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 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 20ppm 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 $\pm$ 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

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

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

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

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

79   This study was aimed at evaluating the acute toxicity of cypermethrin on the survival
80   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 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,

120   which was carried-out in triplicates. Ten fingerlings of *C. gariepinus* fingerlings were

121   introduced into each aquarium containing 1 litre of water. The fingerlings were then exposed

122   to 5 different concentrations (5, 10, 15, 20 and 25ppm) of the toxicant and there was also a

123   control group that were not exposed to any toxicant. The experiment was carried-out using a

124   static non-renewal bioassay for 96hrs. The mortality and general behavior of fish was also

125   observed after 24, 48, 72 and 96 hours of exposure. Fingerlings were considered dead when

126   they cannot move any longer, even when touched with a glass rod. Dead fingerlings were

127   removed immediately and then its mortality recorded.

128   **2.7 Measurement of physico-chemical parameters**

129   Water quality parameters of the culture water was monitored after 24, 48, 72 and 96

130   hours. The culture water for each fish group were tested in-situ for temperature (^{o}C),

131   Conductivity (µs/cm), pH, dissolved oxygen (mg/L) and turbidity (N.T.U) once the toxicant

132   was introduced. The water parameters were then monitored over the 96 hours period of the

133   experiment, and compared to the control water parameters. This was done in order to find out

134   the effect of cypermethrin on the water quality.

135   **Temperature (^{o}C)**
136

137   The surface water temperature was measured in-situ in culture water of each

138   fingerlings group using mercury – in – glass thermometer in degrees Celsius (^{o}c). The

139   thermometer was inserted at a depth of about 2cm from the surface water for about 3 minutes

140   and the reading taken.

141   **Hydrogen ion concentration (pH)**
142

143   The pH of the water was measured in-situ using a model pH-1 pocket-sized pH meter.

144   The meter glass probe was dipped into the culture water and readings taken.
145

147   **Dissolved oxygen (DO) (mg/l)**
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149   The dissolved oxygen was measured in-situ using a dissolved oxygen meter, model

150   DO-5509, calibrated in mg/L (milligrams per litre).
151

152   **Turbidity (N.T.U)**
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154   The turbidity was measured in-situ using a turbidity meter. The meter was inserted

155   2cm from the water surface for about 2 minutes, and then the turbidity of the culture water

156   read to the nearest N.T.U (Nephelometric turbidity unit).
157

158   **Conductivity (µS/cm)**

159

160   Conductivity was measured in-situ using a using Hannah Instrument (Bench meter

161   211 model). The meter was inserted 2cm from the water surface for about 2 minutes, and

162   then the water conductivity value was taken to the nearest µS/cm.

163   **2.8 Data analysis**

164   The mortality data obtained were subjected to probit logarithm transformation.

165   Regression analysis was also performed and the LC_{50} values was computed. The 95%

166   confidence interval was also computed and the slope of the regression line tested using chi-

167   square. Anova was also used to test for the significance of difference in water quality

168   parameters between each concentration group at 0.05 level of significance and at their

169   relevant degree of freedom. Also descriptive statistics (mean and standard deviation) was

170   carried out on the physicochemical parameters of the contaminated culture water and the

171   control group. Graph was plotted using Microsoft excel (MSE) version 2013. Probit analysis

172   was carried-out using predictive analytical software (PASW) version 20.

173

174   **3.   RESULTS** 💬

175   **3.1 Water quality of culture water**

176

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

178   The summary of the temperature alterations of the culture water contaminated with

179   different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 1.

180   After 24 hours of exposure, the water temperature of the culture water had a mean and

181   standard deviation of 29.000 ± 0.000, 29.250 ± 0.353, 28.965 ± 0.091, 28.025 ± 0.035,

182   29.025 ± 0.035 and 29.265 ± 0.332 ^{o}C when exposed to 0 (control), 5, 10, 15, 20 and 30 ppm

183   of cypermethrin respectively. The lowest water temperature was observed in the culture water

184   contaminated with 15ppm of cypermethrin (28.025 ± 0.035 ^{o}C), while the highest water

185   temperature was observed in the culture water contaminated with 30ppm of cypermethrin

186   (29.265 ± 0.332 ^{o}C) (Table 1).

