluted for the desired concentrations. Ten randomly selected juvenile tilapia were stocked into each aquarium. Fish were fed a commercial pellet feed of 30% crude protein twice daily at 3% of their body weight. Feeding was stopped 24 h prior to the initiation of the toxicity test. A preliminary range-finding test (0.5–2.0 ppm) was performed to establish the appropriate concentration range for the definitive experiment.

A static-renewal exposure system was applied to account for the instability of chlorine compounds in aqueous media. At 24-hour intervals, approximately 50% of the test solution was replaced with freshly prepared solution at the respective concentrations. The exposure duration continued for a 96 h period.

2.3 Water Quality Monitoring

Water quality parameters were measured every day using a calibrated multiparameter probe. Water temperature was maintained between 26 and 30 °C, while pH remained stable at approximately 7.0. Dissolved oxygen concentrations varied from 5.0 to 7.0 mg L^{-1}.

2.4 Mortality Assessment, Statistical Analysis and Ethical Compliance

Fish mortality was observed at 12, 24, 48, 72, and 96 h following starting of the experiment. Fish were classified as dead when movement of opercular had ceased and no response to mechanical stimulation. Dead fish were immediately removed from the aquaria. The median lethal concentration (LC_{50}) and corresponding 95% confidence intervals were estimated using Probit analysis in SPSS (version 26.0). Probit analysis was applied because mortality was expressed as a binary response (alive or dead) and the objective was to estimate the NaOCl concentrations associated with 10%, 50%, and 90% mortality (LC_{10}, LC_{50}, and LC_{90}, respectively). The analysis was carried out using the mortality responses observed at the different nominal NaOCl concentrations after 96 h of exposure. All experimental procedures were carried out in strict accordance with the animal ethics guidelines of the International Islamic University Malaysia (IIUM) for toxicity testing in fish. The study complied with institutional standards for the use and care of aquatic organisms, and all efforts were performed to minimize stress and ensure humane treatment throughout the study.

3. Results and Discussion

3.1 Water Quality Parameters

The measured physicochemical parameters remained relatively stable within acceptable limits throughout the 96-h exposure period. Water temperature ranged from 26.2 to 26.5 °C, dissolved oxygen from 5.8 to 6.2 mgL^{-1}, and pH from 7.1 to 7.2, with no statistically significant variations ($p > 0.05$) between the control and NaOCl treatment groups (Table 1). These parameters remained within suitable ranges for warm-water fish (Rahman et al., 2008a, b; Rahman et al., 2009), indicating that the experimental conditions were suitable for the bioassay. The absence of mortality in the control group further supports the suitability and stability of the experimental conditions.

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Table 1: Physicochemical parameters (mean ± standard deviation) of test water during the 96-hour acute toxicity test		
Parameter	**Control (0 ppm)**	**Treatment (1.40-1.60 ppm)**
Temperature (°C)	26.2 ± 0.4	26.5 ± 0.6
pH	7.1 ± 0.2	7.2 ± 0.3
Dissolved oxygen (mg L^{-1})	6.2 ± 0.5	5.8 ± 0.4

In this study, NaOCl concentrations of 0, 1.40, 1.45, 1.50, 1.55, and 1.60 ppm were applied to assess the ecotoxicological responses of juvenile Nile tilapia while key physicochemical parameters were simultaneously evaluated. The stable water-quality parameters and absence of significant variations among treatments indicate that the concentration-dependent mortality observed in the exposed groups was primarily associated with NaOCl exposure rather than substantial variations in temperature, pH, or dissolved oxygen. Therefore, the observed biological responses provide important evidence of the potential ecological effects of chlorine contamination on juvenile Nile tilapia.

