Journal of Agricultural Science and Technology A 15 (2025) 31-50
doi: 10.17265/2161-6256/2025.01.004

Evaluation of Effects of Copper Sulphate and Ferrocene on Nile Tilapia *Oreochromis niloticus* (L.) Fingerlings

Oriaku Ugonma Isdore-Rufus and Chioma Nwakanma

Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike 440101, Nigeria

Abstract: This study was carried out to evaluate the response of Nile tilapia (*Oreochromis niloticus*) fingerlings to acute copper sulphate and ferrocene toxicity. Nile tilapia fingerlings weighing 2.3 ± 0.2 g were acclimated and randomly distributed at a rate of 10 fish per 30 L aquarium. Fish were exposed to a range of copper sulphate and ferrocene concentrations of 4 mg/L, 8 mg/L, 12 mg/L, 16 mg/L and 2.5 mg/L, 5 mg/L, 7.5 mg/L, 10 mg/L respectively. Fish not exposed to any toxicant served as control. Mortality was assessed and median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were calculated. The 96-h LC_{50} values obtained for copper sulphate and ferrocene were 7.49 mg/L (confidence interval CI: 6.35 to 8.57 mg/L) and 3.55 mg/L (CI: 0.98 to 5.17 mg/L) respectively. The LC_{50} decreased with time of exposure implying that toxicity increased over time, however, LT_{50} decreased as concentration increased. The safe concentration for copper sulphate derived was 1.913 mg/L and 1.196 mg/L for ferrocene. Histological analyses were carried out on fish gills and skin. The skin histomorphology showed marked and widespread epidermal loss and widespread mycocytic degeneration in treatments with high concentration for both toxicants. The gill morphology showed moderate to severe hyperplasia of the primary gill epithelia leading to partial or complete loss of the secondary lamellae.

Key words: Acute toxicity test, *Oreochromis niloticus*, copper sulphate, ferrocene, median lethal concentration (LC_{50}), median lethal time (LT_{50}).

1. Introduction

Organometallic compounds are defined as “any member of a class of substances containing at least one metal-to-carbon bond in which the carbon part is an organic group” [1]. Organometallic compounds are persistent—they do not easily decompose but are highly toxic than their elemental form. Increasing concerns for organometallic compounds are as a result of the fact that once they accumulate in different environmental compartment, they can inhibit their functioning and consequently affect the ecosystem, plants and other living organisms and also contaminate the food chain—including that of humans [2]. One of such compounds that fall within this class of organometallic compounds is ferrocene $Fe(C_5H_5)_2$, it is produced from the reaction of cyclopentadienyl with reduced iron in the presence of metal oxides [3]. Due to its use in various industries which include its use as a catalyst for vulcanization, acceleration and polymerization; as anti-knock additive for gasoline; as a coating for missiles and satellites and as a high temperature lubricant, it may find its way into aquatic ecosystems. Its effect on fish can potentially lead to bioaccumulation affecting the aquatic food web [4].

Copper (Cu) is an essential trace metal that is important for the growth and survival of aquatic organisms including fish like the Nile tilapia. It occurs naturally and is used for diverse purposes. It has been used in antifouling paints for centuries because it is effective, available and relatively inexpensive [5]. It is also used for copper-based pesticides, and industrial purposes. Copper can be released into aquatic environments through various human activities, including industrial processes, mining and agricultural runoff. Although copper (Cu) is essential, it is required

Corresponding author: Oriaku Ugonma Isdore-Rufus, M.Sc. in view, research fields: environmental management and toxicology.

in small amounts, elevated copper levels can be toxic to aquatic organisms. It is easily absorbed by the gills and readily stored in the fish affecting their physiology and behaviour [6-9], it can impart lethal effects in tissues that may affect fish growth and development and eventually lead to death [5]. Nile tilapia *O. niloticus*, is a widely cultured freshwater fish species. Due to its sensitivity to many variables in their environment, they play a significant role in the assessment of water quality [10] as a good biological model for toxicological studies.

Copper sulphate and ferrocene are two distinct compounds with significant industrial and academic importance. Their toxicity especially to aquatic life poses serious threat to the aquatic ecosystem and humans at large. Hence, the aim of this study is to evaluate the response of Nile Tilapia, *O. niloticus* to acute copper sulphate and ferrocene toxicity, with the following objectives:

(1) To determine median lethal concentration (LC_{50}) of copper sulphate and ferrocene for *O. niloticus.*

(2) To determine the median lethal time (LT_{50}) of the copper sulphate and ferrocene for *O. niloticus.*

(3) To assess the histological changes in organs of the test specimen treated with ferrocene and copper sulphate.

(4) To detect the safe levels and the threshold values of the copper sulphate and ferrocene.

This study will help to assess potential environmental impacts of copper sulphate and ferrocene as they are often used in various industrial processes and can find their way into aquatic ecosystems. Nile tilapia is a common aquaculture species, and a vital source of protein for many communities, so investigating the impacts of copper and ferrocene on this fish is crucial for ensuring food safety and security. Findings from this research work will inform environmental regulations and guide industries in minimizing their impact on aquatic ecosystems and safeguarding both the environment and human health.

Copper sulphate and ferrocene are common pollutants in ecosystems due to industrial activities, understanding how they affect Nile tilapia, *O. niloticus* will help assess the potential environmental impacts and develop strategies for pollution control. Since it is widely farmed for human consumption, this research is crucial for aquaculture practices as it will help to establish safe levels for copper sulphate and ferrocene exposure to ensure the health and safety of farmed Nile tilapia. This research work will also contribute to our understanding of potential risks to human health as accumulation of copper sulphate and ferrocene in fish tissues may pose health risks to humans through the consumption of contaminated fish. *O. niloticus* is a key species in many aquatic ecosystems; this study will provide insight into potential disruptions of the ecological balance, including effects on other species and the overall health of the ecosystem.

The findings from this study will inform environmental regulations and guidelines regarding copper sulphate and ferrocene discharge limits contributing to the development of policies that aim to protect aquatic ecosystems and aquatic life. Due to its behavioural response to light, it feeds during the daytime. Juveniles and fingerlings are omnivores, feeding mainly on zooplankton and zoobenthos, while the adults are herbivores that thrive on natural food organisms—plankton, some aquatic macrophytes, planktonic and benthic aquatic invertebrates [11]. When these species are introduced outside its native range, they often become invasive. They are known for their ability to survive in a range of water conditions, as they are hardy and resilient. Their tolerance for a wide range of water temperatures and quality make them less prone to diseases and environmental stress [12].

2. Methodology

2.1 Experimental Set Up

The test organisms were exposed to ten different treatments carried out in two replicates including a control which was given separate treatment, for both copper and ferrocene. A total of eighteen (18) aquaria were used for various treatments in the acute toxicity test.

2.2 Test Organism: Description

The test organism *O. niloticus* fingerling is a tilapia species of the Cichlid family. Its common name is Nile tilapia. It is a tropical species that lives in shallow water. They can be found in most type of fresh water habitats like lakes, canals, creeks, rivers and ponds, they can also be found in artificial enclosures as culturable species. It also occurs in brackish water, but is unable to survive long in full salt water. It has a deep-bodied, compressed shape. The most diagnostic features are the regular and definitive stripes on the truncated claudal fin, the red flush of the breeding male and dark margin of the dorsal fin [13]. It also has a disconnected lateral line and can live for more than 10 years.

2.2.1 Collection and Acclimation

The test fish (*O. niloticus*) were purchased from the Michael Okpara University of Agriculture, Umudike, Nigeria (MOUAU) Fish Farm and transported to the Fisheries and Aquatic Resources Management Laboratory of the same institute (MOUAU), where the experiments were carried out. They were acclimated in laboratory conditions for one week before experimentation. The media were renewed every two days until the end of the acclimation. The specimen were fed twice daily with fish feed (coppen) at 3% of their body weight.

2.3 Test Materials

The test materials that were used for the experiment were copper sulphate and ferrocene and they were obtained from Etchukson Scientific supplier Enterprise Nig.

2.4 Toxicity Test

2.4.1 Range-Finding Test

Before the actual biological assay, a range-finding test was carried out, using both test materials separately, The test organisms were subjected to five different concentrations, 5 mg/L, 6 mg/L, 7 mg/L, 8 mg/L, 9 mg/L and a control for ferrocene and 2 mg/L, 4 mg/L, 8 mg/L, 16 mg/L, 32 mg/L and a control for copper sulphate and this was done for 24 h using the static bioassay method. The concentration with fifty percent mortality was used for the serial dilution [14].

2.4.2 Preparation of Stock Solution

The test solution was prepared by dissolving copper sulphate in distilled water. Four concentrations of 4 mg/L, 8 mg/L, 12 mg/L, and 16 mg/L were obtained. For ferrocene, four concentrations 2.5 mg/L, 5 mg/L, 7.5 mg/L and 10 mg/L were also obtained following a geometric series and used for the bioassay following [15].

2.4.3 Preparation of the Bioassay Media

Eighteen (18) aquaria of 30-L capacity were used for the bioassay. Each chamber was prepared from the stock solution to the required concentrations of each treatment respectively and having two replicates and a control. Water used for the test solutions and controls was natural tap water aerated for 48 h to get rid of chlorine. Ten (10) of the experimental fish were randomly selected having the same range of weight and length (2.3 g to 2.5 g and 4.7 cm to 6.7 cm respectively) and carefully placed in each of the aquaria. They were then monitored for the 96-h period.

2.4.4 Bioassay Procedure

The 96-h static bioassays were carried out in the laboratory according to the methods of [16]. Acclimated fish were not fed for a day (24 h) before the start of the experiments until the end of the 96-h experimental period. The controls were not treated with any toxicant. The bioassay lasted for 96 h (four days), during which the test organisms were observed for mortality, general conditions and behavioural changes and these were recorded. They were observed 12-hourly at first, and then 24-hourly to assess mortality until the end of the experiment.

2.4.5 Observation and Assessment of Response (Mortality)

Test organisms were confirmed dead when they floated in the test solution with ventral sides upwards and no motion when gently prodded with a rod and no opercula (gill) movement observed. The dead ones were removed immediately from the test solution. Using the

mortality chart, survival was deduced for the various sets of concentrations of copper sulphate and ferrocene respectively, and then analysed for median lethal concentration (LC_{50}) and median lethal time (LT_{50}).