187   After a 48 hours exposure duration, the water temperature of the culture water had a

188   mean and standard deviation of 28.250 ± 0.353, 29.035 ± 0.049, 28.770 ± 1.032, $30.000 \pm$

189   0.000, 29.750 ± 0.353 and 30.650 ± 0.212 ^{o}C for the culture water contaminated with 0.00

190   (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water

191   temperature was observed in the culture water contaminated with 0.00ppm of cypermethrin

192   (28.250 ± 0.353 ^{o}C), while the highest water temperature was observed in the culture water

193   contaminated with 30ppm of cypermethrin (30.650 ± 0.212 ^{o}C) (Table 1).

194   After a 72 hours exposure duration, the water temperature of the culture water had a
195   mean and standard deviation values of 28.500 ± 0.707, 28.950 ± 0.070, 28.755 ± 0.346,
196   30.300 ± 0.282, 30.025 ± 0.035 and 30.250 ± 0.353 $^{\circ}$C for the culture water contaminated
197   with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water
198   temperature was observed in the culture water contaminated with 0.00ppm of cypermethrin
199   (28.500 ± 0.707 $^{\circ}$C), while the highest water temperature was observed in the culture water
200   contaminated with 20ppm of cypermethrin (0.025 ± 0.035°C) (Table 1).

201   After a period of 96 hours, the water temperature of the culture water had a mean and
202   standard deviation values of 28.500 ± 0.707, 29.150 ± 0.494, 28.750 ± 0.353, 25.250 ± 6.717,
203   29.755 ± 0.360 and 28.950 ± 0.070 $^{\circ}$C for the culture water contaminated with 0.00 (control),
204   5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest water temperature was
205   observed in the culture water contaminated with 15ppm of cypermethrin (25.250 ± 6.717 $^{\circ}$C),
206   while the highest water temperature was observed in the culture water contaminated with
207   20ppm of cypermethrin (29.755 ± 0.360 $^{\circ}$C) (Table 1).

208
209 **Table 1: The alterations in the temperature ($^{\circ}$C) of culture water contaminated with**
210   **different concentrations of cypermethrin**
211

	Temperature ($^{\circ}$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°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]	

213   Values are in mean ± Standard deviation

214   Values with different superscript are significantly different at P<0.05
215

216   The water temperature of the culture water varied across the different treatment group
217   for through-out the observed duration. Statistically, the water temperature varied significantly
218   between the culture water contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin
219   over a 24, 48 and 72 hours period at p<0.05, while that of 96 hour duration did not vary
220   significantly between the 0.00, 5, 10, 15, 20 and 30ppm cypermethrin contaminated group at

221   p>0.05. However, the water temperature of each culture water group through-out the duration

222   observed were all within the WHO acceptable limits (Table 1).

223   **Hydrogen ion concentration (pH)**

224   The summary of the pH alterations of the culture water contaminated with different

225   concentrations of cypermethrin over a 96 hour exposure period is shown in Table 2. After a

226   period of 24 hours, the pH of the culture water had a mean and standard deviation values of

227   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

228   for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of

229   cypermethrin respectively. The lowest pH was observed in the culture water contaminated

230   with 0.00ppm (control) of cypermethrin (5.915 ± 0.021), while the highest pH was observed

231   in the culture water contaminated with 30ppm of cypermethrin (8.005 ± 0.007) (Table 2).

232   **Table 2: The alterations in the pH (^{o}C) of culture water contaminated with different**
233   **concentrations of cypermethrin**
234

	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]	

236   Values are in mean ± Standard deviation

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

238   After a 48 hours exposure duration, the pH of the culture water had a mean and

239   standard deviation values of 5.915 ± 0.021, 6.200 ± 0.565, 6.250 ± 0.353, 6.320 ± 0.014,

240   7.250 ± 0.353 and 7.950 ± 0.070 for the culture water contaminated with 0.00 (control), 5, 10,

241   15, 20 and 30 ppm of cypermethrin respectively. The lowest pH was observed in the culture

242   water contaminated with 0.00ppm (control) of cypermethrin (5.915 ± 0.021), while the

243   highest water pH was observed in the culture water contaminated with 30ppm of

244   cypermethrin (7.950 ± 0.070) (Table 2).

