**original research article**

# TOXICOLOGICAL RESPONSES OF AFRICAN MUD CATFISH (*Clarias gariepinus*, Burchell, 1822) FINGERLINGS EXPOSED TO CULTURE WATER CONTAMINATEDWITH DIFFERENT CONCENTRATIONS OF CYPERMETHRIN


## ABSTRACT

The toxicological effects of cypermethrin on *Clarias gariepinus* fingerlings and its contamination of culture water was studied. Ten fingerlings were stocked in each aquarium and was exposed to 5 different concentrations of cypermethrin and there was a control group. The fingerlings were exposed to 5, 10, 15, 20 and 30ppm of cypermethrin in triplicate. A total of 180 *C. gariepinus* fingerlings with a mean weight of 1.85 ± 0.29g were used throughout the study. The toxicant altered the physico-chemical parameters of culture water. The water temperature, pH, electrical conductivity and turbidity of the contaminated culture water increased with increase in the concentration of cypermethrin, while the dissolved oxygen (DO) decreased with increase in the toxicant concentration. Temperature, conductivity, pH and turbidity values were higher and the DO level was lower in the aquarium contaminated with the highest concentration of the toxicant compared to the control group. Statistically, the physico-chemical parameters varied significantly between the culture waters contaminated with different concentrations of cypermethrin across all exposure durations at $p<0.05$, except for temperature over 96 hours exposure period which was insignificant at $p>0.05$. The water temperature, pH and conductivity of the culture water were within the WHO acceptable limits except the dissolved oxygen (30ppm group over 72 and 96 hour exposure duration) and turbidity (5, 10, 15, 20 and 30ppm group) which were above the World Health Organisation (WHO) permissible limit. The mortality data trend of fingerlings exposed to cypermethrin was concentration and duration dependent. The 96 hours $LC_{50}$ value with 95% confidence limit of *C. gariepinus* fingerlings exposed to the toxicant was 9.332ppm ± 0.839, and was significant with a determination coefficient ($r^2$) of 0.88 at $P<0.05$. The low $LC_{50}$ value for the fingerlings exposed to the pesticide indicated its high toxicity. In conclusion, contamination of culture water with cypermethrin led to the mortality of *C. gariepinus* fingerlings and the alteration of the physico-chemical parameters of the culture water. As a result, more similar research should be carried-out involving haemathological, reproductive, histological and other physiological alterations when fishes are exposed to cypermethrin so as to further reveal the toxic and harmful potentials of pesticides.



**KEYWORDS:** Toxicological, responses, concentrations, *Clarias gariepinus* and fingerlings


## 1. INTRODUCTION

Cypermethrin is globally used for the control of pest, in order to improve food productivity [1], but their use could create a risk of food contamination as well as affects non-target aquatic species like; invertebrates and vertebrates [2]. It is a synthetic pyrethroid, with a very high activity and stability [3]. Of all the pesticides available in the market, pyrethroids make about 25% of global pesticides sale [4]. The usefulness of the pesticide has always

marked its toxic effects on the aquatic environment [5]. Over 200 types of synthetic pesticides exist [6] and they all contain several heavy metals. These metals enter the water bodies, thereby affecting growth, physiology, reproduction and survival of fish [7].

Pesticides occupy a unique position among many chemicals which are encountered daily by man. Pesticides are deliberately added to the environment for pest control in homes and on farmlands. They are used in large quality by agro-farmers which in turn pollute our aquatic environment [8]. The toxicity of pyrethroids varies between biological species, due to the difference in elimination and metabolic degradation from the body [9]. Globally, Cypermethrin is used for the control of cotton, fruits and vegetables pest [9], copepod parasite infestation [10], aquatic and terrestrial ectoparasites [11] and for illegal fishing [9]. Agricultural run-off happens to be the main route of entry of cypermethrin into the aquatic eco-system, and this affects the non-target species [12]. Residues of these toxic chemicals found in water, sediment, fish and other aquatic biota, can pose a risk to organisms, predators and human being at high concentration (Lethal concentration), and are known to reduce the survival, growth, reproduction of fish and produce many visible effects on fish [13].

The rapid advancement of industrialization and green revolution has led to a number of environmental problems, with aquatic pollution being the most prominent. In Nigeria, effluents from industries, wastes from household activities and agricultural runoffs are directly discharged into streams, ponds and other aquatic bodies. These pollutants contain infectious pathogens, oil, hydrocarbon, radioactive substances, heavy metals, pesticides, herbicides and different corrosive substances such as acids and bases [14]. Yet these water sources are used for supplying water to the local masses and culturing of economically important and luscious fish species [14].

Water covers about 70% of the earth, and happens to be the most essential natural resources [15]. Despite this awareness of the essentiality of water, humans have ignored its importance by polluting it [16]. The advancement in industrialization has coincided with the problem of aquatic pollution. The use of mechanical and biological means of pest control has been abandoned for an easier and faster use of agricultural pesticides for control of pest, in order to generate massive crop yield, so as to meet-up with the ever growing human population [17, 18, 19]. The careless and indiscriminate use of these synthetic pesticides has led to the global pollution of water bodies [20, 21] leading to mortality of aquatic organisms and a general deterioration of the aquatic ecosystem [22, 23].

This study was aimed at evaluating the acute toxicity of cypermethrin on the survival of *C. gariepinus* fingerlings and the alterations in the water quality of the culture water.

*Clarias. gariepinus* was chosen for the study because it is the most common cultured fish in Nigeria, as a result their fingerlings can be easily seen and purchased. Also, they are able to withstand stress and are more suitable for research of this kind.

## 2. MATERIALS AND METHODS

### 2.1 Test Chemical

Cypermethrin used for this study was purchased from Cross River State Ministry of Agriculture, Barracks Road, Calabar.

