

# Effects of Pretilachorpyribenzoxim Pollution on the Water Quality, Serum Biochemical indices, and Behavioural Response of *Oreochromis niloticus* Juveniles

## ABSTRACT

**Aims:** The effects of Pretilachorpyribenzoxim (PP) pollution on the water quality, serum biochemical indices, and behavioural response of *Oreochromis niloticus* Juveniles were studied.

**Study design:** The completely randomized design was used in this study.

**Place and Duration of Study:** The experiment was carried out in the Central Laboratory of the Fisheries and Aquaculture Department, Faculty of Agriculture, Adekunle Ajasin University Akungba-Akoko, Nigeria, between January and March 2023.

**Methodology:** The experiment was carried out in 30 L of non-chlorinated and aerated water in rectangular plastic tanks (45cm x 30cm x 30cm). 250 juveniles *O. niloticus* of both sexes were collected from Federal University of Technology, Akure, Ondo state and acclimatized for 14 days in the laboratory. During rearing period, the fish were fed copen feed at 2% body weight. In a definitive test, a group of 10 fish specimen (11.3-17.5cm length and 10.5-32.3g weight) were treated at random to nominal PP doses of 0.0, 0.10, 0.125, 0.150, 0.175, and 0.2 ml being diluted in 30 litres of water and labelled T1, T2, T3, T4, T5 and T6, respectively in triplicate. The LC<sub>50</sub> of PP, test water's physicochemical, behavioural responses and serum biochemical indices were determined.

**Results:** The dissolved oxygen depleted ( $P < 0.05$ ) in T5 and T6, compared to T1 and the rest of the treatments. The water conductivity increases ( $P < 0.05$ ) with an increased concentration of PP contamination. The water's total dissolved solids levels increased progressively with an increase in PP concentration. The total dissolved solids in T5 and T6 were similar ( $P > 0.05$ ) to T4 but lower ( $P < 0.05$ ) than T1 and T2. Juveniles in treatments 2, 3, 4, 5 and 6 shows behavioural responses such as loss of reflex, fin deformation, erratic swimming, air gulping, and moulting. The serum total protein of the juveniles in treatments 2, 3, 4, 5 and 6 was lower ( $P < 0.05$ ) than T1. Serum creatinine level increased with increased PP contamination from treatments 1 to 6. The serum aspartate aminotransferase (AST) of the juvenile in T3, T4, T5 and T6 were higher ( $P < 0.05$ ) than in T1. The serum Alanine aminotransferase (ALT) of the juvenile in T2, T3, T4, T5 and T6 were significantly ( $P < 0.05$ ) higher than T1.

**Conclusion:** The exposure of juvenile *O. niloticus* to PP contamination affected the physicochemical properties of water, behaviour and Serum biochemicals of juvenile *O. niloticus* fish. Hence, leaching of pp into water bodies through Agricultural activities should be monitored to reduce loss of fish which can lead to economic detune.

**Keywords:** behavioural changes, *O. niloticus*; Pretilachorpyribenzoxim, serum biochemical indices; toxicity, water quality

## 1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

One of the most popular chloroacetanilide herbicides for controlling annual grasses in rice fields and several broadleaf weeds is pretilachorpyribenzoxim [1]. It is a significant herbicide used for rice weed management in Nigeria [2]. Pretilachorpyribenzoxim and other herbicides were also used in seed beds, seed transplant fields, and some crop fields such as wheat, barley, cotton, vegetables, and peanuts [3]. Herbicides escape and leached into aquatic environment because, agricultural country sides are close to bodies of water. The organisms in the waterbodies such as fish and other aquatic species are negatively impacted by the repeated and negligent use of herbicides, careless handling, accidental spills, and the release of untreated effluents into natural waterways [3]. The pesticide can disintegrate quickly, but in the

24 absence of adequate microbial degraders and under conditions of low temperature, low moisture, high alkalinity, and  
25 persistent biological activity, it may persist in soils for a very long time [3,4].