245   After a 72 hours exposure duration, the pH of the culture water had a mean and

246   standard deviation values of 5.700 ± 0.282, 6.475 ± 0.063, 6.950 ± 0.070, 7.425 ± 0.530,

247   7.900 ± 0.000 and 7.950 ± 0.070 for the culture water contaminated with 0.00 (control), 5, 10,

248   15, 20 and 30 ppm of cypermethrin respectively. The lowest pH was observed in the culture

249 water contaminated with 0.00ppm (control) of cypermethrin (5.700 ± 0.282), while the

250 highest water temperature was observed in the culture water contaminated with 30ppm of

251 cypermethrin (7.950 ± 0.070) (Table 2).

252   After a period of 96 hours, the pH of the culture water had a mean and standard

253 deviation values of 5.950 ± 0.070, 6.950 ± 0.070, 7.840 ± 0.014, 7.875 ± 0.035, 8.125 ± 0.035

254 and 8.955 ± 0.063 for the culture water contaminated with 0.00 (control), 5, 10, 15, 20 and 30

255 ppm of cypermethrin respectively. The lowest pH was observed in the culture water

256 contaminated with 0.00ppm (control) of cypermethrin (5.950 ± 0.070), while the highest pH

257 was observed in the culture water contaminated with 30ppm of cypermethrin (8.955 ± 0.063)

258 (Table 2).

259   The pH of the culture water varied across the different treatment group, increasing

260 with increase in the concentration of the toxicant through-out the observed duration.

261 Statistically, the pH varied significantly between the culture water contaminated with 0.00, 5,

262 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96 hours period at $p < 0.05$.

263 However, the pH of each culture water group through-out the duration observed were all

264 within the WHO acceptable limits, except for the 30ppm group over 96 hours duration (Table

265 2).

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

267   The summary of the dissolved oxygen (DO) alterations of the culture water

268 contaminated with different concentrations of cypermethrin over a 96 hour exposure period is

269 shown in Table 3. After a period of 24 hours, the DO of the culture water had a mean and

270 standard deviation values of 6.960 ± 0.042, 6.950 ± 0.000, 6.855 ± 0.035, 6.580 ± 0.148,

271 6.560 ± 0.070 and 6.460 ± 0.212 mg/L for the culture water contaminated with 0.00 (control),

272 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the

273 culture water contaminated with 30ppm of cypermethrin (6.460 ± 0.212 mg/L), while the

274 highest DO was observed in the culture water contaminated with 0.00ppm (control) of

275 cypermethrin (6.960 ± 0.042 mg/L) (Table 3).

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

289 Values are in mean ± Standard deviation

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

291 After a 48 hours duration, the DO of the culture water had a mean and standard
292 deviation values of 6.960 ± 0.084, 6.875 ± 0.063, 6.775 ± 0.035, 6.505 ± 0.120, 6.465 ± 0.077
293 and 6.020 ± 0.268 mg/L for the culture water contaminated with 0.00 (control), 5, 10, 15, 20
294 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the culture water
295 contaminated with 30ppm of cypermethrin (6.020 ± 0.268 mg/L), while the highest DO was
296 observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (6.960 ±
297 0.084 mg/L) (Table 3).

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

305 After a period of 96 hours, the DO of the culture water had a mean and standard
306 deviation values of 6.555 ± 0.035, 6.435 ± 0.021, 6.375 ± 0.007, 6.365 ± 0.035, 6.355 ± 0.205
307 and 4.415 ± 0.558 mg/L for the culture water contaminated with 0.00 (control), 5, 10, 15, 20
308 and 30 ppm of cypermethrin respectively. The lowest DO was observed in the culture water
309 contaminated with 30ppm of cypermethrin (4.415 ± 0.558 mg/L), while the highest DO was
310 observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (6.555 ±
311 0.035 mg/L) (Table 3).

312   The DO of the culture water varied across the different treatment group, decreasing

313 with increase in the concentration of the toxicant through-out the observed duration.

314 Statistically, the DO varied significantly between the culture water contaminated with 0.00, 5,

315 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96 hours period at p<0.05.

316 However, the DO of each culture water group through-out the duration observed were all

317 within the WHO acceptable limits, except for the 30ppm group over 72 and 96 hours

318 observed duration (Table 3).