2.5 Biological Parameters

The lengths of the test fish were determined using a metre rule on a measuring board calibrated in centimeters (cm). A sensitive weigh balance, Model AR2140 was used to weigh them in grammes (g). Other parameters including body movement, buoyancy, period of quiescence, and mortality were also observed visually.

2.6 Physico-Chemical Parameters

The test solution was analyzed daily for temperature, hydrogen ion concentration (pH), dissolved oxygen (DO), total hardness (TH), and total alkalinity (TA) using standard methods.

2.7 Histological Studies

At the end of the treatments, fish samples from each group of bioassay and control were collected and dissected; gills and skin sections were then extracted and fixed in 10% formalin for 24 h and taken for histological analyses.

2.8 Statistical Analysis

Data obtained were represented as means and standard deviation. The significance of difference was tested with analysis of variance (ANOVA). To obtain the dose-response (mortality), data of the acute toxicity test of the toxicants against the test organisms were analysed using probit analysis method [17] to calculate the median lethal concentration LC_{50} with the aid of the Statistical Package for the Social Sciences (SPSS) statistical software (version 25). The median lethal time LT_{50} for each of a series of concentrations was calculated. A toxicity curve was drawn by plotting median survival times against concentrations on logarithmic paper; this was to reveal any unusual features of toxicity. This would help to obtain a lethal threshold concentration [18].

3. Result

3.1 Acute Toxicity Result for O. niloticus Treated with Copper Sulphate

3.1.1 Physicochemical Parameters for *O. niloticus* Treated with Copper Sulphate

The values obtained for the physicochemical parameters for *O. niloticus* exposed to copper sulphate in the 96-h bioassay are presented in Table 1. DO range of 4.61-4.65 was obtained from the test aquaria, this varied from the control which had a mean value of 4.88 ± 0.11. The aquarium with the highest concentration of 16 mg/L had the lowest DO value with a mean of 4.75 ± 0.04 mg/L, while the aquarium having a concentration of 12 mg/L had the highest DO value.

For the pH, a range of 6.71 to 6.80 was obtained from the test aquaria. There was a slight variation from the control which had a mean value of 6.93 ± 0.06. The highest pH value was observed in the concentration of 12 mg/L with a mean value of 6.80 ± 0.01 and the lowest pH value in the aquarium with the highest concentration of 16 mg/L with a mean value of 6.71 ± 0.08. There was a very slight difference in temperature. The lowest value 25.88 ± 0.01 ℃ was observed in the aquaria with concentrations of 4 mg/L and 8 mg/L. The highest value was in the 12 mg/L concentration with a mean value of 28.8 ± 0.04 ℃. Total alkalinity had variations as well; the highest mean value of 58.48 ± 0.01 was seen in the aquarium with the lowest concentration (4 mg/L) with the lowest mean value of 58.17 ± 0.01 being the control. Total hardness had variations as well; the highest mean value of 117.33 ± 0.08 was seen in the control; the aquarium with a concentration of 12 mg/L had the lowest mean value of 116.49 ± 0.12 mg/L.

3.1.2 Mortality

The data on mortality of *O. niloticus* exposed to copper sulphate is represented in Figs. 1-5. Fig. 1 shows the effect of copper sulphate on fish mortality over 24 h.

Table 1 Mean and standard deviation of physico-chemical properties of copper sulphate solution for *O. niloticus.*

Concentration	DO (mg/L)	pH	TH (mg/L)	Temp. (℃)	TA (mg/L)
Control	4.88 ±0.11	6.93 ±0.06	117.33 ±0.08	28.83 ±0.05	58.17 ±0.01
4 mg/L	4.65 ±0.05	6.77 ±0.11	117.14 ±0.22	28.88 ±0.01	58.48 ±0.01
8 mg/L	4.66 ±0.08	6.8 ±0.09	117.17 ±0.81	28.88 ±0.01	58.42 ±0.01
12 mg/L	4.75 ±0.04	6.8 ±0.01	116.49 ±0.12	28.81 ±0.03	58.33 ±0.01
16 mg/L	4.61 ±0.07	6.71 ±0.08	116.80 ±0.83	28.83 ±0.04	58.46 ±0.01

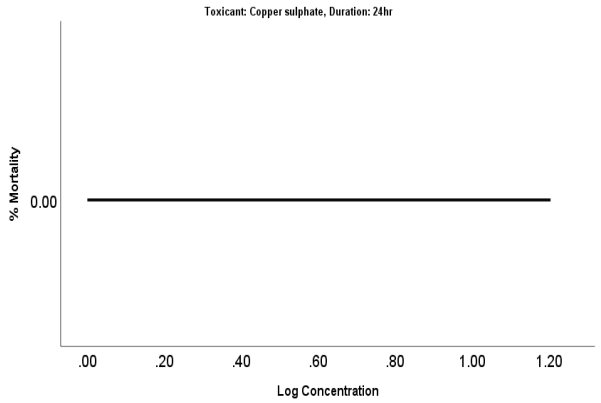

Fig. 1 Plot of percent mortality against log concentration for *O. niloticus* exposed to copper sulphate after 24 h.

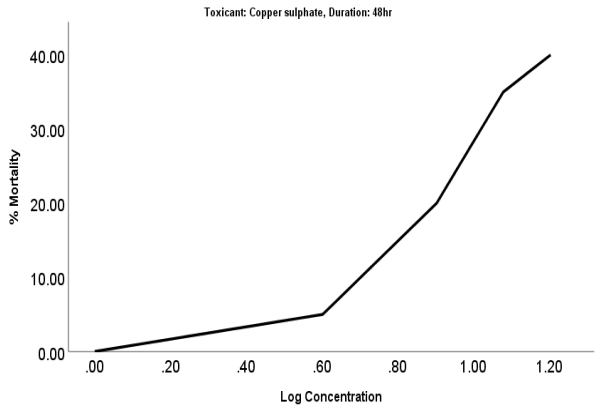

Fig. 2 Plot of percent mortality against log concentration for *O. niloticus* exposed to copper sulphate after 48 h.

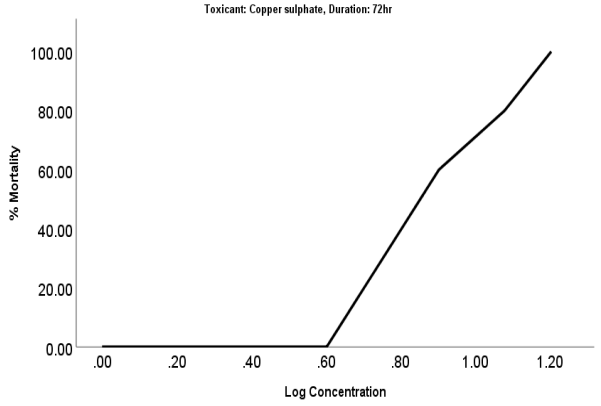

Fig. 3 Plot of percent mortality against log concentration for *O. niloticus* exposed to copper sulphate after 72 h.

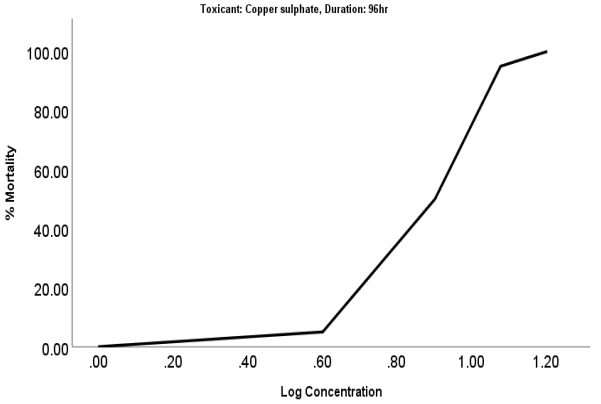

Fig. 4 Plot of percent mortality against log concentration for *O. niloticus* exposed to copper sulphate after 96 h.

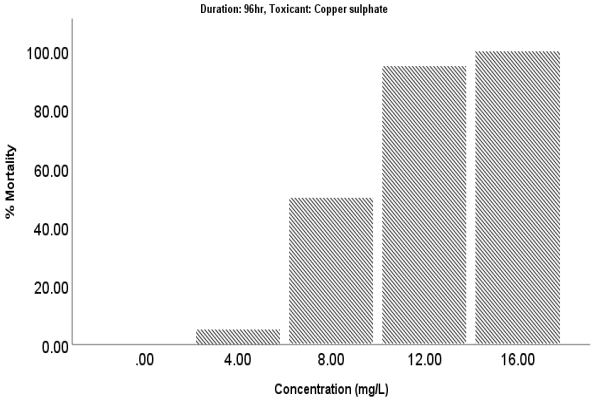

Fig. 5 Plot of percent mortality against various concentrations of copper sulphate after 96 h.

Across the range of concentrations, no mortality was observed, indicating that within this concentration range, copper sulphate did not cause any lethal effects within the 24-h duration. In Fig. 2 after 48 h as concentration increased, the mortality percentage increased, reaching up to 40%. This indicates that higher concentrations of copper sulphate led to increased mortality rates within the 48-h duration. Figs. 3 and 4 show the effect of copper sulphate on fish mortality over 72 and 96 h respectively, as the log concentration increases from 0.00 to 1.20, the mortality

percentage increased to 100%. This indicates that higher concentrations of copper sulphate led to increased mortality rates within the duration. The bar graph in Fig. 5 illustrates the impact of different copper sulphate concentrations on mortality over a 96-h period. At 0.00 mg/L, no mortality was observed. As the concentration increased, mortality rates also rose: 4.00 mg/L showed 20%-40% mortality, 8.00 mg/L had around 60% mortality, and 16.00 mg/L reached close to 100% mortality.