### 2.2 Collection and transportation of test fish

*Clarias gariepinus* fingerlings were collected from the University of Calabar fish farm, Calabar, Cross River State using a scoop net in the early hours of the morning to avoid heat, high intensity and stress. The collected fingerlings were then transported to the Zoology and Environmental Biology laboratory using a plastic bucket containing a well aerated habitat water.

### 2.3 Acclimatization and maintenance of test fish

Once the fingerlings samples were taken to the laboratory, they were stored in a 30 x 30 x 80 cm tank containing a well aerated water and allowed to acclimate for 14 days in order to get used to the laboratory conditions. During the acclimation, the fingerlings were fed twice daily with coppens feed at 5% of their body weight. The water (borehole water) was changed every 48 hours to avoid contamination of water due to accumulated toxic waste metabolites and food particles. An aerator was also used in order to ensure adequate dissolved oxygen through-out the acclimatization period. Feeding of the fingerlings was stopped 48 hours to the commencement of the experiment.

### 2.4 Preparation of stock solution

The stock solution was prepared by dissolving 6mL of cypermethrin with 96.8% purity in 994 mL of water in a conical flask, which resulted in a 1000mL of the stock solution. The stock solution was then diluted serially to 5, 10, 15, 20 and 30ppm concentrations.

### 2.5 Range finding test

A range finding test was carried-out using the test chemical, in order to determine the most appropriate range of concentration. A wide range of concentration was used for this purpose, including the concentration that killed all within 24 hours and another that

did not kill the test organism within 96 hours. Through this, the most appropriate concentrations were selected for the experiment proper.

**2.6 Test procedure**

Eighteen aquaria measuring 60 X 30 X 30 $cm^3$ were used for the experiment. A total of 180 *C. gariepinus* fingerling weighing 1.85 ± 0.29g were used through-out the study, which was carried-out in triplicates. Ten fingerlings of *C. gariepinus* fingerlings were introduced into each aquarium containing 1 litre of water. The fingerlings were then exposed to 5 different concentrations (5, 10, 15, 20 and 30ppm) of the toxicant and there was also a control group that were not exposed to any toxicant. The experiment was carried-out using a static non-renewal bioassay for 96hrs. The mortality and general behavior of fish was also observed after 24, 48, 72 and 96 hours of exposure. Fingerlings were considered dead when they cannot move any longer, even when touched with a glass rod. Dead fingerlings were removed immediately and then its mortality recorded.

**2.7 Measurement of physico-chemical parameters**

Water quality parameters of the culture water was monitored after 24, 48, 72 and 96 hours. The culture water for each fish group were tested in-situ for temperature ($^oC$), Conductivity (µs/cm), pH, dissolved oxygen (mg/L) and turbidity (N.T.U) once the toxicant was introduced. The water parameters were then monitored over the 96 hours period of the experiment, and compared to the control water parameters. This was done in order to find out the effect of cypermethrin on the water quality.

**Temperature ($^oC$)**

The surface water temperature was measured in-situ in culture water of each fingerlings group using mercury – in – glass thermometer in degrees Celsius ($^oc$). The thermometer was inserted at a depth of about 2cm from the surface water for about 3 minutes and the reading taken.

**Hydrogen ion concentration (pH)**

The pH of the water was measured in-situ using a model pH-1 pocket-sized pH meter. The meter glass probe was dipped into the culture water and readings taken.

**Dissolved oxygen (DO) (mg/l)**

The dissolved oxygen was measured in-situ using a dissolved oxygen meter, model DO-5509, calibrated in mg/L (milligrams per litre).

**Turbidity (N.T.U)**

The turbidity was measured in-situ using a turbidity meter. The meter was inserted 2cm from the water surface for about 2 minutes, and then the turbidity of the culture water read to the nearest N.T.U (Nephelometric turbidity unit).

**Conductivity (µS/cm)**

Conductivity was measured in-situ using a using Hannah Instrument (Bench meter 211 model). The meter was inserted 2cm from the water surface for about 2 minutes, and then the water conductivity value was taken to the nearest µS/cm.

**2.8 Data analysis**

The mortality data obtained were subjected to probit logarithm transformation. Regression analysis was also performed and the $LC_{50}$ values was computed. The 95% confidence interval was also computed and the slope of the regression line tested using chi-square. Anova was also used to test for the significance of difference in water quality parameters between each concentration group at 0.05 level of significance and at their relevant degree of freedom. Also descriptive statistics (mean and standard deviation) was carried out on the physicochemical parameters of the contaminated culture water and the control group. Graph was plotted using Microsoft excel (MSE) version 2013. Probit analysis was carried-out using predictive analytical software (PASW) version 20.

## 3. RESULTS

### 3.1 Water quality of culture water

**Water temperature ($^{o}$C)**

The summary of temperature alterations of the culture water contaminated with different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 1. After 24 hours of exposure, the water temperature of the culture water had a mean and standard deviation of 29.000 ± 0.000, 29.250 ± 0.353, 28.965 ± 0.091, 28.025 ± 0.035, 29.025 ± 0.035 and 29.265 ± 0.332 $^{o}$C when exposed to 0 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water temperature was observed in the culture water contaminated with 15ppm of cypermethrin (28.025 ± 0.035 $^{o}$C), while the highest water temperature was observed in the culture water contaminated with 30ppm of cypermethrin (29.265 ± 0.332 $^{o}$C) (Table 1).