319

320 **Water conductivity (µs/cm)**

321   The summary of the water conductivity alterations of the culture water contaminated

322 with different concentrations of cypermethrin over a 96 hour exposure period is shown in

323 Table 4. After a period of 24 hours, the conductivity of the culture water had a mean and

324 standard deviation values of 165.000 ± 0.000, 165.500 ± 0.707, 166.500 ± 0.707, 168.000 ±

325 1.414, 168.500 ± 0.707 and 170.500 ± 0.707 µs/cm for the culture water contaminated with

326 0.00 (control), 5, 10, 15, 20

327

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

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	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]	

332 Values are in mean ± Standard deviation

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

334

335 and 30 ppm of cypermethrin respectively. The lowest conductivity was observed in the

336 culture water contaminated with 0.00ppm (control) of cypermethrin (165.000 ± 0.000 µs/cm),

337 while the highest DO was observed in the culture water contaminated with 30ppm of

338 cypermethrin (170.500 ± 0.707 µs/cm) (Table 4).

339   After a 48 hours exposure duration, the conductivity of the culture water had a mean

340 and standard deviation values of 165.000 ± 0.000, 167.000 ± 1.414, 168.000 ± 0.000, 168.500

341 ± 0.707, 172.500 ± 3.535 and 177.000 ± 2.828 μs/cm for the culture water contaminated with

342 0.00(control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest

343 conductivity was observed in the culture water contaminated with 0.00ppm (control) of

344 cypermethrin (165.000 ± 0.000 μs/cm), while the highest conductivity was observed in the

345 culture water contaminated with 30ppm of cypermethrin (177.000 ± 2.828 μs/cm) (Table 4).

346 After a 72 hours exposure duration, the conductivity of the culture water had a mean

347 and standard deviation values of 165.500 ± 0.707, 166.000 ± 0.000, 171.500 ± 0.707, 176.500

348 ± 0.707, 178.500 ± 2.120 and 180.500 ± 0.707 μs/cm for the culture water contaminated with

349 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest

350 conductivity was observed in the culture water contaminated with 0.00ppm (control) of

351 cypermethrin (165.500 ± 0.707 μs/cm), while the highest conductivity was observed in the

352 culture water contaminated with 30ppm of cypermethrin (180.500 ± 0.707 μs/cm) (Table 4).

353 After a period of 96 hours, the conductivity of the culture water had a mean and

354 standard deviation values of 165.000 ± 0.000, 170.500 ± 0.707, 171.500 ± 0.707, 185.500 ±

355 0.707, 189.000 ± 1.414 and 189.500 ± 0.707 μs/cm for the culture water contaminated with

356 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest

357 conductivity was observed in the culture water contaminated with 0.00ppm (control) of

358 cypermethrin (165.000 ± 0.000 μs/cm), while the highest conductivity was observed in the

359 culture water contaminated with 30ppm of cypermethrin (189.500 ± 0.707 μs/cm) (Table 4).

360 The conductivity of the culture water varied across the different treatment group,

361 increasing with increase in the concentration of the toxicant through-out the observed

362 duration. Statistically, the conductivity varied significantly between the culture water

363 contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96

364 hours period at $p < 0.05$. However, the conductivity of each culture water group were all

365 within the WHO acceptable limits (Table 4).

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

367 The summary of the turbidity alterations of the culture water contaminated with

368 different concentrations of cypermethrin over a 96 hour exposure period is shown in Table 5.

369 After a period of 24 hours, the turbidity of the culture water had a mean and standard

370 deviation values of 3.600 ± 0.000, 9.850 ± 0.212, 19.100 ± 0.141, 19.650 ± 0.212, 39.900 ±

371 0.141 and 40.650 ± 0.212 Nephelometric turbidity unit (N.T.U) for the culture water

372 contaminated with 0.00 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The

373 lowest turbidity was observed in the culture water contaminated with 0.00ppm (control) of

374 cypermethrin (3.600 ± 0.000 N.T.U), while the highest conductivity was observed in the

375 culture water contaminated with 30ppm of cypermethrin (0.650 ± 0.212 N.T.U) (Table 5).

