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original research article

Comment [WU1]:

TOXICOLOGICAL RESPONSES OF AFRICAN MUD CATFISH (*Clarias gariepinus*, Burchell, 1822) FINGERLINGS EXPOSED TO CULTURE WATER CONTAMINATED WITH 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 30 (why the leap jump to 30 from 20?) ppm 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 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 **26** temperature, pH and conductivity of the culture water were within the WHO acceptable limits **27** except for the dissolved oxygen (30ppm group over 72 and 96 hour exposure duration) and turbidity (5, 10, 15, 20 and 20??ppm group) which were above the 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 **35** physico-chemical parameters of the culture water. As a result, more **similar** research should **36** be carried-out involving haemathological, reproductive, histological and other physiological **37** alterations when fishes are exposed to cypermethrin so as to further reveal the toxic and **38** harmful potentials of pesticides.

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UNDER PEER REVIEW

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 quantity**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].

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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.

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

C 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

UNDER PEER REVIEW

to get used to the laboratory conditions. During the acclimation, the fingerlings were fed twice daily with coppens 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 various 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 25 (note that this was 30 in the abstract) ppm) author should establish reasons for chosen this test range 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.

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2.7 Measurement of physico-chemical parameters

UNDER PEER REVIEW

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 (°C), 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 (°C)

The surface water temperature was measured in-situ in culture water of each fingerlings group using mercury – in – glass thermometer in degrees Celsius (°c). 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.

UNDER PEER REVIEW

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 (^oC)

The summary of the 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 ^oC 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 ^oC), while the highest water temperature was observed in the culture water contaminated with 30ppm of cypermethrin (29.265 ± 0.332 ^oC) (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 ^oC 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 ^oC), while the highest water temperature was observed in the culture water contaminated with 30ppm of cypermethrin (30.650 ± 0.212 ^oC) (Table 1).

After a 72 hours exposure duration, the water temperature of the culture water had a

UNDER PEER REVIEW

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

UNDER PEER REVIEW

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 (^oC) 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,

UNDER PEER REVIEW

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

UNDER PEER REVIEW

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(25?) ppm of cypermethrin respectively. The lowest DO was observed

UNDER PEER REVIEW

in the culture water contaminated with 30(or 25)ppm 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 (25?)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 30(25?)ppm 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

UNDER PEER REVIEW

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

UNDER PEER REVIEW

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 30(25?)ppm 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(25?) 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

UNDER PEER REVIEW

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	30(25?)ppm	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

UNDER PEER REVIEW

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

UNDER PEER REVIEW

25?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
3025?	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 *Clarias 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

UNDER PEER REVIEW

447

448

449 **Table 8: Results of regression analysis of 96 Hrs Log Concentration–probit relationship**
450 **of *Clarias gariepinus* fingerlings exposed to different concentrations of** 451
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			

UNDER PEER REVIEW

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

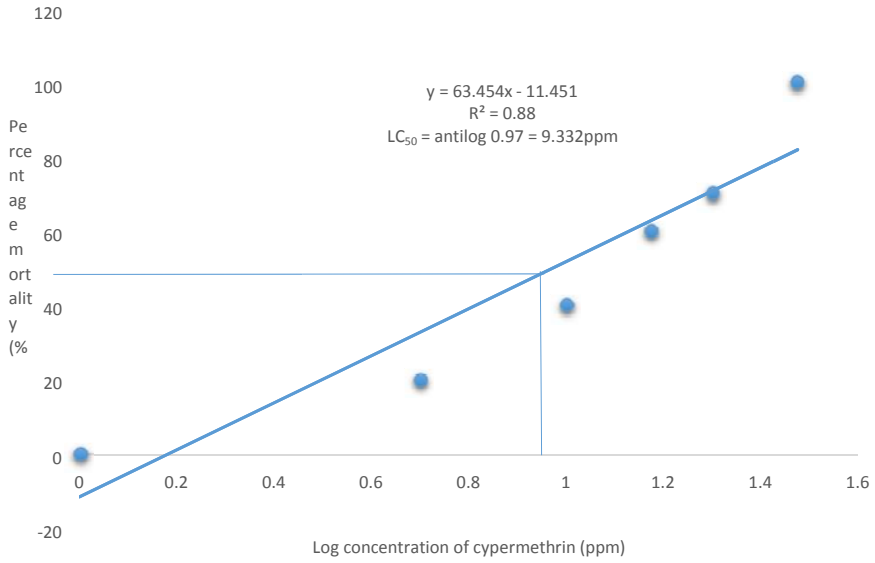

Fig 1: Probit transformation graph of *Clarias gariepinus* fingerlings exposed to different concentrations of cypermethrin

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).