Figs. 6-8 show the probit transformed responses to different log concentrations of copper sulphate over 48, 72 and 96 h respectively. The data points follow a linear trend, indicating a linear relationship between the log concentration and probit values. This suggests that as the exposure duration and log concentration of copper sulphate increased, the probit values also increased. The log concentration at which the probit value is approximately 0.5 will give us an estimate of the LC_{50}. From the graph, the probit value of 0.5 appears to intersect the line of best fit around a log concentration of approximately 1.0 and 0.95. This suggests that the LC_{50} for copper sulphate over 72 h (Fig. 7) and 96 h (Fig. 8) is at a log concentration of about 1.0 and 0.95 respectively.

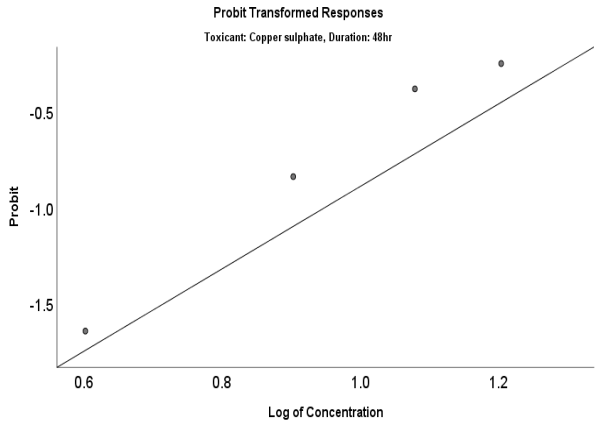

Fig. 6 Plot of probit mortality against log concentration for *O. niloticus* exposed to copper sulphate after 48 h.

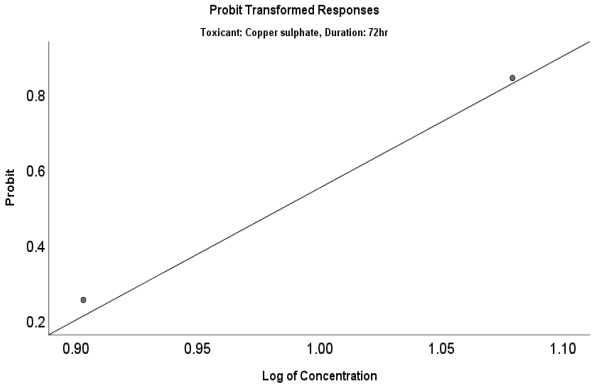

Fig. 7 Plot of probit mortality against log concentration for *O. niloticus* exposed to copper sulphate after 72 h.

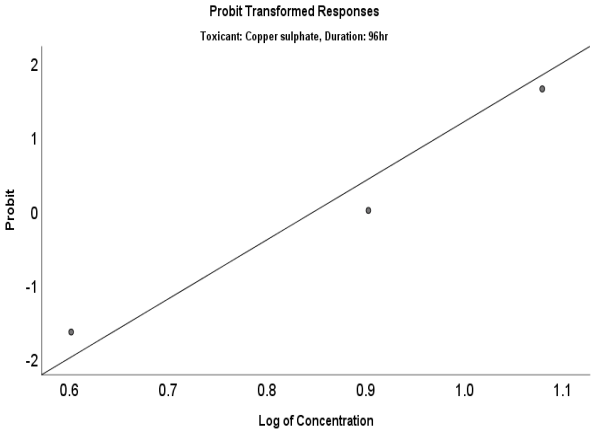

Fig. 8 Plot of probit mortality against log concentration for *O. niloticus* exposed to copper sulphate after 96 h.

3.2 Interrelationship between Physicochemical and Biological Parameters for O. niloticus Treated with Copper Sulphate

In Table 2, copper sulphate exhibited significant correlations with various water quality parameters. The concentration of copper sulphate negatively correlates with DO (r = -0.553) and pH (r = -0.642), indicating that higher concentrations of copper sulphate reduce both DO and pH levels. Additionally, there is a positive correlation between copper sulphate concentration and total alkalinity (TA) (r = 0.521), suggesting that increased copper sulphate levels enhance the buffering capacity of the water. Most notably, the mortality rate (% Mortality)

Table 2 Correlation of physicochemical properties and % mortality for *O. niloticus* exposed to copper sulphate.

	Conc.	DO (mg/L)	pH	TH (mg/L)	Temp. (℃)	TA (mg/L)	% Mortality
Conc.	1						
DO (mg/L)	-0.553	1					
pH	-0.642*	9.912**	1				
TH (mg/L)	-0.498	0.170	0.343	1			
Temp. (℃)	-0.264	-0.159	0.078	0.133	1		
TA (mg/L)	0.521	-0.923**	-0.763*	-0.113	0.278	1	
% Mortality	0.961**	-0.356	-0.480	-0.536	-0.403	0.311	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 3 Mean and standard deviation of physico-chemical properties of ferrocene solution for *O. niloticus.*

Concentration	DO (mg/L)	pH	TH (mg/L)	Tempt. (℃)	TA (mg/L)
Control	4.83 ±0.04	6.78 ±0.28	117.13 ±0.22	28.8 ±0.01	58.32 ±0.03
2.5 mg/L	4.65 ±0.09	6.82 ±0.04	117.49 ±0.01	28.87 ±0.01	58.44 ±0.02
5 mg/L	4.56 ±0.08	6.70 ±0.05	117.52 ±0.03	28.79 ±0.03	58.39 ±0.01
7.5 mg/L	4.80 ±0.11	6.90 ±0.01	117.54 ±0.05	28.81 ±0.03	58.35 ±0.03
10 mg/L	4.66 ±0.14	6.76 ±0.01	117.50 ±0.01	28.85 ±0.05	58.42 ±0.01

shows a strong positive correlation with copper sulphate concentration ($r = 0.961$, $p < 0.01$), highlighting the toxic effects of copper sulphate on aquatic organisms.

3.3 Acute Toxicity Result for O. niloticus Treated with Ferrocene

3.3.1 Physicochemical Parameters for *O. niloticus* Treated with Ferrocene

The values obtained for the physicochemical parameters for O. niloticus exposed to ferrocene in the 96-h bioassay are presented in Table 3. DO range of 4.56-4.66 was obtained from the test aquaria, this varied from the control which had a mean value of 4.83±0.04. The aquarium with a concentration of 5mg/L had the lowest DO value with a mean of 4.56±0.08, while the control had the highest DO value of 4.83±0.04.The highest pH value was observed in the concentration of 7.5mg/L with a mean value of 6.90±0.01and the lowest pH value in the aquarium with the concentration of 5mg/L with a mean value of 6.70±0.05.There was a very slight fluctuation in temperature value. The highest value 28.79±0.05 as observed in the aquaria with the highest concentration of 10mg/L and the lowest value was in the 2.5mg/L concentration with a mean value of 28.87±0.01.Total alkalinity (TA) had variations as well; the highest mean value of 58.44±0.02 was seen in the aquarium with the lowest concentration (2.5mg/L) with the lowest mean value of 58.32±0.03 being the control. The highest total hardness (TH) value was in the 7.5 mg/L concentration with a mean value of 117.54±0.05 and a lowest mean value of 117.49±0.01 in the aquarium, with the lowest concentration (2.5mg/L).

In summary, there were minor fluctuations of the DO of the test solutions across the concentrations. pH, total hardness and temperature remained relatively stable, while total alkalinity (TA) increased slightly.

3.3.2 Mortality

The data on mortality are represented in Figs. 9-13. Over 24 h, across the range of concentrations, no mortality was observed, indicating that within this concentration range, ferrocene did not cause any lethal effects within the 24-h duration (Fig. 9). Fig. 10 shows the effect of different log concentrations of ferrocene on mortality over 48 h. As concentration increased, the mortality increased, reaching up to 40%. This indicates that higher concentrations of ferrocene led to increased mortality rates within the 48-h duration. Figs. 11 and 12 show the effect of ferrocene on fish mortality over 72 and 96 h respectively. As the log concentration increases from 0.00 to 1.20, the mortality percentage

increased to 60% and 80% after 72 and 96 h respectively. Fig. 13 shows the effect of different ferrocene concentrations on mortality over 96 h. At 0.00 mg/L, no mortality was observed. As the concentration increased, mortality rates also rose: 2.50 mg/L showed around 20% mortality, 5.00 mg/L had around 40% mortality, 7.50 mg/L showed around 60% mortality, and 10.00 mg/L reached close to 80% mortality.

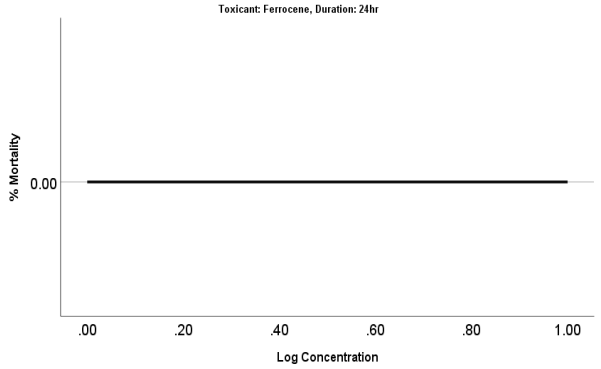

Fig. 9 Plot of percent mortality against log concentration for *O. niloticus* exposed to ferrocene after 24 h.

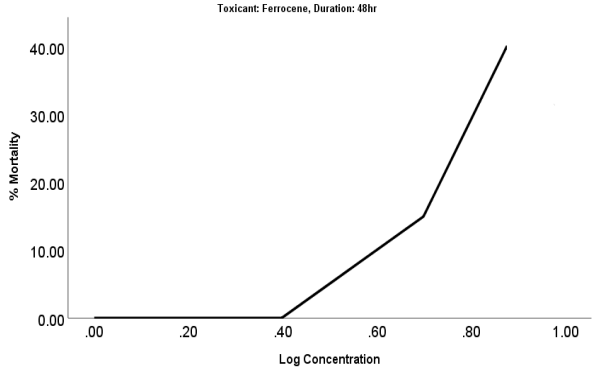

Fig. 10 Plot of percent mortality against log concentration for *O. niloticus* exposed to ferrocene after 48 h.