376 After a 48 hours exposure duration, the turbidity of the culture water had a mean and

377 standard deviation values of 3.600 ± 0.000, 10.800 ± 0.141, 19.850 ± 0.070, 19.950 ± 0.707,

378 40.505 ± 0.007 and 41.750 ± 0.353 N.T.U for the culture water contaminated with

379 0.00(control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest

380 conductivity was observed in the culture water contaminated with 0.00ppm (control) of

381 cypermethrin (3.600 ± 0.000 N.T.U), while the highest turbidity was observed in the culture

382 water contaminated with 30ppm of cypermethrin (41.750 ± 0.353 N.T.U) (Table 5).

383

384 **Table 5: The alterations in the Turbidity (N.T.U) of culture water contaminated with**
385 **different concentrations of cypermethrin**
386

	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]	

387

388 Values are in mean ± Standard deviation

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

390

391 After a 72 hours exposure duration, the turbidity of the culture water had a mean and

392 standard deviation values of 3.650 ± 0.070, 12.750 ± 0.070, 19.850 ± 0.070, 19.950 ± 0.707,

393 41.750 ± 0.353 and 42.250 ± 0.353 N.T.U for the culture water contaminated with 0.00

394 (control), 5, 10, 15, 20 and 30 ppm of cypermethrin respectively. The lowest turbidity was

395 observed in the culture water contaminated with 0.00ppm (control) of cypermethrin (3.650 ±

396 0.070 N.T.U), while the highest turbidity was observed in the culture water contaminated

397 with 30ppm of cypermethrin (42.250 ± 0.353 N.T.U) (Table 5).

398 After a period of 96 hours, the turbidity of the culture water had a mean and standard

399 deviation values of 3.700 ± 0.494, 16.260 ± 0.339, 26.500 ± 0.282, 27.010 ± 0.014, 47.475 ±

400 0.601 and 47.545 ± 0.643 N.T.U for the culture water contaminated with 0.00 (control), 5, 10,

401 15, 20 and 30 ppm of cypermethrin respectively. The lowest turbidity was observed in the

402   culture water contaminated with 0.00ppm (control) of cypermethrin (3.700 ± 0.494 N.T.U),

403   while the highest turbidity was observed in the culture water contaminated with 30ppm of

404   cypermethrin (47.545 ± 0.643 N.T.U) (Table 5).

405   The turbidity of the culture water varied across the different treatment group,

406   increasing with increase in the concentration of the toxicant through-out the observed

407   duration. Statistically, the turbidity varied significantly between the culture water

408   contaminated with 0.00, 5, 10, 15, 20 and 30ppm of cypermethrin over a 24, 48, 72 and 96

409   hours period at $p < 0.05$. However, the turbidity of each culture water group were all above the

410   WHO acceptable limits except for the control group (Table 5).

411   **3.2 Mortality and survival profile of *Clarias gariepinus* fingerlings**

412   The summary of the survival and mortality profile of *Clarias gariepinus* fingerlings

413   exposed to different concentrations of cypermethrin is shown in Table 6. The *C. gariepinus*

414   fingerlings exposed to 0.00ppm (control) concentration of cypermethrin had 10 survivors

415   (100% survival). No fingerlings mortality was recorded in the control group (0% mortality).

416   The 5ppm concentration of the toxicant recorded 8 survivors (80% survivor) and a mortality

417   of 2 (20% mortality). The 10ppm toxicant concentration recorded 6 survivors (60% survivor),

418   with a mortality of 4 (40% mortality). The 15ppm concentration of cypermethrin recorded 4

419   survivors (40% fingerlings), while mortality of 6 was recorded (60% fingerlings mortality).

420   The 20ppm concentration of the toxicant recorded 3 survivor (30% fingerlings survivor) and

421   a mortality of 7 (70% fingerlings mortality). No fingerlings survived in the 30ppm

422   cypermethrin treatment group (0% survival), but all the fingerlings died after 96 hours of

423   exposure (100% mortality) (Table 6).