After a 48 hours exposure duration, the water temperature of the culture water had a mean and standard deviation of 28.250 ± 0.353, 29.035 ± 0.049, 28.770 ± 1.032, 30.000 ±

0.000, 29.750 ± 0.353 and 30.650 ± 0.212 $^{o}$C for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water temperature was observed in the culture water contaminated with 0.00ppm of cypermethrin (28.250 ± 0.353 $^{o}$C), while the highest water temperature was observed in the culture water contaminated with 30ppm of cypermethrin (30.650 ± 0.212 $^{o}$C) (Table 1).

After a 72 hours exposure duration, the water temperature of the culture water had a mean and standard deviation values of 28.500 ± 0.707, 28.950 ± 0.070, 28.755 ± 0.346, 30.300 ± 0.282, 30.025 ± 0.035 and 30.250 ± 0.353 $^{o}$C for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water temperature was observed in the culture water contaminated with 0.00ppm of cypermethrin (28.500 ± 0.707 $^{o}$C), while the highest water temperature was observed in the culture water contaminated with 20ppm of cypermethrin (0.025 ± 0.035$^{o}$C) (Table 1).

After a period of 96 hours, the water temperature of the culture water had a mean and standard deviation values of 28.500 ± 0.707, 29.150 ± 0.494, 28.750 ± 0.353, 25.250 ± 6.717, 29.755 ± 0.360 and 28.950 ± 0.070 $^{o}$C for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water temperature was observed in the culture water contaminated with 15ppm of cypermethrin (25.250 ± 6.717 $^{o}$C), while the highest water temperature was observed in the culture water contaminated with 20ppm of cypermethrin (29.755 ± 0.360 $^{o}$C) (Table 1).

**Table 1: The alterations in the temperature ($^{o}$C) of culture water contaminated with different concentrations of cypermethrin**

| | Temperature ($^{o}$C) | | | | | | |
|---|---|---|---|---|---|---|---|
| Exposure Duration | 0.00 ppm (control) | 5ppm | 10ppm | 15ppm | 20ppm | 30ppm | WHO limit |
| 24 Hours | 29.000 ± 0.000$^{a}$ | 29.250 ± 0.353$^{b}$ | 28.965 ± 0.091$^{c}$ | 28.025 ± 0.035$^{d}$ | 29.025 ± 0.035$^{e}$ | 29.265 ± 0.332$^{f}$ | |
| 48 Hours | 28.250 ± 0.353$^{a}$ | 29.035 ± 0.049$^{b}$ | 28.770 ± 1.032$^{c}$ | 30.000 ± 0.000$^{d}$ | 29.750 ± 0.353$^{e}$ | 30.650 ± 0.212$^{f}$ | 20 – 32$^{o}$C |
| 72 Hours | 28.500 ± 0.707$^{a}$ | 28.950 ± 0.070$^{b}$ | 28.775 ± 0.346$^{c}$ | 30.300 ± 0.282$^{d}$ | 30.025 ± 0.035$^{e}$ | 30.250 ± 0.353$^{f}$ | |
| 96 Hours | 28.500 ± 0.707$^{a}$ | 29.000 ± 0.494$^{a}$ | 28.750 ± 0.353$^{a}$ | 25.250 ± 6.717$^{a}$ | 29.755 ± 0.360$^{a}$ | 28.950 ± 0.070$^{a}$ | |

Values are in mean ± Standard deviation

Values with different superscript are significantly different at P<0.05

The water temperature of the culture water varied across the different treatment group for through-out the observed duration. Statistically, the water temperature varied significantly between the culture water contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48 and 72 hours period at $p<0.05$, while that of 96 hour duration did not vary significantly between the 0.00, 5, 10, 15, 20 and 30ppm cypermethrin contaminated group at $p>0.05$. However, the water temperature of each culture water group through-out the duration observed were all within the WHO acceptable limits (Table 1).

**Hydrogen ion concentration (pH)**

The summary of the pH alterations of the culture water contaminated with different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 2. After a period of 24 hours, the pH of the culture water had a mean and standard deviation values of 5.915 ± 0.021, 6.435 ± 0.544, 6.510 ± 0.014, 6.855 ± 0.077, 7.905 ± 0.007 and 8.005 ± 0.007 for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest pH was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (5.915 ± 0.021), while the highest pH was observed in the culture water contaminated with 30ppm of cypermethrin (8.005 ± 0.007) (Table 2).

**Table 2: The alterations in the pH ($^{o}$C) of culture water contaminated with different concentrations of cypermethrin**

| | pH values | | | | | | |
|---|---|---|---|---|---|---|---|
| Exposure Duration | 0.00 ppm (control) | 5ppm | 10ppm | 15ppm | 20ppm | 30ppm | WHO limit |
| 24 Hours | 5.915 ± 0.021 [a] | 6.435 ± 0.544 [b] | 6.510 ± 0.014 [c] | 6.855 ± 0.077 [d] | 7.905 ± 0.007 [e] | 8.005 ± 0.007 [f] | |
| 48 Hours | 5.915 ± 0.021 [a] | 6.200 ± 0.565 [b] | 6.250 ± 0.353 [c] | 6.320 ± 0.014 [d] | 7.250 ± 0.353 [e] | 7.950 ± 0.070 [f] | 6.5 – 8.5 |
| 72 Hours | 5.700 ± 0.282 [a] | 6.475 ± 0.063 [b] | 6.950 ± 0.070 [c] | 7.425 ± 0.530 [d] | 7.900 ± 0.000 [e] | 7.950 ± 0.070 [f] | |
| 96 Hours | 5.950 ± 0.070 [a] | 6.950 ± 0.070 [b] | 7.840 ± 0.014 [c] | 7.875 ± 0.035 [d] | 8.125 ± 0.035 [e] | 8.955 ± 0.063 [f] | |

Values are in mean ± Standard deviation

Values with different superscript are significantly different at $P<0.05$

After a 48 hours exposure duration, the pH of the culture water had a mean and standard deviation values of 5.915 ± 0.021, 6.200 ± 0.565, 6.250 ± 0.353, 6.320 ± 0.014, 7.250 ± 0.353 and 7.950 ± 0.070 for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest pH was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (5.915 ± 0.021), while the

highest water pH was observed in the culture water contaminated with 30ppm of cypermethrin (7.950 ± 0.070) (Table 2).