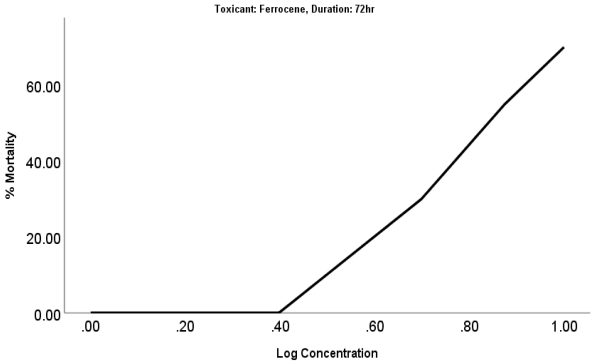

Fig. 11 Plot of percent mortality against log concentration for *O. niloticus* exposed to ferrocene after 72 h.

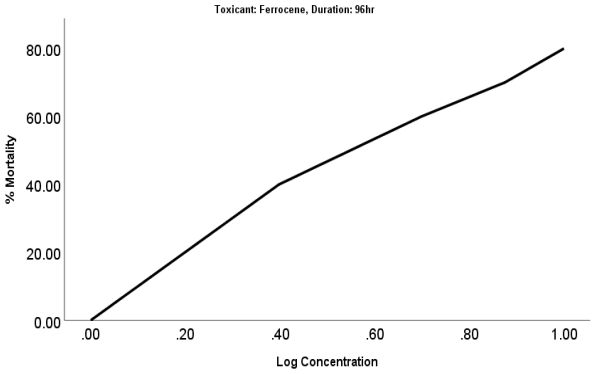

Fig. 12 Plot of percent mortality against log concentration for *O. niloticus* exposed to ferrocene after 96 h.

Figs. 14-16 show the probit transformed responses to different log concentrations of ferrocene over 48, 72 and 96 h respectively. The data points follow a linear trend, indicating a linear relationship between the log concentration and probit values. This suggests that as the exposure duration and log concentration of ferrocene increased, the probit values also increased. The log concentration at which the probit value is

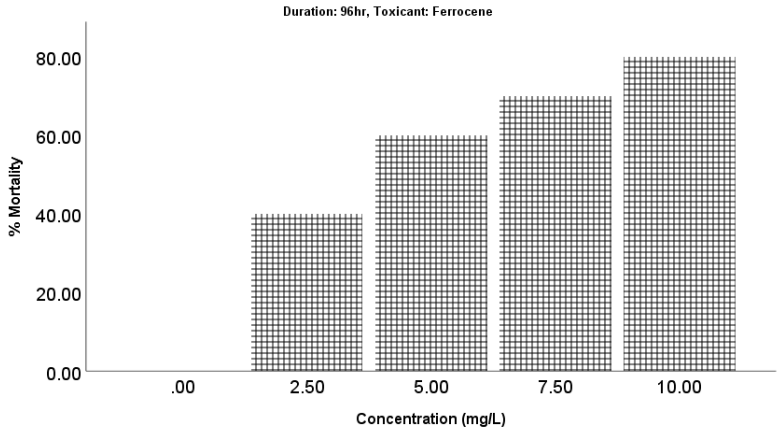

Fig. 13 Plot of percent mortality against various concentrations of ferrocene after 96 h.

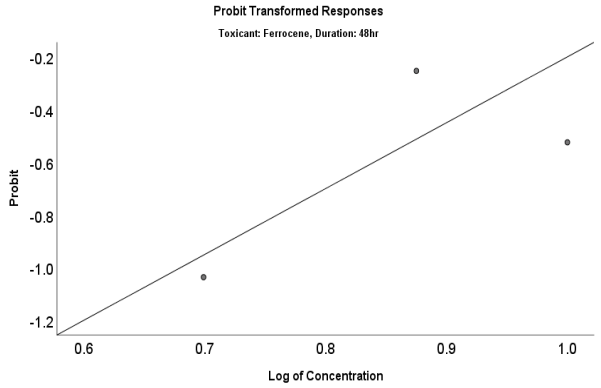

Fig. 14 Plot of probit mortality against log concentration for *O. niloticus* exposed to ferrocene after 48 h.

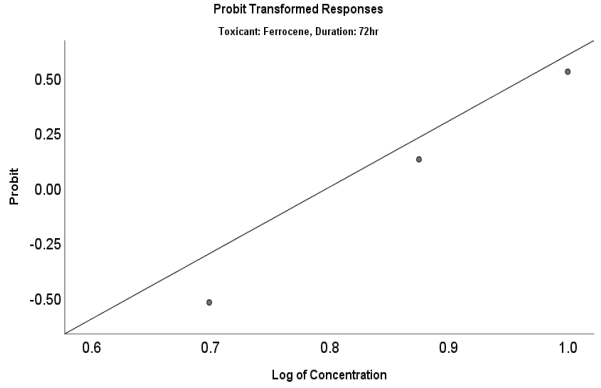

Fig. 15 Plot of probit mortality against log concentration for *O. niloticus* exposed to ferrocene after 72 h.

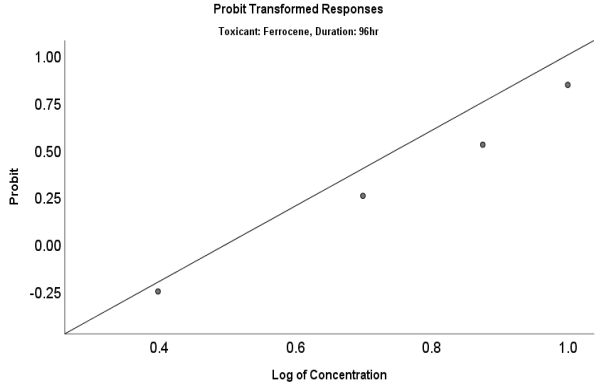

Fig. 16 Plot of probit mortality against log concentration for *O. niloticus* exposed to ferrocene after 96 h.

approximately 0.5 will give us an estimate of the LC_{50}. From the graph, the probit value of 0.5 appears to intersect the line of best fit around a log concentration of approximately 1.0 and 0.7. This suggests that the LC_{50} for ferrocene over 72 h (Fig. 15) and 96 h (Fig. 16) is at a log concentration of about 1.0 and 0.7 respectively.

The safe concentration of ferrocene for O. niloticus was calculated using the application factor of 0.1 as 1.196 mg/L from the LC_{50} value of 11.96 mg/L (Table 7).

3.4 Interrelationship between Physicochemical and Biological Parameters for O. niloticus Treated with Ferrocene

In Table 4, ferrocene concentration negatively correlated with DO ($r = -0.211$, $p = 0.047$), indicating that higher ferrocene levels reduce oxygen availability in the water. There is a positive correlation between ferrocene concentration and total hardness (TH) ($r = 0.663$, $p = 0.037$), suggesting that ferrocene may increase the concentration of calcium and magnesium ions in the water. The mortality rate (% Mortality) shows a strong positive correlation with ferrocene concentration ($r = 0.957$, $p < 0.01$).

In Table 5, the 24-h LC_{50} value of copper sulphate for *O. niloticus* is not specified (NC), indicating that data for this time frame are not available. At 48 h, the LC_{50} was 19.13 mg/L, with a confidence interval of 13.34 to 92.92 mg/L, suggesting moderate toxicity. The toxicity increased over time, with the 72-h LC_{50} dropping to 8.01 mg/L (confidence interval: 6.7 to 9.14 mg/L), and further to 7.49 mg/L at 96 h (confidence interval: 6.35 to 8.57 mg/L). These values indicate that copper sulphate becomes more toxic with prolonged exposure, significantly affecting aquatic organisms.

Ferrocene also showed increasing toxicity over time. The 24-h LC_{50} value was not calculated (NC), similar to copper sulphate. At 48 h, the LC_{50} was 11.96 mg/L, with a confidence interval of 8.8 to 42.98 mg/L, indicating higher toxicity compared to copper sulphate at this time frame. The 72-h LC_{50} was 7.17 mg/L (confidence interval: 6.01 to 8.79 mg/L), and the 96-h LC_{50} was significantly lower at 3.55 mg/L (confidence interval: 0.98 to 5.17 mg/L). These values demonstrate that ferrocene is highly toxic to aquatic organisms, with its toxicity increasing markedly over extended exposure periods.

Table 4 Correlation of physicochemical properties and %mortality for *O. niloticus* exposed to ferrocene.

	Concentration	DO (mg/L)	pH	TH (mg/L)	Temp. (℃)	TA (mg/L)	% Mortality
Conc.	1	-0.211	0.064	0.663*	0.179	0.332	0.957**
DO (mg/L)	-0.211	1	0.374	-0.419	-0.124	-0.637*	-0.322
pH	0.064	0.374	1	0.379	0.233	-0.241	0.067
TH (mg/L)	0.663*	-0.419	0.379	1	0.313	0.459	0.812**
Temp. (℃)	0.179	-0.124	0.233	0.313	1	0.558	0.221
TA (mg/L)	0.332	-0.637*	-0.241	0.459	0.558	1	0.464
% Mortality	0.957**	-0.322	0.067	0.812**	0.221	0.464	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 5 Median lethal concentration (LC_{50}) for *O. niloticus* exposed to copper sulphate and ferrocene.

	Toxicant	
	Copper sulphate	Ferrocene
24-h LC_{50}	NC	NC
48-h LC_{50}	19.13 (13.34-92.92)	11.96 (8.8-42.98)
72-h LC_{50}	8.01 (6.7-9.14)	7.17 (6.01-8.79)
96-h LC_{50}	7.49 (6.35-8.57)	3.55 (0.98-5.17)

NC: Not calculated.

Table 6 The median lethal time (LT_{50}) for *O. niloticus* exposed to copper sulphate and ferrocene.

Toxicant	Concentration	LT_{50}	95% Confidence interval	
Copper sulphate	4 mg/L	NC	NC	NC
	8 mg/L	66.3	59.2	75.6
	12 mg/L	49.2	43.7	54.6
	16 mg/L	45.9	40.9	50.6
Ferrocene	2.5 mg/L	156.6	103.8	117,783.4
	5 mg/L	90.5	83.5	100.5
	7.5 mg/L	61.1	55.0	68.4
	10 mg/L	58.0	55.2	61.0

NC: Not calculated.