424   **Table 6: A 96 Hrs survival and mortality profile of *Clarias gariepinus* fingerlings**

425   **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

426

3.3 A 96 hours probit transformation

428   The summary of the probit transformation mortality data for *C. gariepinus* exposed to
429 different concentration of cypermethrin is shown in Table 7. The mortality data trend of
430 fingerlings exposed to cypermethrin were concentration dependent (Table 6). The fingerlings
431 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

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

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

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

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			

452
453 **Table 9: Chi-square Tests of *Clarias gariepinus* fingerlings exposed to different**
454 **concentrations of cypermethrin**
455

	Chi square	df^a	Sig.
PROBIT Pearson Goodness-of-FitTest	1.884	3	0.88^a

456
457
458 **Table 10: LC_{50} with 95% confidence limits of *Clarias gariepinus* fingerlings exposed to**
459 **concentrations of cypermethrin**

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

460
461

462
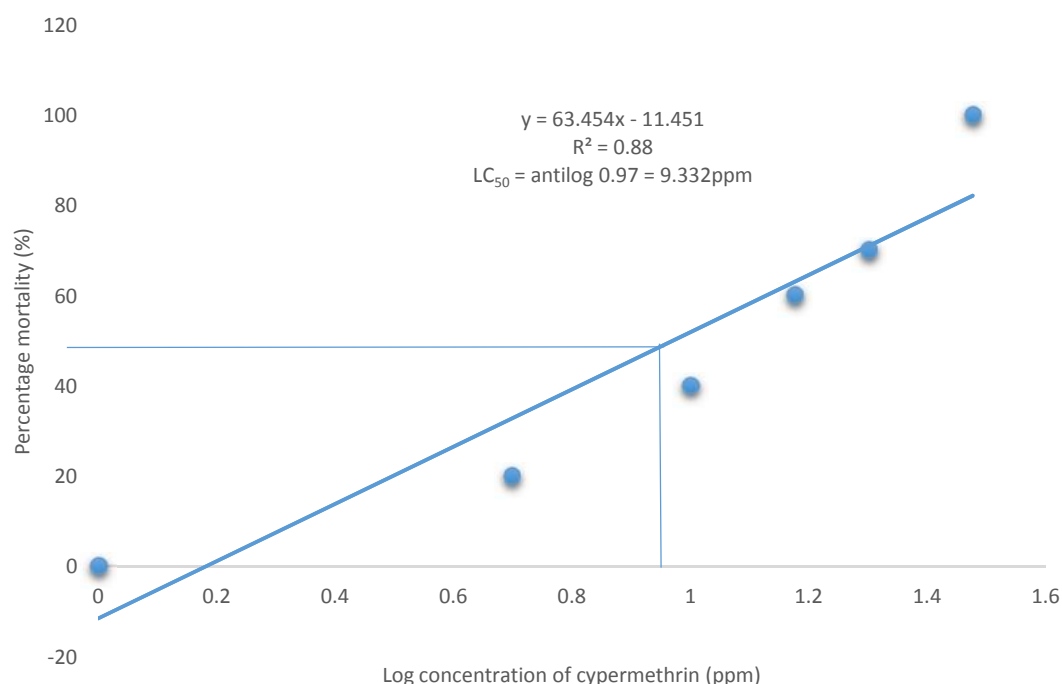
463 **Fig 1: Probit transformation graph of *Clarias gariepinus* fingerlings exposed to different**
464 **concentrations of cypermethrin**

465 value of 1.884 (Table 9), and a 96 hours LC_{50} with 95% confidence limit of 9.332ppm ±
466 0.839 (Figure 1) (Table 10) and a lower and upper limit values of 12.76 and 14.44
467 respectively (Table 10).

468 **4.DISCUSSION**

469   Cypermethrin are deliberately added to the environment in large quality by agro-
470   farmers to control pest, and this in turn pollute our aquatic environment [8]. The presence of
471   environmental stress such as low dissolved oxygen, high temperature and high ammonia
472   reduces the ability of organisms to maintain its internal environment (i.e. metabolism,
473   catabolism) [24]. Fish growth depends on water quality to boost its production and
474   physicochemical parameters are known to affect the biotic components of an aquatic
475   environment in various ways. Cypermethrin is a globally used for the control of pest, in order
476   to improve food productivity [1], but their use creates risk of food contamination as well as
477   affects the non-target aquatic species like fish [2]. It is a synthetic pyrethroid, with a very
478   high activity and stability [3]. The response of fish to variety of metal and organic pollutants
479   are transient and are dependent on species, enzymes and single or mixed contaminants [25].
480   Water pollution affects organisms and plants that lives in these water bodies and in almost all
481   cases, the effect is damaging not only to the individual specie and populations, but also to the
482   natural biological communities [26]. When pesticides are applied on farmlands, only 1% gets
483   to the target organism, as most of these chemicals remain in the environment, and as such the
484   pollution of the environment on the long run is inevitable [27].