3.2 Mortality Dynamics and Median Lethal Concentration (LC50)

No mortality was recorded in the control treatment throughout the 96-h exposure period, whereas mortality in the NaOCl-treated groups increased progressively with increasing NaOCl concentration (Table 2), indicating a clear concentration–response relationship. At the lowest concentration of 1.40 ppm, mortality was the lowest, with 70% survival at the end of the exposure period. With increasing NaOCl concentration, mortality became increasingly pronounced. At 1.50 ppm, mortality increased progressively between 48 and 72 h of exposure, whereas the highest NaOCl concentration of 1.60 ppm produced the most rapid toxic response, with a substantial proportion of mortality occurring within the first 36 h. Alomost similar concentration-dependent increase in mortality was observed by Akpang et al. (2017) in African catfish (*Clarias gariepinus*) fingerlings exposed to NaOCl. However, responses of toxicity may vary among species and developmental stages because of variations in water chemistry, exposure conditions, and chlorine speciation (Ullah et al., 2015; Andem et al., 2016). Therefore, comparisons among studies need to be interpreted cautiously.

Table 2: Mortality (%) of juvenile tilapia exposed to various cconcentrations of NaOCL (ppm) during the 96-hour acute toxicity test	
Concentration of NaOCL (ppm)	**Mortality (%) of juvenile tilapia**
0.00	0
1.40	30
1.45	40
1.50	40
1.55	50
1.60	60

Probit analysis further confirmed the strong concentration–response relationship, with the regression equation expressed as probit(p) = −5.77 + 3.73C, where p indicates the probability of mortality and C is the NaOCl concentration. The model indicated an excellent fit (R^2 = 0.98), indicating a highly consistent relationship between NaOCl concentration and mortality in juvenile fish. The observed 96-h LC_{10}, LC_{50}, and LC_{90} values were 1.42, 1.55, and 1.62 ppm, respectively, with a relatively narrow 95% confidence interval for the LC_{50} (1.52–1.58 ppm) (Figure 1). The mortality curve indicated a pronounced increase between 1.50 and 1.60 ppm, showing a steep concentration–response relationship. Thus, a relatively small increase of only 0.10 ppm in NaOCl concentration was related to a significant increase in mortality. This finding is particularly relevant to the management of chlorine-containing discharges because relatively small diferrences in residual chlorine concentrations may result in significantly different biological responses.

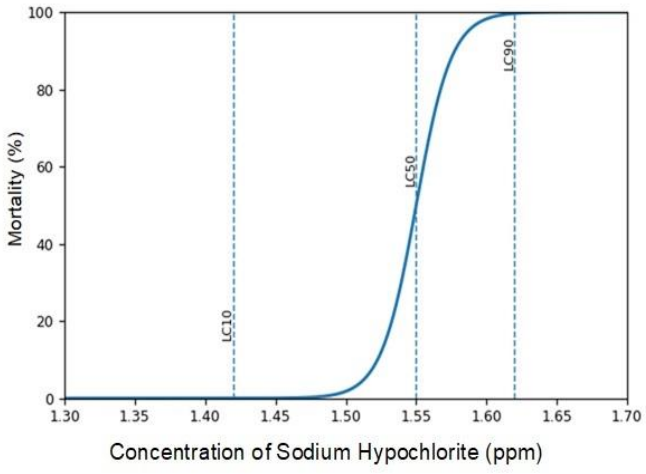


Figure 1: Regression line of NaOCL (ppm) vs. mortality (%) of juvenile tilapia.

The 96-h LC_{50} of 1.55 ppm obseved in the present study provides baseline ecotoxicological information on the sensitivity of juvenile tilapia to NaOCl under the tested conditions. Direct comparison with published studies is limited because relatively few studies have reported comparable LC_{50} values for juvenile Nile tilapia. Nevertheless, the present value is largely consistent with the 1.54 ppm LC_{50} reported by Akpang et al. (2017) for African catfish fingerlings. The relatively close LC_{50} values between these two species indicate that NaOCl may pose a substantial toxicological risk to freshwater fish at similar exposure concentrations, although species-specific variations should be considered when interpreting such comparisons.