In Table 6, for copper sulphate concentration of 4 mg/L, the LT_{50} is not calculated (NC). However, as the concentration increases, the LT_{50} becomes estimable. At 8 mg/L, the LT_{50} was approximately 66.3 h (95% confidence interval: 59.2 to 75.6 h). Similarly, at 12 mg/L, the LT_{50} decreased to approximately 49.2 h (95% CI: 43.7 to 54.6 h), and at 16 mg/L, it further reduced to around 45.9 h (95% CI: 40.9 to 50.6 h). For ferrocene, at 2.5 mg/L, the LT_{50} was approximately 156.6 h, albeit with a wide confidence interval (103.8 to 117,783.4 h). As the concentration increased, the LT_{50} decreased. At 5 mg/L, the LT_{50} was approximately 90.5 h (95% CI: 83.5 to 100.5 h). At 7.5 mg/L, the LT_{50} further decreased to approximately 61.1 h (95% CI: 55.0 to 68.4 h), and at 10 mg/L, it was 58.0 h (95% CI: 55.2 to 61.0 h).

From Table 7, the threshold (no effect values) of an organism is the highest concentration that has no effect on the organism; the maximum exposure when toxicity does not occur [20]. For copper sulphate, the safe concentration value is 0.749mg/L and ferrocene is 0.355mg/L which are the upper limits. The maximum threshold values for both toxicants are the 48-h LC50since 48-hLC50was higher than 96-h LC50.

Table 7 Threshold (no-effect) values and safe concentrations of copper sulphate and ferrocene for *O. niloticus*.

Toxicant	LC_{50} (mg/L)	Threshold (mg/L) (0.05-0.1)	Safe concentration (mg/L) from 0.1
Copper sulphate	19.13 (48-h)	0.9565-1.913	1.913
	8.01 (72-h)	0.4005-0.801	0.801
	7.49 (96-h)	0.3745-0.749	0.749
Ferrocene	11.96 (48-h)	0.598-1.196	1.196
	7.17 (72-h)	0.3585-0.717	0.717
	3.55 (96-h)	0.1775-0.355	0.355

3.5 Histological Analyses of O. niloticus Treated with Copper Sulphate

The histological analyses of some organs of *O. niloticus* treated with copper sulphate are presented in Figs. 17 and 18. Fig. 17 represents the gill morphology, while Fig. 18 represents the skin histomorphology at various concentrations of copper sulphate. Examining the plates, Fig. 17 shows that there were significant differences between the gills of the fish treated with the various concentrations of copper sulphate and the control. The skin histomorphology also showed significant variance with marked, widespread epidermal loss at high concentrations compared to the control which had a normal piscine skin histomorphology. At the concentration of 16 mg/L and 12 mg/L , the sections of the gills presented showed mild-to-moderate hyperplasia of the primary gill epithelia (arrow) leading to partial or complete loss of secondary gill lamella. The sections of the gills presented in the group exposed to copper sulphate concentrations of 8 mg/L and 4 mg/L both showed mild hyperplasia of the primary gill epithelia (arrow) which also led to partial or complete loss of secondary gill lamella. Comparatively, the sections of the gills presented in the control group (0 mg/L) showed the normal gill histomorphology of the primary and secondary gill lamella. For the skin histomophology, the sections of the skin presented in the group with the highest concentration (16 mg/L) showed marked, widespread, epidermal loss, the subdermal muscle tissues (M) were normal. The groups with 12 mg/L and 8 mg/L concentration also showed marked, widespread, epidermal loss but the subdermal muscle tissues (M) showed a widespread myocytic degeneration and necrosis with the muscle fibers shrunken and fragmented: Epidermis (E); dermis (D); hypodermis (H) and muscle (M). The subdermal muscle tissues (M) were normal. HE ×400. Similarly, the sections of the skin presented in the group with 4 mg/L concentration showed marked, widespread, epidermal loss, the subdermal muscle tissues (M) were normal. However, a thin layer of epidermal tissues (one cell layer thick) were occasionally observed (arrow). All sections of the skin in the treated groups showed significant difference compared to that of the control (0 mg/L) which showed the normal piscine skin histomorphology, with normal histomorphology of the epidermis (E); dermis (D); and muscle (M) and the subdermal muscle tissues (M) were also normal.

3.6 Histological Analyses of O. niloticus Treated with Ferrocene

The histological analyses of some organs of *O. niloticus* treated with ferrocene are presented in Figs. 19 and 20. Fig. 19 represents the gill morphology, while Fig. 20 represents the skin histomorphology at various concentrations of ferrocene. Examining the plates, Fig. 19 shows that there were significant differences between the gills of the fish treated with the various concentrations of ferrocene and the control. The skin histomorphology also show same with marked, widespread epidermal loss at high concentrations compared to the control which had a normal piscine skin histomorphology (Fig. 20). The sections of the gills presented in the groups with a ferrocene concentration of 10 mg/L and 7.5 mg/L showed

moderate to severe hyperplasia of the primary gill epithelia (arrow) leading to partial or complete loss of secondary gill lamella: Primary lamella (P); Secondary lamella (S); Cartilage plate (*). HE ×400. The sections of the gills presented in the third group (5 mg/L) showed mild hyperplasia of the primary gill epithelia (arrow) leading to partial or complete loss of secondary

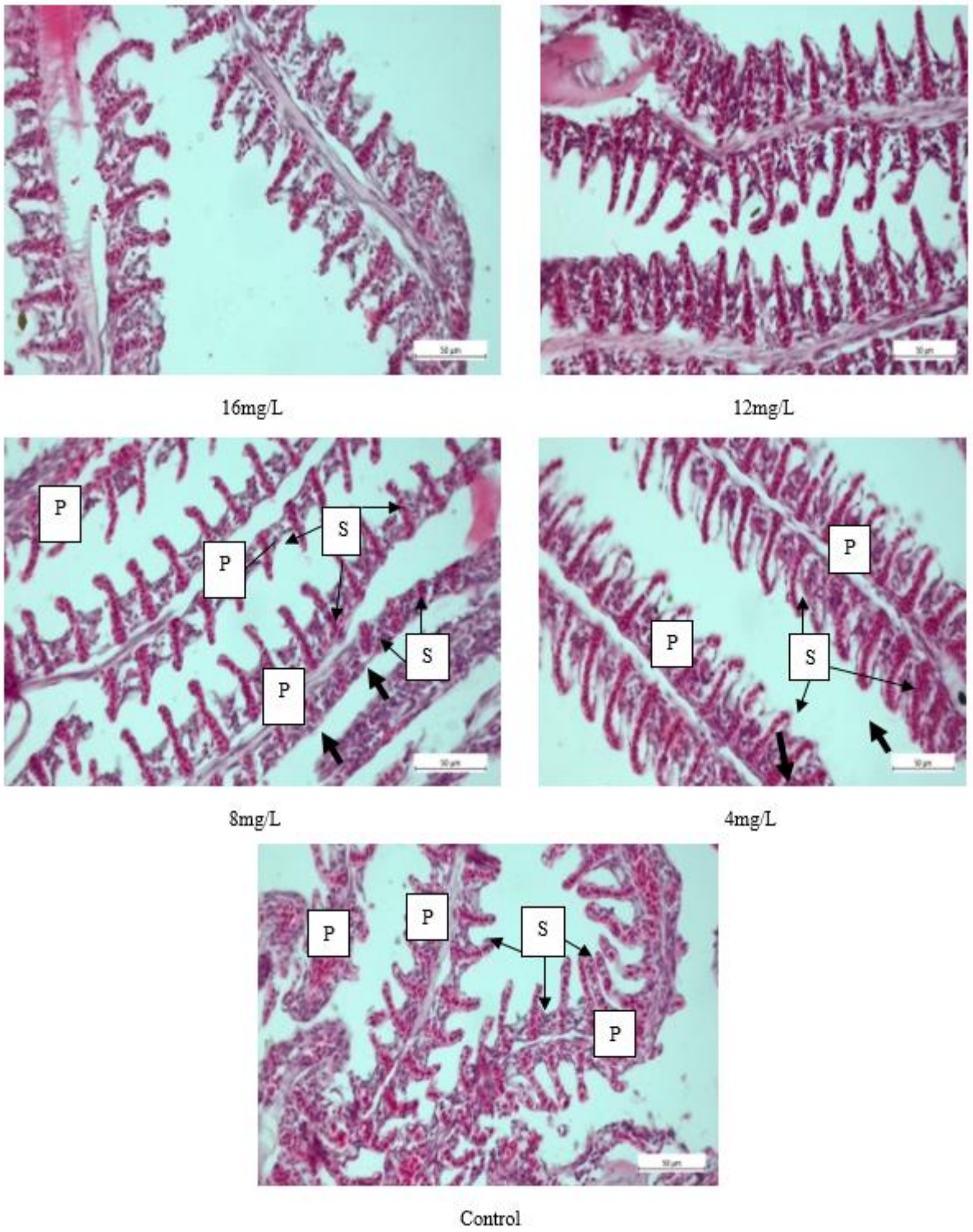

Fig. 17 Changes in gill morphorlogy of *O. niloticus* after 96-h exposure to copper sulphate.