UNDER PEER REVIEW

4.DISCUSSION

Cypermethrin are deliberately added to the environment in large quantity **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 culture water 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 water quality unclear with increase in toxicant concentration. On the other way round, dissolved oxygen (DO) decreased with increase in the toxicant concentration, this corroborated with the reports 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, and 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,

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UNDER PEER REVIEW

pH values were higher and the DO level was lower in the aquarium contaminated with the highest concentration of the toxicant (25 or 30ppm ?? cypermethrin concentration) than in the control aquarium. **Statistically, t**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 can you mention type of toxicants used by other authors???. The water temperature, pH and conductivity of the culture water were within the WHO acceptable limits except the dissolved oxygen (25 or 30ppm group over 72 and 96 hour duration) and turbidity (control group) which were above the WHO permissible limit, and as a result, the toxicant made the water contaminated and unconducive for the fingerlings thereby causing mortality. Even as most of the water parameters were within the WHO acceptable standard after 96 hours of contamination with the toxicant, there is a high tendency of a chronic contamination of the water over a long period of time, leading to its pollution. Apart from the alteration of the water and fingerlings mortality, the fish (biological organisms) could accumulate the toxicants **from the toxicant** into their tissues, which are consumed by humans, leading to a lot of health challenges.

The toxicity of cypermethrin on *Clarias gariepinus* fingerlings observed for the present study was concentration and duration dependent, with mortality increasing with increase in the concentration of the toxicant as well as exposure duration and this corroborated with the findings of [30, 31, 32]. What was their findings??? The fingerlings of *C. gariepinus* exposed to different concentrations of the cypermethrin showed abnormal

UNDER PEER REVIEW

behaviours changes and 525 appearence like; repeated darting movement within an hour of introduction, darkening in the 526 eye and skin, spiral swimming, death, erratic swimming and loss of balance due to impaired 527 metabolism and nervous disorder (respiratory impairment), and this was similar to the 528 findings of [33, 34, 35, 31, 32, 36, 37, 38, 39, 31], who all reported similar changes in

529 behaviour of fingerlings of which species of fish?? when exposed to chemicals. The respiratory distress of test 530 fingerlings exposed to the cypermethrin may be due to decrease in the dissolved oxygen 531 contents in the culture water [40].

532 As observed in the present study, no mortality was observed in the control group, but 533 mortality was recorded for the 5ppm group upwards and similar result was observed by [41]. 534 The 96 hours LC_{50} with 95% confidence limit for *C. gariepinus* exposed to different 535 concentrations of cypermethrin was 9.332ppm, indicating its high toxicity. The 96 hours LC_{50} 536 value observed for cypermethrin on *C. gariepinus* in the present study was higher than those

537 reported by [31] (1.80ppm) who evaluated the toxicological and histopathological changes of

538 *C. gariepinus* exposed to cypermethrin, [42] (0.04ppm) who carried-out a histological study 539 on the intestine and liver tissues of *Oreochromis mossambucus* exposed to cypermethrin and 540 [41] (0.60ppm) who studied the acute toxicity of mercury to *C. gariepinus*. These

541 discrepancies in the 96 hours LC_{50} value of the different study could be due to the difference 542 in components of the toxicant, difference in toxicant, toxicity of the chemicals, fish species

543 and age of fingerlings used. The difference could also be due to the fact that the response of

544 fish to variety of metal and organic pollutants are transient and are dependent on species,

545 enzymes and single or mixed contaminants [25]. Also, the difference in the toxicity of 546 cypermethrin in the present study compared to that observed in the aforementioned findings 547 could be due to difference in biological species, difference in elimination and metabolic

548 degradation from the body [9]. The relatively low LC_{50} value observed for the present study 549 denotes that cypermethrin are highly toxic to *Clarias gariepinus* fingerlings causing the 550 mortality of the fingerlings, bio-accumulation in the fish tissues, resulting in high risk to 551 public health for the consumers of such contaminated aquatic resources.

UNDER PEER REVIEW

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. Permission was also received from the relevant bodies for the use of fish for this experiment.

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