We acknowledge that NaOCl is unstable in aqueous systems and that the actual residual chlorine concentration may decrease during the 96-h exposure period because of degradation and reactions with other constituents in the test water. Therefore, the observed LC_{10}, LC_{50}, and LC_{90} values should be explained as toxicity estimates based on nominal NaOCl concentrations under the experimental conditions. However, the progressive mortality observed in the present study suggests that inadequate control of residual chlorine could negatively affect fish survival and potentially affect aquatic community structure. Although chlorine-based compounds are widely applied for the treatment of water and wastewater, their environmental risks should be considered alongside their treatment benefits. Proper management should therefore include appropriate control of disinfectant dosing, adequate treatment or dechlorination of chlorine-containing effluents before discharge, and regular monitoring of residual chlorine in receiving waters. The LC_{10}, LC_{50}, and LC_{90} values observed in this study can serve as important biological reference points for environmental risk evaluation and for informing the development or refinement of protective water-quality criteria. These measures are important for reducing chlorine-based ecological risks and protecting the biological integrity and long-term sustainability of freshwater environments. However, the LC_{50} and related toxicity estimates should be considered baseline ecotoxicological information and biological reference points that can contribute to environmental risk assessment, rather than proposed regulatory standards or water-quality limits. Additional information, including species-specific sensitivity to NaOCl, is required to support the development of appropriate water-quality criteria.

3.3 Implications for Water Quality Management

The narrow LC_{10}–LC_{90} range (1.42–1.62 ppm) indicates that chlorine concentrations within the range of potential industrial and municipal discharge changes can cause significant ecological impacts. This finding

has direct implications for water quality monitoring programs, as it suggests that:

I. Regular chlorine monitoring in receiving waters should target concentrations well below 1.42 ppm to prevent acute toxicity.

II. Discharge permits for chlorine-based effluents should incorporate safety margins based on observed toxicity data.

III. Real-time monitoring of residual chlorine is important in areas with intermittent discharge events.

The results support the application of the USEPA's water quality criteria development framework (USEPA, 2002), indicating that surrounding chlorine concentrations should remain below the LC_{10} threshold to protect aquatic life.

4. Conclusion

The 96-hour LC_{50} of 1.55 ppm NaOCl for juvenile tilapia provides useful evidence for establishing protective water quality thresholds. The steep concentration–response relationship, with an LC_{10} of only 1.42 ppm, suggests that chlorine discharge events exceeding this concentration can pose acute risks to freshwater biota. These findings suggest the implementation of proper monitoring and discharge controls for chlorine-containing effluents. Key recommendations include (i) implementing dechlorination before discharging to remove residual chlorine and minimize its potential toxicity to aquatic biota and freshwater environments, (ii) incorporating available toxicity data into local water quality guidelines, (iii) implementing continuous or regular monitoring of residual chlorine concentrations in receiving waters, and (iv) exploring alternative disinfection methods or treatment technologies particularly using UV, ozone, peracetic acid to minimize chlorine discharge, and (v) developing early warning systems to detect elevated chlorine concentrations, thereby reducing risks to aquatic biota. Overall, this study provides to the growing body of evidence that chlorine contamination warrants active management to safeguard freshwater environments, particularly in tropical regions where fisheries and aquaculture are important for food security.

Declarations

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Conflict Of Interest Statement

All authors declare that they have no competing interests.

Author Contributions

Study design, preparation of materials, manuscript drafting were carried out by Kamaruzzaman Yunus. Mohammad Mustafizur Rahman was responsible for data analysis, critical review, and manuscript editing. Nur Najihah Farhana Junaidan, Siti Waznah Abdurahman, Mohd Masrol Masduki and Asnor Azrin Sabuti contributed to data curation. Ong Meng Chuan, Defri Yona, and Siripon Pradit contributed to the review of the manuscript.

Data Availability Statement

Data of this study are available upon responsible request.

Informed Consent

Informed consent was not required for this study because it did not involve human participants or identifiable personal data.

Artificial Intelligence (AI) Use

The authors declare that no artificial intelligence (AI) tools were used in the preparation of this manuscript.

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