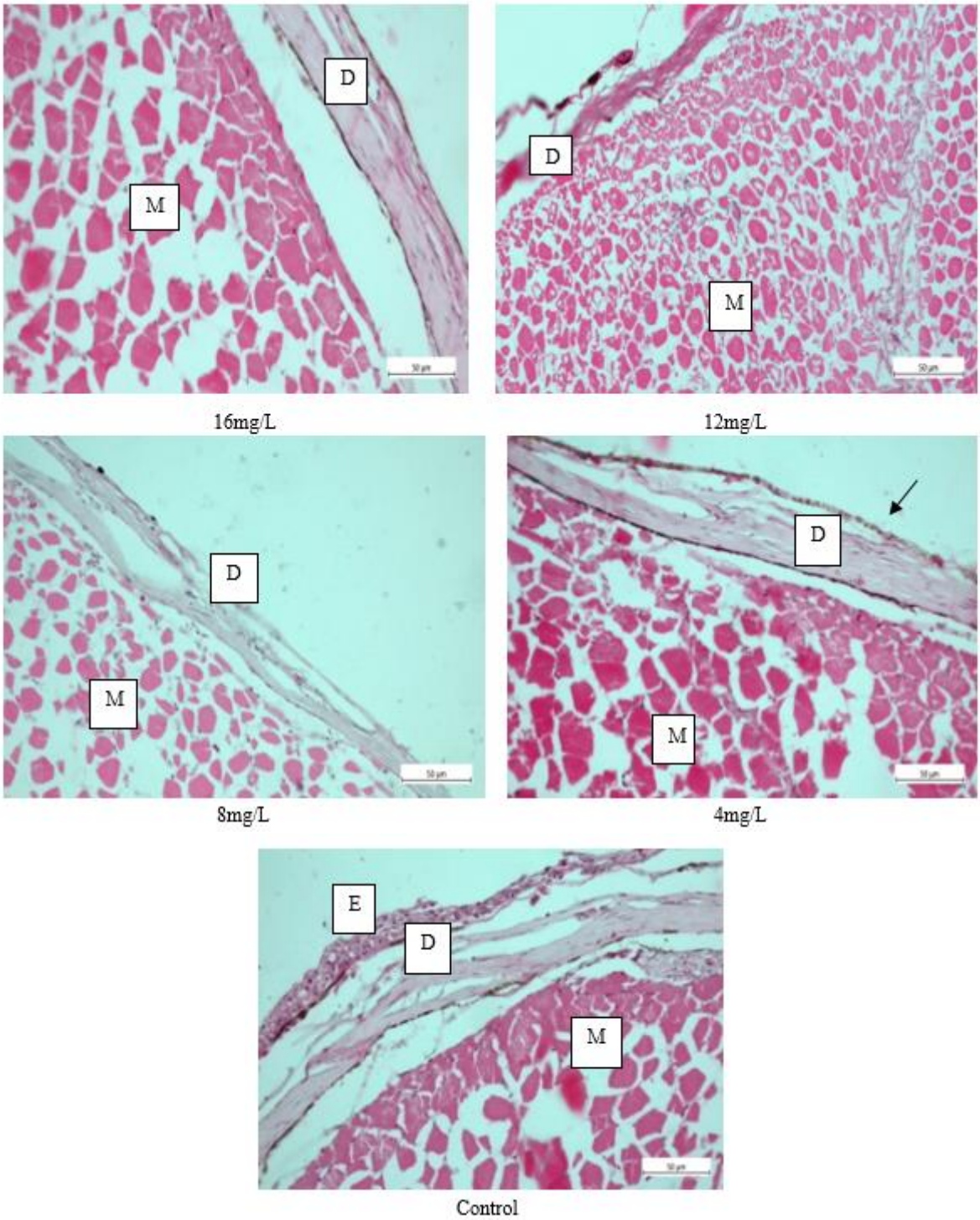

Fig. 18 Changes in skin morphorlogy of *O. niloticus* after 96-h exposure to copper sulphate.

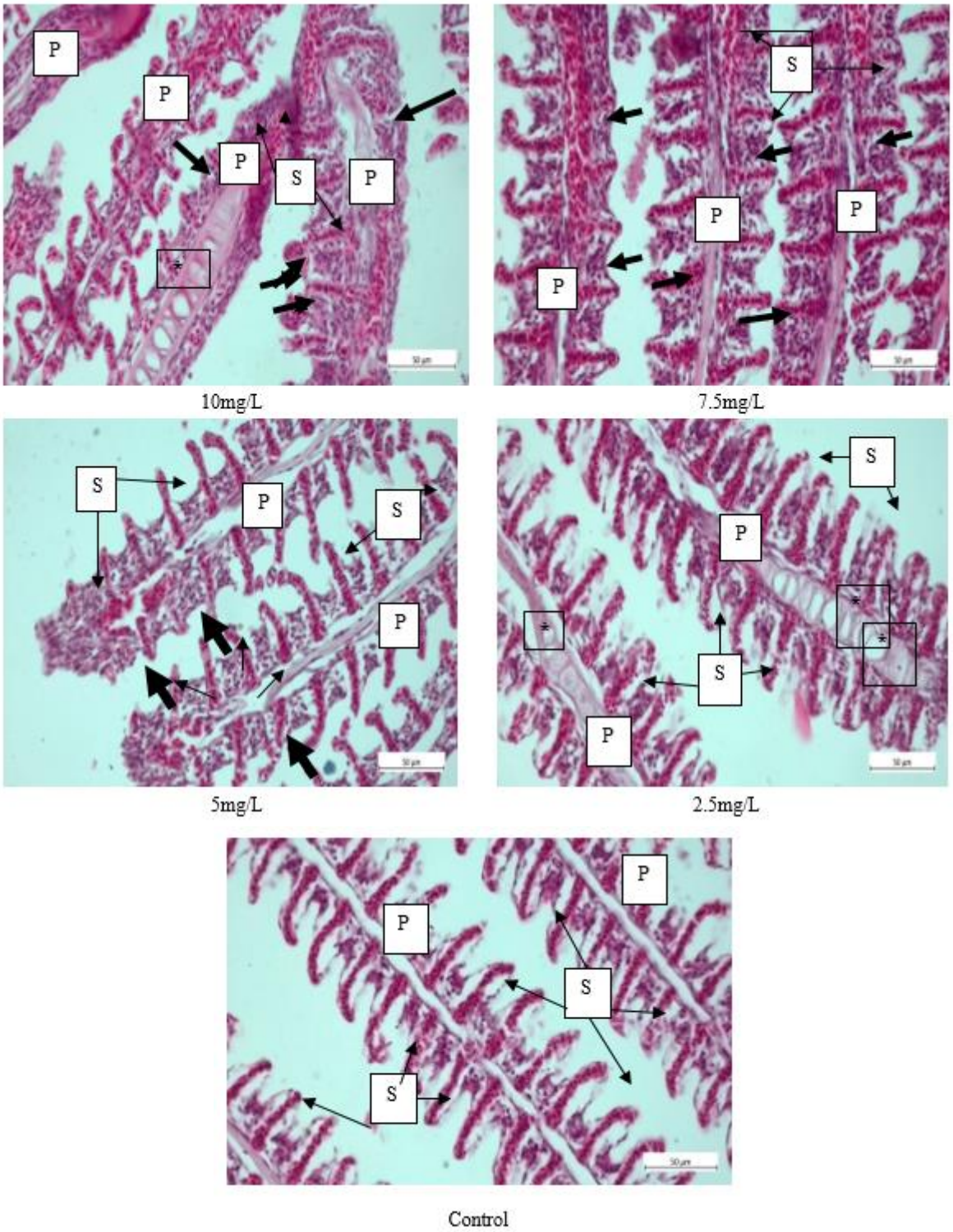

Fig. 19 Changes in gill morphorlogy of *O. niloticus* after 96-h exposure to ferrocene.

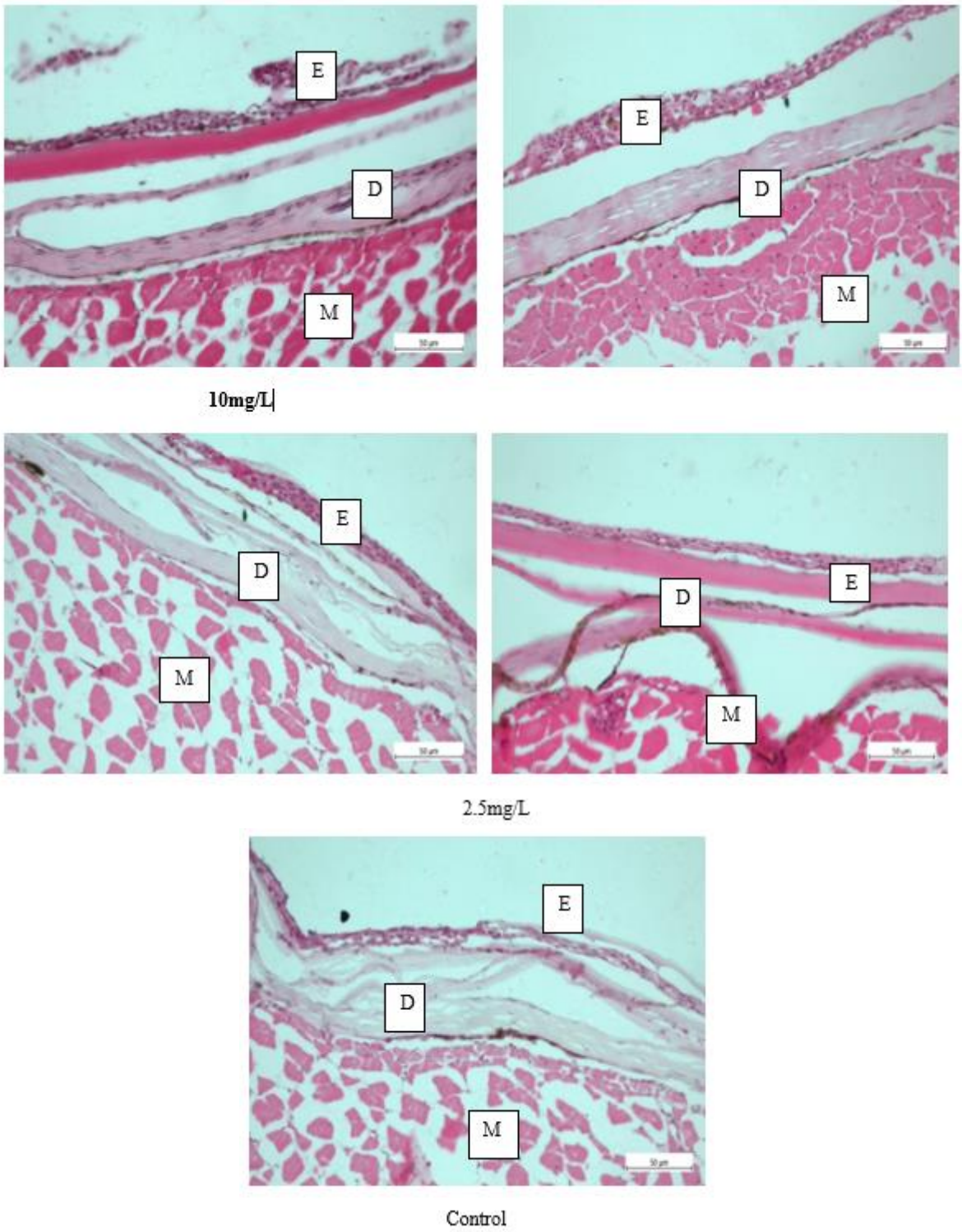

Fig.20 Changes in skin morphorlogy of *O.niloticus* after 96-h exposure to ferrocene.

gill lamella while sections of the gills presented in the group with the lowest concentration (2.5 mg/L) and also the control were similar as they both showed the normal histomorphology of the primary and secondary gill lamella. For the skin histomorphology, skin sections of all the treated groups showed significant variations from those of the control group as they all exhibited moderate-to-severe epidermal loss; the

epidermal layers were markedly thinned, reflecting a marked decrease in the number of the epidermal cells but the subdermal muscle tissues (M) were normal (Fig. 20). The control group (0 mg/L), however, showed the normal piscine skin histomorphology—normal histomorphology of the epidermis (E); dermis (D); hypodermis (H) and muscle (M). The subdermal muscle tissues (M) were also normal.