After a 72 hours exposure duration, the pH of the culture water had a mean and standard deviation values of 5.700 ± 0.282, 6.475 ± 0.063, 6.950 ± 0.070, 7.425 ± 0.530, 7.900 ± 0.000 and 7.950 ± 0.070 for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest pH was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (5.700 ± 0.282), while the highest water temperature was observed in the culture water contaminated with 30ppm of cypermethrin (7.950 ± 0.070) (Table 2).

After a period of 96 hours, the pH of the culture water had a mean and standard deviation values of 5.950 ± 0.070, 6.950 ± 0.070, 7.840 ± 0.014, 7.875 ± 0.035, 8.125 ± 0.035 and 8.955 ± 0.063 for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest pH was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (5.950 ± 0.070), while the highest pH was observed in the culture water contaminated with 30ppm of cypermethrin (8.955 ± 0.063) (Table 2).

The pH of the culture water varied across the different treatment group, increasing with increase in the concentration of the toxicant through-out the observed duration. Statistically, the pH varied significantly between the culture water contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96 hours period at $p<0.05$. However, the pH of each culture water group through-out the duration observed were all within the WHO acceptable limits, except for the 30ppm group over 96 hours duration (Table 2).

**Dissolved oxygen (DO) (mg/L)**

The summary of the dissolved oxygen (DO) alterations of the culture water contaminated with different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 3. After a period of 24 hours, the DO of the culture water had a mean and standard deviation values of 6.960 ± 0.042, 6.950 ± 0.000, 6.855 ± 0.035, 6.580 ± 0.148, 6.560 ± 0.070 and 6.460 ± 0.212 mg/L for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the culture water contaminated with 30ppm of cypermethrin (6.460 ± 0.212 mg/L), while the highest DO was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (6.960 ± 0.042 mg/L) (Table 3).

**Table 3: The alterations in the dissolved oxygen (mg/l) of culture water contaminated with different concentrations of cypermethrin**

| | Dissolved Oxygen (mg/l) | | | | | | |
|---|---|---|---|---|---|---|---|
| Exposure Duration | 0.00 ppm (control) | 5ppm | 10ppm | 15ppm | 20ppm | 30ppm | WHO limit |
| 24 Hours | 6.960 ± 0.042 [a] | 6.950 ± 0.000 [b] | 6.855 ± 0.035 [c] | 6.580 ± 0.148 [d] | 6.560 ± 0.070 [e] | 6.460 ± 0.212 [f] | |
| 48 Hours | 6.960 ± 0.084 [a] | 6.875 ± 0.063 [b] | 6.775 ± 0.035 [c] | 6.505 ± 0.120 [d] | 6.465 ± 0.077 [e] | 6.020 ± 0.268 [f] | >6 |
| 72 Hours | 6.875 ± 0.035 [a] | 6.825 ± 0.035 [b] | 6.700 ± 0.028 [c] | 6.440 ± 0.056 [d] | 6.205 ± 0.007 [e] | 4.620 ± 0.862 [f] | |
| 96 Hours | 6.555 ± 0.035 [a] | 6.435 ± 0.021 [b] | 6.375 ± 0.007 [c] | 6.365 ± 0.035 [d] | 6.355 ± 0.205 [e] | 4.415 ± 0.558 [f] | |

Values are in mean ± Standard deviation

Values with different superscript are significantly different at $P<0.05$

After a 48 hours duration, the DO of the culture water had a mean and standard deviation values of 6.960 ± 0.084, 6.875 ± 0.063, 6.775 ± 0.035, 6.505 ± 0.120, 6.465 ± 0.077 and 6.020 ± 0.268 mg/L for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the culture water contaminated with 30ppm of cypermethrin (6.020 ± 0.268 mg/L), while the highest DO was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (6.960 ± 0.084 mg/L) (Table 3).

After a 72 hours duration, the DO of the culture water had a mean and standard deviation values of 6.875 ± 0.035, 6.825 ± 0.035, 6.700 ± 0.028, 6.400 ± 0.056, 6.205 ± 0.007 and 4.620 ± 0.862 mg/L for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the culture water contaminated with 30ppm of cypermethrin (4.620 ± 0.862 mg/L), while the highest pH was observed in the culture water contaminated with 0.00ppm of cypermethrin (6.875 ± 0.035 mg/L) (Table 3).

After a period of 96 hours, the DO of the culture water had a mean and standard deviation values of 6.555 ± 0.035, 6.435 ± 0.021, 6.375 ± 0.007, 6.365 ± 0.035, 6.355 ± 0.205 and 4.415 ± 0.558 mg/L for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the culture water contaminated with 30ppm of cypermethrin (4.415 ± 0.558 mg/L), while the highest DO was

observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (6.555 ± 0.035 mg/L) (Table 3).

The DO of the culture water varied across the different treatment group, decreasing with increase in the concentration of the toxicant through-out the observed duration. Statistically, the DO varied significantly between the culture water contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96 hours period at $p<0.05$. However, the DO of each culture water group through-out the duration observed were all within the WHO acceptable limits, except for the 30ppm group over 72 and 96 hours observed duration (Table 3).