485   The present study revealed variations and alterations in the physico-chemical
486   parameters of contaminated with different concentrations of cypermethrin. The water
487   temperature, pH, electrical conductivity and turbidity of the contaminated culture water
488   increased with increase in the concentration of the toxicant, this corroborated with the report
489   of [28] who also reported an increase in water quality with increase in toxicant concentration.
490   On the other way round, dissolved oxygen (DO) decreased with increase in the toxicant
491   concentration, this corroborated with the reports of [29] who also observed a decrease in the
492   DO values of culture water when contaminated with cypermethrin. The decrease in the DO
493   and increase in pH, turbidity could be due to the increase in the microbial activities and bio-
494   chemical oxygen demand as a result of the introduction of the toxicant. Also, the increase in
495   the conductivity of culture water with increased toxicant concentration could be due to the
496   increased chemical ions associated with the chemical. In general, temperature, conductivity,
497   pH values were higher and the DO level was lower in the aquarium contaminated with the
498   highest concentration of the toxicant (30ppm cypermethrin concentration) than in the control
499   aquarium. Statistically, the physico-chemical parameters varied significantly across the
500   culture water group contaminated with different concentrations of toxicant over all durations
501   of contamination at $p<0.05$, except for water temperature over 96 hours exposure period

502 which was insignificant at p>0.05 and this was contrary to the report of [30] who reported

503 insignificant alterations in all physico-chemical parameters but dissolved oxygen.

504   The range of the water temperature, dissolved oxygen, pH, turbidity and DO of

505 culture water observed in the culture water contaminated with cypermethrin in the present

506 study were not within the same range reported by [28, 29]. The pH and conductivity range of

507 the present study was lower, but temperature and DO range were higher than that reported by

508 [28, 29]. The variation between the findings could be due to the difference in the toxicants,

509 concentration of the toxicants and differences in chemical components of the test toxicant.

510 The water temperature, pH and conductivity of the culture water were within the WHO

511 acceptable limits except the dissolved oxygen (30ppm group over 72 and 96 hour duration)

512 and turbidity (control group) which were above the WHO permissible limit, and as a result,

513 the toxicant made the water contaminated and unconducive for the fingerlings thereby

514 causing mortality. Even as most of the water parameters were within the WHO acceptable

515 standard after 96 hours of contamination with the toxicant, there is a high tendency of a

516 chronic contamination of the water over a long period of time, leading to its pollution. Apart

517 from the alteration of the water and fingerlings mortality, the fish (biological organisms)

518 could accumulate the toxicants from the toxicant into their tissues, which are consumed by

519 humans, leading to a lot of health challenges.

520   The toxicity of cypermethrin on *Clarias gariepinus* fingerlings observed for the

521 present study was concentration and duration dependent, with mortality increasing with

522 increase in the concentration of the toxicant as well as exposure duration and this

523 corroborated with the findings of [30, 31, 32]. The fingerlings of *C. gariepinus* exposed to

524 different concentrations of the cypermethrin showed abnormal 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 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.

552 **5. CONCLUSION**

553 In conclusion, the cypermethrin caused significant alterations in the physicochemical

554 parameters of water, compared to the control aquarium water, increasing in some cases

555 (temperature, pH, turbidity and conductivity), and reducing in some cases (DO). Also, the

556 toxicant raised some water parameters to undesired levels, leading to the bio-accumulation of

557 toxicants in the fingerlings. The toxicological effects of the toxicant was concentration and

558 duration dependent**.** The cypermethrin was highly toxic to the fingerlings, causing mortality

559 in the process, as a result, more research of this kind should be carried-out involving

560 haemathological, reproductive, histological and other physiological alterations due to

561 exposure of *C. gariepinus* to cypermethrin, so as to further reveal the toxic and harmful

562 potentials of pesticides.

563 **ETHICAL CONSIDERATION**

564 The authors ensured that all ethical and other basic principles underlying behavior and

565 advancing welfare for the use of animals in research, including handling, relevant laws and

566 regulations were considered before proceeding with the research. Permission was also

567 received from the relevant bodies for the use of fish for this experiment.

568

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