3.7 Biological Parameters Observed (Fish Behaviour)

After about 30 min of administering copper sulphate with the highest concentration, two fish tried to jump out of the aquarium, this was quickly prevented by using mosquito mesh-sized net to prevent fish from jumping out. There were visible signs of distress in swimming pattern of fish treated with both copper sulphate and ferrocene. After some hours, compared to the fish in the control, the treated fish showed unstable swimming, restlessness and air gulping with loss of balance and equilibrium, and then there was absence of fin and body movement. Opercula hemorrhage and excess mucus secretion were also observed, Secretion of mucus helps to keep the skin surface free from pathogens [21]. However, when secretion is in excess it is possibly as a result of irritation of the skin due to direct contact with the toxicant as the mucus forms a layer between the body and the toxicant in order to minimize the irritating effect [22]. The dead fish sank to the bottom of the aquarium with mouth and gill open, no opercula movement and having very weak skin pigmentation and lying ventral side upwards. The internal organs and muscles were also observed to be pale during dissection. Similar behaviours were observed in the fish treated with ferrocne, except that there was neither pale pigmentation nor excess mucus secretion.

4. Discussion

The results from the study show that water quality fell within the recommended limits in both test solutions. Although variations occurred within the physicochemical parameters, they were within tolerable ranges throughout the bioassay.

The Unites States Environmental Protection Agency (USEPA) [23] gives the recommended limit of pH as 6.5-8.5, and the range of pH for both copper sulphate and ferrocene test solutions fell within this limit. For the DO, World Health Organisation (WHO) [24] recommended 4-6 mg/L as its limit and the DO from this study fell within the tolerable range. The total alkalinity was within tolerable limits of 200 mg/L [23]. The recommended limit for temperature is 20-30 ℃ for WHO and 20-25 ℃ for USEPA, although the temperature varied, they were within the permissible limits. Total hardness and total alkalinity were within the tolerable range of 200-500 mg/L [25] and 200 mg/L (USEPA). The values from the physicochemical parameters show that they did not adversely affect the water quality or mortality rather the mortality that occurred was due to the effect of the toxicants.

Generally, mortality has been used as the endpoint of toxicity tests [26]. Hence it is the main biological parameter measured in this study, along with other parameters. The mortality rates increased gradually with increase in concentration in both test solutions. For both copper sulphate and ferrocene, no death occurred in their controls, which translates that the mortalities that occurred were prompted by the toxicants introduced. For *O. niloticus* treated with copper sulphate, after the 96-h bioassay the concentration of 4.00 mg/L had 40% mortality, which was lower than that of 8.00 mg/L with about 60% mortality, and the highest concentration of 16.00 mg/L reached 100% mortality. The same progressive increase was observed in the fish exposed to ferrocene which showed 20% mortality at 2.50 mg/L concentration; 5.00 mg/L had around 40% mortality, 7.50 mg/L showed around 60% mortality, and the highest concentration of 10.00 mg/L reached close to 80% mortality.

The LC_{50} value of copper sulphate and ferrocene obtained for *O. niloticus* at 96-h was 7.49 mg/L (confidence interval, CI: 6.35 to 8.57 mg/L) and 3.55

mg/L (CI: 0.98 to 5.17 mg/L) respectively. Other median lethal concentrations, LC_{50} obtained were 48-h LC_{50} of 19.13 mg/L (CI: 13.34 to 92.92 mg/L), and 72-h LC_{50} 8.01 mg/L (CI: 6.7 to 9.14 mg/L) for copper sulphate and for ferrocene, 48-h LC_{50} 11.96 mg/L (CI: 8.8 to 42.98 mg/L) and 72-h LC_{50} 7.17 mg/L (CI: 6.01 to 8.79 mg/L). It is however worthy to note that for ferrocene, the 96-h LC_{50} was significantly lower than that of copper sulphate and these values demonstrate that ferrocene is highly toxic than copper sulphate to aquatic organisms, with its toxicity increasing markedly over extended exposure periods in conformity with Jiraungkoorskul, Sahaphong, and Kangwanrangsan [27] who stated that copper toxicity is dependent on length of exposure. From the results, we see that LC_{50} values decreased with time of exposure for both copper sulphate and ferrocene, implying that toxicity increased over time. The results also show that the LT_{50} of both toxicants reduced with increased concentration. *O. niloticus* treated with ferrocene recorded longer LT_{50} than those treated with copper sulphate showing that fish treated with ferrocene will require more time to die than those treated with copper sulphate.

The toxicity test result for copper sulphate with a 96-h LC_{50} value of 7.49 mg/L (confidence interval, CI: 6.35 to 8.57 mg/L) from this study is close to the results of Vasconcelos et al.'s [28] study on Nile tilapia juveniles with a 96-h LC_{50} value for copper sulphate as 6.96 mg/L and a median LC_{50} value of $CuSO_4$ for blackfish (*Copoeta fusca*) as 6.928 mg/L [29]. Other works have also been done on the 96-h toxicity of copper sulphate with varying LC_{50} values for different species. Elahe, Elnaz, and Kasalkhe [30] showed 96-h LC_{50} of copper sulphate for Gray mullet (*Mugil cephalus*) 39.68 ppm, and Oliva et al. [31] showed 96-h LC_{50} 0.32 mg/L Senegal sole (*Solea senegalensis*). A test conducted on *Rasbora sumantrana* (cyprinidae) and *Poecilia reticula* (guppy) revealed their LC_{50} values for copper sulphate as 5.6 μg/L and 37.9 μg/L respectively [32] whereas, African catfish (*Clarias garipinus*) 96-h LC_{50} value for copper was 0.67 mg/L [8]. Although these values differ from our results, this could be due to difference in species, age and size of specimens, test methods and physicochemical factors such as hardness of water, levels of pH and temperature which affect toxicity caused by copper and ferroene [33, 34]. It also indicates that different organisms have different sensitivities to heavy metals. For ferrocene, LC_{50} obtained at 96-h was 3.55 mg/L (CI: 0.98 to 5.17 mg/L), because there are not so many works on ferrocene so there was no value to compare it with.

Mortalities observed in this study could be attributed to respiratory stress and asphyxiation resulting from badly damaged gills; gill hemorrhages were observed at death. The gill tissues also had hyperplasia, which could be the reason why the fish died with mouths open as they gasped for air due to gas exchange failure at the gill surfaces as confirmed by Alkobaby and Abd El-Wahed [35]. The gill morphology of the fish exposed to copper sulphate showed severe hyperplasia of the primary gill epithelia leading to partial or complete loss of the secondary gill lamella and in line with observations by Pelgrom et al. [36], which stated that the most sensitive parameters affected by chemicals are the gills and plasma levels.

Many other authors reported similar gill epithelial damage, hyperplasia, lamella fusion and edema [37]; epithelial lesions and epithelial hyperplasia of both primary and secondary lamellae [35]; filament cell proliferation, increase in intercellular spaces, epithelial lifting and thickening of filament and lamellar epithelium were observed in an investigation by Jiraungkoorskul, Sahaphong, and Kangwanrangsan [27] on copper toxicity in butterfish (*Poronotus triacanthus*). Hoseini and Al-Sulivany [38] in his work on common carp stated that copper exposure induces stress in fish gills. Heavy metals accumulated in gills will affect the respiration and osmoregulation processes, causing cellular damage to gill cells.

There were also very significant differences in the skin sections of the fish exposed to high concentrations

of copper sulphate as they showed widespread epidermal loss leading to partial or complete loss of the secondary gill lamella, myocyctic degeneration and necrosis with muscle fibre shrunken and fragmented, which varied from the control and corresponds with Padrilah et al. [39], who noted that histopathological changes in animal tissue especially fish are powerful indicators for prior exposure of aquatic environmental stressors.

The fish treated with ferrocene also showed differences in the gills with hyperplasia of primary gill epithelia leading to partial or complete loss of secondary lamella as observed by Singh, Kumar, Bilal, and Chandra [40] in his study on toxic effect of organometallic pollutants on Stinging catfish (*Heteropneustes fossilis*) and sludgeworm (*Tubifex tubifex*) which showed resultant changes in primary and secondary lamella. The skin morphology however showed severe epidermal loss in fish treated with high concentrations of ferrocene.

5. Conclusion and Recommendations

Aquatic habitats are most vulnerable to any kind of water pollution because all of the industrial waste, weathering of soil and urban mining is discharged into the water bodies which in turn affect the aquatic biota.

The observed toxicity levels of ferrocene suggest that even low concentrations can pose a significant threat to Nile tilapia, which could lead to serious implications for aquaculture operations and the health of natural water bodies, while copper sulphate was less toxic in comparison, its potential to cause sub-lethal effects at lower concentrations still raises concerns about its long-term impact on fish health.

Given the acute toxicity of copper sulpahate and ferrocene, it is essential to establish strict guidelines for their permissible levels in aquaculture systems. There are limiting data specifically on the lethal and sub-lethal effects of ferrocene on Nile tilapia, highlighting a need for further studies to explore the chronic effects of these compounds and assess their potential bioaccumulation in fish tissues.