**Water conductivity (µs/cm)**

The summary of the water conductivity alterations of the culture water contaminated with different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 4. After a period of 24 hours, the conductivity of the culture water had a mean and standard deviation values of 165.000 ± 0.000, 165.500 ± 0.707, 166.500 ± 0.707, 168.000 ± 1.414, 168.500 ± 0.707 and 170.500 ± 0.707 µs/cm for the culture water contaminated with 0.00 (control), 5, 10, 15, 20

**Table 4: The alterations in the conductivity (µs/cm) of culture water contaminated with different concentrations of cypermethrin**

| | Conductivity (µs/cm) | | | | | | |
|---|---|---|---|---|---|---|---|
| Exposure Duration | 0.00 ppm (control) | 5ppm | 10ppm | 15ppm | 20ppm | 30ppm | WHO limit |
| 24 Hours | 165.000 ± 0.00[a] | 165.500 ± 0.71[b] | 166.500 ± 0.71[c] | 168.000 ± 1.41[d] | 168.500 ± 0.71[e] | 170.500 ± 0.71[f] | |
| 48 Hours | 165.000 ± 0.00[a] | 167.000 ± 0.41[b] | 168.000 ± 0.00[c] | 168.500 ± 0.71[d] | 172.500 ± 3.53[e] | 177.00 ± 2.828[f] | 250 µs/cm |
| 72 Hours | 165.500 ± 0.70[a] | 166.000 ± 0.00[b] | 171.500 ± 0.71[c] | 176.500 ± 0.71[d] | 178.500 ± 2.12[e] | 180.500 ± 0.71[f] | |
| 96 Hours | 165.000 ± 0.00[a] | 170.500 ± 0.71[b] | 171.500 ± 0.71[c] | 185.500 ± 0.71[d] | 189.000 ± 1.41[e] | 189.500 ± 0.71[f] | |

Values are in mean ± Standard deviation

Values with different superscript are significantly different at $P<0.05$

and 30 ppm of cypermethrin respectively. The lowest conductivity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (165.000 ± 0.000 µs/cm), while the highest DO was observed in the culture water contaminated with 30ppm of cypermethrin (170.500 ± 0.707 µs/cm) (Table 4).

After a 48 hours exposure duration, the conductivity of the culture water had a mean and standard deviation values of 165.000 ± 0.000, 167.000 ± 1.414, 168.000 ± 0.000, 168.500 ± 0.707, 172.500 ± 3.535 and 177.000 ± 2.828 µs/cm for the culture water contaminated with 0.00(control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest conductivity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (165.000 ± 0.000 µs/cm), while the highest conductivity was observed in the culture water contaminated with 30ppm of cypermethrin (177.000 ± 2.828 µs/cm) (Table 4).

After a 72 hours exposure duration, the conductivity of the culture water had a mean and standard deviation values of 165.500 ± 0.707, 166.000 ± 0.000, 171.500 ± 0.707, 176.500 ± 0.707, 178.500 ± 2.120 and 180.500 ± 0.707 µs/cm for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest conductivity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (165.500 ± 0.707 µs/cm), while the highest conductivity was observed in the culture water contaminated with 30ppm of cypermethrin (180.500 ± 0.707 µs/cm) (Table 4).

After a period of 96 hours, the conductivity of the culture water had a mean and standard deviation values of 165.000 ± 0.000, 170.500 ± 0.707, 171.500 ± 0.707, 185.500 ± 0.707, 189.000 ± 1.414 and 189.500 ± 0.707 µs/cm for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest conductivity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (165.000 ± 0.000 µs/cm), while the highest conductivity was observed in the culture water contaminated with 30ppm of cypermethrin (189.500 ± 0.707 µs/cm) (Table 4).

The conductivity of the culture water varied across the different treatment group, increasing with increase in the concentration of the toxicant through-out the observed duration. Statistically, the conductivity varied significantly between the culture water contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96 hours period at $p<0.05$. However, the conductivity of each culture water group were all within the WHO acceptable limits (Table 4).

**Turbidity (N.T.U)**

The summary of the turbidity alterations of the culture water contaminated with different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 5. After a period of 24 hours, the turbidity of the culture water had a mean and standard deviation values of 3.600 ± 0.000, 9.850 ± 0.212, 19.100 ± 0.141, 19.650 ± 0.212, 39.900 ± 0.141 and 40.650 ± 0.212 Nephelometric turbidity unit (N.T.U) for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The

lowest turbidity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (3.600 ± 0.000 N.T.U), while the highest conductivity was observed in the culture water contaminated with 30ppm of cypermethrin (0.650 ± 0.212 N.T.U) (Table 5).

After a 48 hours exposure duration, the turbidity of the culture water had a mean and standard deviation values of 3.600 ± 0.000, 10.800 ± 0.141, 19.850 ± 0.070, 19.950 ± 0.707, 40.505 ± 0.007 and 41.750 ± 0.353 N.T.U for the culture water contaminated with 0.00(control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest conductivity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (3.600 ± 0.000 N.T.U), while the highest turbidity was observed in the culture water contaminated with 30ppm of cypermethrin (41.750 ± 0.353 N.T.U) (Table 5).

**Table 5: The alterations in the Turbidity (N.T.U) of culture water contaminated with different concentrations of cypermethrin**

| | Turbidity (N.T.U) | | | | | | |
|---|---|---|---|---|---|---|---|
| Exposure Duration | 0.00 ppm (control) | 5ppm | 10ppm | 15ppm | 20ppm | 30ppm | WHO limit |
| 24 Hours | 3.600 ± 0.000[a] | 9.850 ± 0.212[b] | 19.100 ± 0.141[c] | 19.650 ± 0.212[d] | 39.900 ± 0.141[e] | 40.650 ± 0.212[f] | |
| 48 Hours | 3.600 ± 0.000[a] | 10.800 ± 0.141[b] | 19.850 ± 0.070[c] | 19.950 ± 0.070[d] | 40.505 ± 0.007[e] | 41.750 ± 0.353[f] | 5 |
| 72 Hours | 3.650 ± 0.070[a] | 12.750 ± 0.070[b] | 19.850 ± 0.070[c] | 19.900 ± 0.707[d] | 41.750 ± 0.353[e] | 42.250 ± 0.353[f] | |
| 96 Hours | 3.700 ± 0.494[a] | 16.260 ± 0.339[b] | 26.500 ± 0.282[c] | 27.010 ± 0.014[d] | 47.475 ± 0.601[e] | 47.545 ± 0.643[f] | |

Values are in mean ± Standard deviation

Values with different superscript are significantly different at $P<0.05$

After a 72 hours exposure duration, the turbidity of the culture water had a mean and standard deviation values of 3.650 ± 0.070, 12.750 ± 0.070, 19.850 ± 0.070, 19.950 ± 0.707, 41.750 ± 0.353 and 42.250 ± 0.353 N.T.U for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest turbidity was observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (3.650 ± 0.070 N.T.U), while the highest turbidity was observed in the culture water contaminated with 30ppm of cypermethrin (42.250 ± 0.353 N.T.U) (Table 5).

After a period of 96 hours, the turbidity of the culture water had a mean and standard deviation values of 3.700 ± 0.494, 16.260 ± 0.339, 26.500 ± 0.282, 27.010 ± 0.014, 47.475 ± 0.601 and 47.545 ± 0.643 N.T.U for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest turbidity was observed in the

culture water contaminated with 0.00ppm (control) of cypermethrin (3.700 ± 0.494 N.T.U), while the highest turbidity was observed in the culture water contaminated with 30ppm of cypermethrin (47.545 ± 0.643 N.T.U) (Table 5).

The turbidity of the culture water varied across the different treatment group, increasing with increase in the concentration of the toxicant through-out the observed duration. Statistically, the turbidity varied significantly between the culture water contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96 hours period at $p<0.05$. However, the turbidity of each culture water group were all above the WHO acceptable limits except for the control group (Table 5).

### 3.2 Mortality and survival profile of *Clarias gariepinus* fingerlings

The summary of the survival and mortality profile of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin is shown in Table 6. The *C. gariepinus* fingerlings exposed to 0.00ppm (control) concentration of cypermethrin had 10 survivors (100% survival). No fingerlings mortality was recorded in the control group (0% mortality). The 5ppm concentration of the toxicant recorded 8 survivors (80% survivor) and a mortality of 2 (20% mortality). The 10ppm toxicant concentration recorded 6 survivors (60% survivor), with a mortality of 4 (40% mortality). The 15ppm concentration of cypermethrin recorded 4 survivors (40% fingerlings), while mortality of 6 was recorded (60% fingerlings mortality). The 20ppm concentration of the toxicant recorded 3 survivor (30% fingerlings survivor) and a mortality of 7 (70% fingerlings mortality). No fingerlings survived in the 30ppm cypermethrin treatment group (0% survival), but all the fingerlings died after 96 hours of exposure (100% mortality) (Table 6).

**Table 6: A 96 Hrs survival and mortality profile of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin**

| Cypermethrin Concentration (ppm) | Survival | % Survival | Mortality | % Mortality |
|---|---|---|---|---|
| 0 (control) | 10 | 100 | 0 | 0 |
| 5 | 8 | 80 | 2 | 20 |
| 10 | 6 | 60 | 4 | 40 |
| 15 | 4 | 40 | 6 | 60 |
| 20 | 3 | 30 | 7 | 70 |
| 30 | 0 | 0 | 10 | 100 |

### 3.3 A 96 hours probit transformation

The summary of the probit transformation mortality data for *C. gariepinus* exposed to different concentration of cypermethrin is shown in Table 7. The mortality data trend of fingerlings exposed to cypermethrin were concentration dependent (Table 6). The fingerlings of *C. gariepinus*

**Table 7: A 96 Hrs Probit Transformation of mortality data of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin**

| Conc (ppm) | Log Conc (x) | N | R | P | $M_R$ | Y | $R_P$ | *P* |
|---|---|---|---|---|---|---|---|---|
| 0 (control) | 0.00 | 10 | 0 | 0.00 | 0 | 0.00 | 0.00 | 0.00 |
| 5 | 0.699 | 10 | 2 | 0.20 | 20 | 1.428 | 0.572 | 0.143 |
| 10 | 1.000 | 10 | 4 | 0.40 | 40 | 4.417 | -0.417 | 0.442 |
| 15 | 1.176 | 10 | 6 | 0.60 | 60 | 6.525 | -0.525 | 0.653 |
| 20 | 1.301 | 10 | 7 | 0.70 | 70 | 7.807 | -0.807 | 0.781 |
| 30 | 1.447 | 10 | 10 | 1.00 | 100 | 9.055 | 0.945 | 0.905 |

n = Number of fish fingerling tested at each concentration, r = Number of fish fingerling responding, p = Response rate, r/n, $M_R$ = Mortality rate, Y = Expected probit from visual regression line, $R_P$ = Residual probit, *P* = Probability

showed signs of stress, erratic behaviour and gasping for air when exposed to different concentrations of cypermethrin, due to respiratory impairment.