Additionally, strategies such as water treatment and monitoring should be implemented to mitigate the risks posed by these toxicants in aquaculture environments.

References

[1] Shriver, D. F. 2023. *Coordination Compounds: Organomettalic Compounds.* Chicago: Encyclopaedia Britinnica.

[2] Kovacs, M. E., and Kovacs, E. D. 2017. *Recent Progress in Organometallic Chemistry*, edited by M. Rahman. London: IntechOpen.

[3] Cohrssen, B., and Bingham, E. (eds.). 2012. *Patty's Toxicology* (Vol. 6). New York: Wiley, pp. 26-7.

[4] Özgeris, B., Yeltekin, C. A., Ucar, A., Cağlar, Ö., Parlark, V., Arslan, M. E., Tűrkez, H., Atamanalp, M., and Alak, G. 2023. "Toxic Releases and Exposure Assessment: A Multi-endpoint Approach in Fish for Ferrocene Toxicity." *Journal of Process Safety and Environmental Protection* 169: 636-45.

[5] Al Ghais, M. S., Bhardway, V., and Kumbhar, P. 2020. "Sublethal Effects of Anti-fouling Paints on Marine Organism." *International Journal of Fisheries and Aquatic Studies* 8 (2): 92-7.

[6] Wani, M. A., Shammi, Q., and Singh, J. 2014. "Evaluation of Heavy Metal Iron on a Fresh Water Fish, *Labeorohita* and Its Behavioural Impacts." *Journal of Pharmaceutical Biological, and Chemical Sciences.*

[7] Al Ghais, M. S., Bhardway, V., Kumbhar, P., and Shehhi, O. A. 2019. "Effects of Copper Nanoparticles and Organometallic Compounds (Dibutylin) on Tilapia Fish." *Journal of Basic and Applied Zoology* 80: 32.

[8] Olaifa, F. E., Olaifa, A. K., and Onwude, T. E. 2004. "Lethal and Sub-lethal Effects of Copper to the African Cat Fish (*Clarias garipinus*) Juveniles." *African Journal of Biomedical Research* 7 (2): 65-70.

[9] Kulesza, A., Leonard, E. M., and McClelland, G. B. 2020. "Influence of 96 Hour Sublethal Copper Exposure on Aerobic Scope and Recovery from Exhaustive Exercise in Killifish (*Fundulus heteroclitus*)." *Aquatic Toxicology* 218: 105373.

[10] Al Ghais, M. S. 2013. "Acetylchlorinesterase, Glutathoinic and Hepatosomatic Index Potential Biomkakers and Sewage Pollution and Depuration in Fish." M*arine Pollution Bulletin* 74 (1): 183-6.

[11] Tesfahun, A., and Tesmesgem, M. 2018. "Food and Feeding Habits of Nile Tilapia *Oreochromis niloticus* (L.) in Ethiopian Water Bodies: A Review." *Int'l Journal of Fisheries and Aquatic Studies* 6 (1): 43-7.

[12] Vajarah, M. F. 2021. "A Review of the Physiology and Biology of Nile Tilapia (*Oreochromis niloticus*)." *Journal of Aquaculture and Marine Biology* 10 (5): 244-6.

[13] Fredrick, S. B. 2002. *Aquaculture Pathophysiology—Description of Major Farmed Aquatic Animal Species* (vol. 1). Finfish Diseases: Academic Press.

[14] Kopperdahl, F. R. 1976. "Guidelines for Performing Static Acute Toxicity Fish Bioassays in Municipal and Industrial Wastewaters."

[15] Organisation for Economic Cooperation and Development (OECD). 2019. *TEST No. 203: Fish, Acute Toxicity Test, OECD Guidelines for the Testing of Chemicals (Section 2).* Paris: OECD Publishing.

[16] APHA (The American Public Health Association). 2017. *Standard Methods for the Examination of Water and Wastewater* (23rd ed.). Washington DC: American Public Health Association.

[17] Halmi, M. I. E., Abd Rahim, M. B. H., and Othman, A. R. 2018. "Estimation of LC_{50} and Its Confidence Interval for the Effect of Ferrous Sulphate on *Catla catla*." *Journal of Environmental Microbiology and Toxicology* 16 (1): 22-23.

[18] Sprague, J. B. 1969. "Measurement of Pollutant Toxicity to Fish." *Bioassay Methods for Acute Toxicity* 3 (11): 793-821.

[19] Joshi, A., and Munisamy, S. D. 2020. "Enhancement of Performance Parameter of Cloud Using Dynamic Degree Balanced with Membership Value Algorithm." *International Journal of Advanced Research in Engineering and Technology* 11 (8): 664-76.

[20] Aldridge, W. N. 1995. "Defining Thresholds in Occupational and Environmental Toxicology." *Toxicology Letters* 17 (1-3): 109-18.

[21] Elliot, D. G. 2011. "The Many Functions of Fish Integument." In *Encyclopedia of Fish Physiology. From Genome to Environment* (vol. 1), edited by A. P. Farrell. San Diego: Academic Press, pp. 471-5.

[22] Sharma, M. 2019. "Behavioural Responses in Effect of Chemical Stress in Fish: A Review." *International Journal of Fisheries and Aquatic Studies* 7 (1): 1-5.

[23] USEPA (Unites States Environmental Protection Agency). 2012. *Edition of the Drinking Water Standards and Health Advisories.* Washington D.C.: Office of Water, USEPA.

[24] WHO (World Health Organisation). 2017. *World Health Guidelines for Drinking Water Quality* (4th ed.). Geneva: WHO Publications.

[25] Department of Environment (DOE). 1997. *Environment Conservation Rules.* Bangladesh, Dhaka: Department of Environment of Ministry of Environment and Forest, pp. 212-4.

[26] Handson, L., and Ritter, L. 2010. "Toxicity and Safety Evaluation of Pesticides." In *Hayes Handbook of Pesticide Toxicology* (3rd ed.), edited by R, Krieger. New York: Academic Press, pp. 2319-42.

[27] Jiraungkoorskul, W., Sahaphong, S., and Kangwanrangsan, N. 2007. "Toxicity of Copper in Butterfish (*Poronotus triacanthus*): Tissues Accumulation and Ultrastructural Changes." *Environmental Toxicology: An International Journal* 22 (1): 92-100.

[28] Vasconcelos, R. T., Copatti, C. E., Albinati, A. C., Campeche, D. F. B., Bonfà, H., and Melo, J. 2024. "Waterborne Copper Sulfate Toxicity in Nile Tilapia (*Oreochromis niloticus*) Juveniles after Survival, Growth and Physiology." *Journal of Applied Aquaculture* 36 (1): 129-50.

[29] Zarei, I., Pourkhabaz, A., Allpour, H., and Khazael, S. H. 2012. "Acute Toxicity and Effects of Copper Sulphate ($CuSO_4$ $5H_20$) on the Behaviour of Blackfish (*Copoeta fusca*)." *Iranian Journal of Toxicology* 6 (19): 771-8.

[30] Elahe, E., Elnaz, E., and Kasalkhe, N. 2018. "Acute Toxicity and Effects of Copper Sulphate ($CuSO_4$ $5H_2O$) on the Behaviour of the Gray Mullet (*Mugil Cephalus*)." *International Journal of Scientific Research in Environmental Science and Toxicology* 3 (2): 1-4.

[31] Oliva, M., Garrido, M. C., Màrquez, D. S., and De Canalas, M. L. G. 2009. "Sublethal and Lethal Toxicity in Juvenile Senegale Sole (*Solea Senegalensis)* Exposed to Copper: A Preliminary Toxicity Range-Finding Test." *Experimental and Toxicologic Pathology* 61 (2): 113-21.

[32] Suhaimi-Othman M., Nadzifah, A. K., and Ahmad. 2010. "Toxicity of Copper and Cadmium to Freshwater Fishes." *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering* 4 (5): 319-21.

[33] Malhotra, N., Ger, T., Uapipatanakul, B., Huang, J., Chen, K. H. C., and Hsiao, C. 2020. "Review of Copper and Copper Nanoparticle Toxicity in Fish." *Nanomaterials* 10 (6): 1126.

[34] Çoğun, H. Y., and Kargun, F. 2004. "Effects of pH on the Mortality and Accumulation of Copper in Tissues of *Oreochromis niloticus.*" *Chemosphere* 55 (2): 279-82.

[35] Alkobaby, A. I., and Abd El-Wahed, R. K. 2017. "The Acute Toxicity of Copper to Nile Tilapia (*Oreochromis niloticus*) Fingerlings and Its Effects on Gill and Liver Histology." *Journal of Aquaculture Research and Development* 8 (1): 1-6.

[36] Pelgrom, S. M. G. J., Lock, R. A. C., Balm, P. H. M., and Weldelaar Bonga, S. E. 1995. "Integrated Physiological Response of Tilapia, *Oreochromis mossambicus*, to Sublethal Copper Exposure." *Journal of Aquatic Toxicology* 32 (4): 302-20.

[37] Khabbazi, M., Harsij, M., Hedayati, S. A. A., Gerami, M. H., and Ghafari-Farsani, H. 2005. "Histopathology of Rainbow Trout Gills after Exposure to Copper." *Iranian Journal of Ichkyology* 1 (3): 191-6.

[38] Hoseini, S. M., and Al-Sulivany, B. S. A. 2024. "Copper and Microplastic Exposure Affects the Gill Gene

Expression of Common Carp during Saltwater Challenge." *Science Journal of University of Zakho* 12 (3): 382-7.

[39] Padrilah, S. N., Sabullah, M. K., Abd Shukur, M. Y., Yasid, N. A., Shamaan, N. A., and Ahmad, S. A. 2018. "Toxicity Effects of Histopathology on Copper Accumulation." *Pertanika Journal of Tropical agricultural Science* 41 (2): 519-40.

[40] Singh, A. K., Kumar, A., Bilal, M., and Chandra, R. 2021. "Organometallic Pollutants of Paper Mill Wastewater and Their Toxicity Assessment on Stinging Catfish and Sludge Worm." *Environmental Technology and Innovation* 24: 101831.