The regression equation for the probit transformation of *C. gariepinus* fingerlings exposed to different concentration of cypermethrin was y = 63.454X – 11.45 (Table 8) and was significant at $P<0.05$, yielding a determination coefficient ($r^2$) of 0.88 (Table 8), a chi-square

**Table 8: Results of regression analysis of 96 Hrs Log Concentration–probit relationship of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin**

| Conc. (Log Unit) | Response rate, p | Equation | Co-efficient of determination, $r^2$ | Significant level, α |
|---|---|---|---|---|
| 0.00 | 0.00 | | | |
| 0.699 | 0.20 | | | |
| 1.000 | 0.40 | Y = 63.454X – 11.451 | 0.88 | 0.05 (Sig) |
| 1.176 | 0.60 | | | |
| 1.301 | 0.70 | | | |
| 1.477 | 1.00 | | | |

**Table 9: Chi-square Tests of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin**

| | Chi square | df[a] | Sig. |
|---|---|---|---|
| PROBIT Pearson Goodness-of-FitTest | 1.884 | 3 | 0.88[a] |

**Table 10: $LC_{50}$ with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to concentrations of cypermethrin**

| $LC_{50}$ with ± 95%CL | Confidence limits | |
|---|---|---|
| | Lower | Upper |
| 9.332ppm ± 0.839 | 12.76 | 14.44 |

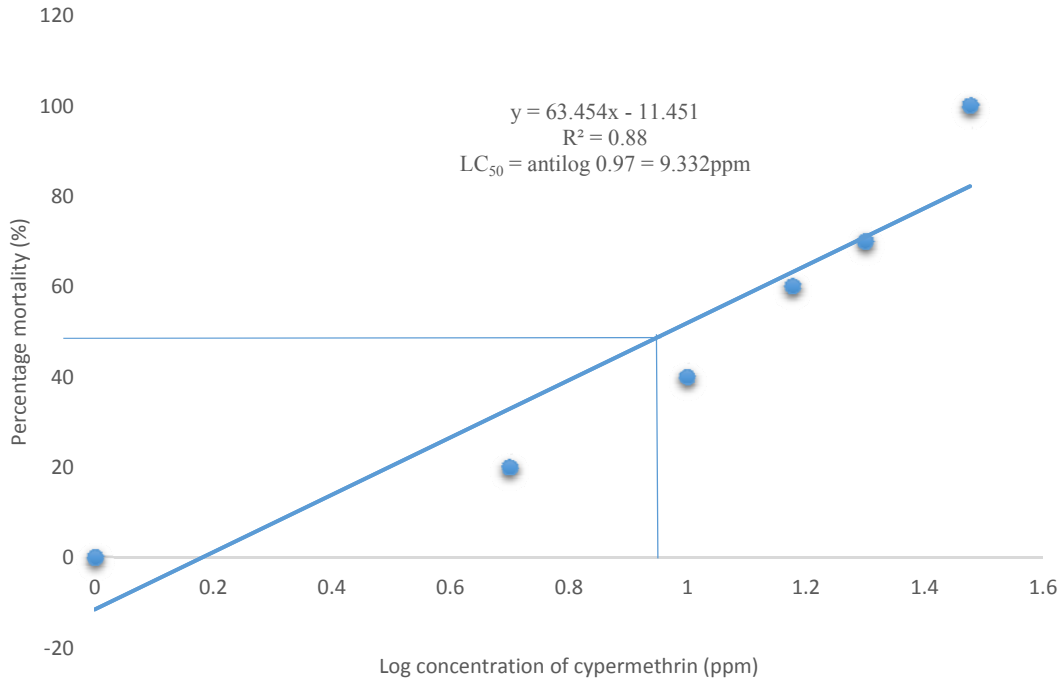


**Fig 1: Probit transformation graph of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin**

value of 1.884 (Table 9), and a 96 hours $LC_{50}$ with 95% confidence limit of 9.332ppm ± 0.839 (Figure 1) (Table 10) and a lower and upper limit values of 12.76 and 14.44 respectively (Table 10).

## 4.DISCUSSION

Cypermethrin are deliberately added to the environment in large quality by agro-farmers to control pest, and this in turn pollute our aquatic environment [8]. The presence of environmental stress such as low dissolved oxygen, high temperature and high ammonia reduces the ability of organisms to maintain its internal environment (i.e. metabolism, catabolism) [24]. Fish growth depends on water quality to boost its production and physicochemical parameters are known to affect the biotic components of an aquatic environment in various ways. Cypermethrin is a globally used for the control of pest, in order to improve food productivity [1], but their use creates risk of food contamination as well as affects the non-target aquatic species like fish [2]. It is a synthetic pyrethroid, with a very high activity and stability [3]. The response of fish to variety of metal and organic pollutants are transient and are dependent on species, enzymes and single or mixed contaminants [25]. Water pollution affects organisms and plants that lives in these water bodies and in almost all

cases, the effect is damaging not only to the individual specie and populations, but also to the natural biological communities [26]. When pesticides are applied on farmlands, only 1% gets to the target organism, as most of these chemicals remain in the environment, and as such the pollution of the environment on the long run is inevitable [27].

The present study revealed variations and alterations in the physico-chemical parameters of contaminated with different concentrations of cypermethrin. The water temperature, pH, electrical conductivity and turbidity of the contaminated culture water increased with increase in the concentration of the toxicant, this corroborated with the report of [28] who also reported an increase in pH, electrical conductivity and turbidity with increase in toxicant concentration. Moreover, dissolved oxygen (DO) decreased with increase in the toxicant concentration, this corroborated with the findings of [29] who also observed a decrease in the DO values of culture water when contaminated with cypermethrin. The decrease in the DO and increase in pH, turbidity could be due to the increase in the microbial activities and bio-chemical oxygen demand as a result of the introduction of the toxicant. Also, the increase in the conductivity of culture water with increased toxicant concentration could be due to the increased chemical ions associated with the chemical. In general, temperature, conductivity, pH values were higher and the DO level was lower in the aquarium contaminated with the highest concentration of the toxicant (30ppm cypermethrin concentration) than in the control aquarium. Statistically, the physico-chemical parameters varied significantly across the culture water group contaminated with different concentrations of toxicant over all durations of contamination at $p<0.05$, except for water temperature over 96 hours exposure period which was insignificant at $p>0.05$ and this was contrary to the report of [30] who reported insignificant alterations in all physico-chemical parameters but dissolved oxygen.

The range of the water temperature, dissolved oxygen, pH, turbidity and DO of culture water observed in the culture water contaminated with cypermethrin in the present study were not within the same range reported by [28, 29]. The pH and conductivity range of the present study was lower, but temperature and DO range were higher than that reported by [28, 29]. The variation between the findings could be due to the difference in the toxicants, concentration of the toxicants and differences in chemical components of the test toxicant [30]. The water temperature, pH and conductivity of the culture water were within the WHO acceptable limits except the dissolved oxygen (30ppm group over 72 and 96 hour duration) and turbidity (control group) which were above the WHO permissible limit, and as a result,

512 the toxicant made the water contaminated and unconducive for the fingerlings thereby
513 causing mortality. Even as most of the water parameters were within the WHO acceptable
514 standard after 96 hours of contamination with the toxicant, there is a high tendency of a
515 chronic contamination of the water over a long period of time, leading to its pollution. Apart
516 from the alteration of the water and fingerlings mortality, the fish (biological organisms)
517 could accumulate the toxicants from the toxicant into their tissues, which are consumed by
518 humans, leading to a lot of health challenges.

519 The toxicity of cypermethrin on *Clarias gariepinus* fingerlings observed for the
520 present study was concentration and duration dependent, with mortality increasing with
521 increase in the concentration of the toxicant as well as exposure duration and this
522 corroborated with the findings of [30, 31, 32], who also observed that toxicity of test
523 toxicants were concentration and duration dependent. The fingerlings of *C. gariepinus*
524 exposed to different concentrations of the cypermethrin showed abnormal behaviours
525 changes and appearence like; repeated darting movement within an hour of introduction,
526 darkening in the eye and skin, spiral swimming, death, erratic swimming and loss of balance
527 due to impaired metabolism and nervous disorder (respiratory impairment), and this was
528 similar to the findings of [30, 31, 32] for *C. gariepinus*, [33] for *Thevetia nerifolia*, [34] for
529 *Thevetia peruviana*, [35] for *Azardirachta indica*, [36, 37] for *C. gariepinus*, [38] for
530 *Oreochromis mossambicus* and [39] for *C. gariepinus*, who all reported similar changes in
531 behaviour of fingerlings when exposed to chemicals. The respiratory distress of test
532 fingerlings exposed to the cypermethrin may be due to decrease in the dissolved oxygen
533 contents in the culture water [40].

534 As observed in the present study, no mortality was observed in the control group, but
535 mortality was recorded for the 5ppm group upwards and similar result was observed by [41].
536 The 96 hours $LC_{50}$ with 95% confidence limit for *C. gariepinus* exposed to different
537 concentrations of cypermethrin was 9.332ppm, indicating its high toxicity. The 96 hours $LC_{50}$
538 value observed for cypermethrin on *C. gariepinus* in the present study was higher than those
539 reported by [30] (1.80ppm) who evaluated the toxicological and histopathological changes of
540 *C. gariepinus* exposed to cypermethrin, [42] (0.04ppm) who carried-out a histological study
541 on the intestine and liver tissues of *Oreochromis mossambucus* exposed to cypermethrin and
542 [41] (0.60ppm) who studied the acute toxicity of mercury to *C. gariepinus*. These
543 discrepancies in the 96 hours $LC_{50}$ value of the different study could be due to the difference
544 in components of the toxicant, difference in toxicant, toxicity of the chemicals, fish species

and age of fingerlings used. The difference could also be due to the fact that the response of fish to variety of metal and organic pollutants are transient and are dependent on species, enzymes and single or mixed contaminants [25]. Also, the difference in the toxicity of cypermethrin in the present study compared to that observed in the aforementioned findings could be due to difference in biological species, difference in elimination and metabolic degradation from the body [9]. The relatively low $LC_{50}$ value observed for the present study denotes that cypermethrin are highly toxic to *Clarias gariepinus* fingerlings causing the mortality of the fingerlings, bio-accumulation in the fish tissues, resulting in high risk to public health for the consumers of such contaminated aquatic resources.

## 5. CONCLUSION

In conclusion, the cypermethrin caused significant alterations in the physicochemical parameters of water, compared to the control aquarium water, increasing in some cases (temperature, pH, turbidity and conductivity), and reducing in some cases (DO). Also, the toxicant raised some water parameters to undesired levels, leading to the bio-accumulation of toxicants in the fingerlings. The toxicological effects of the toxicant was concentration and duration dependent**.** The cypermethrin was highly toxic to the fingerlings, causing mortality in the process, as a result, more research of this kind should be carried-out involving haemathological, reproductive, histological and other physiological alterations due to exposure of *C. gariepinus* to cypermethrin, so as to further reveal the toxic and harmful potentials of pesticides.

## ETHICAL CONSIDERATION

The authors ensured that all ethical and other basic principles underlying behavior and advancing welfare for the use of animals in research, including handling, relevant laws and regulations were considered before proceeding with the research. Bio-ethical clearance for use of animals for laboratory studies was issued out by Animal Use and Care Committee of the national and veterinary research institute of Nigeria, with the code number of nuriAUCC/F035/18.

## COMPETING INTEREST

Authors have declared that no competing interests exist, but rather the research was a collective effort of all the authors.

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