26 Recent research has shown the toxicity of Pretilachorpyribenzoxim and other herbicides on fish and other aquatic  
27 animals, this has drawn the attention of researchers to the xenobiotic contamination of aquatic habitats [3, 5]. Fish are the  
28 most notable of the non-target creatures and also crucial in determining the possible risk of contamination in aquatic  
29 environments [6]. Since chloroacetamide herbicides were found to be likely carcinogens, the poisoning of aquatic  
30 environments by chloroacetamide herbicides like pretilachlor has drawn more attention [7]. Pretilachlor (2-chloro-2',6'-  
31 diethyl-N-[2-propoxyethyl] acetanilide) is a systemic herbicide that is widely used to suppress weeds in rice fields. This  
32 herbicide prevents the synthesis of long-chain fatty acids, which slows cell division [8]. Herbicide-induced behavioural  
33 changes in fish are still very rare [7], but sensitive early warnings are provided by behavioural changes brought on by  
34 chemical exposure than by traditional diagnostic procedures. Behavioural toxicity of fish most frequently used  
35 measurements are swimming-oriented reactions, opercular movement, feeding attempts, jerk swimming, and schooling  
36 [7].

37 The Nile Tilapia, also known as *O. niloticus*, is a significant fish in aquaculture and catch fisheries. It is frequently  
38 widespread and present in Nigerian water bodies [9]. Up to 3.5kg has been seen as its maximum size as it grows to a  
39 respectable size [10]. The knowledge on the effects of Pretilachorpyribenzoxim on several local fish species, such  
40 as *O. niloticus* is scarce, even though farmers often use it in farmlands and there may be an ecotoxicological impact  
41 associated with its uses. In light of the aforementioned, the current study was conducted to assess the impact of the  
42 herbicide pretilachlor on juvenile *O. niloticus* by analysing their behavioural activities and serum biochemical indices  
43 following exposure to the herbicide.

## 44 2. MATERIAL AND METHODS

### 45 2.1 Experimental Site

46  
47 The experiment was carried out in the Central Laboratory of the Fisheries and Aquaculture Department, Faculty of  
48 Agriculture, AdekunleAjasin University Akungba-Akoko, Nigeria; using a plastic tank of 50 litres capacity (45cm x 30cm x  
49 30cm). Each tank was filled with 30 litres of non-chlorinated water.

### 50 2.2 Fish Collection and Experiment

51 Two hundred and fifty (250) healthy *O. niloticus* juveniles of 11.3-17.5cm length and 10.5-32.3g weight were purchased  
52 from a reputable hatchery in Akure, Nigeria. The AdekunleAjasin University's Animal Ethics Committee established  
53 guidelines for the care of laboratory animals, and these guidelines were followed when treating the fish. The health status  
54 of selected fish was assessed based on the presence or absence of physical injuries and other morphological deformities.  
55 The juveniles were acclimatized under laboratory conditions for 14 days before the commencement of the experiment.  
56 During the acclimatization period, the fish were fed daily with coppen feed at 2% body weight (BW) and covered with  
57 netting materials to prevent jumping out of the water. The water was changed daily, uneaten feed and faecal matters were  
58 siphoned out and dead fish were also removed to minimize contamination of water.

### 59 2.3 Source and Processing of PretiolachortPyribenzoxim

60 320 g/l of Pretiolachorpyribenzoxim (PP) with Batch No ERW2021030-1 and NAFDAC Reg No A5-1272 was purchased in  
61 an Agro-Chemical shop at Ikare Market, Ondo State, Nigeria. The Pretiolachorpyribenzoxim was mixed directly with 30  
62 litres of water for each treatment (T) as follows:

63 T1: Control, no PP

64 T2: 0.10 ml of PP dissolved in 30 litres of water

65 T3: 0.125 ml of PP dissolved in 30 litres of water

66 T4: 0.150 ml of PP dissolved in 30 litres of water

67 T5: 0.175 ml of PP dissolved in 30 litres of water

68 T6: 0.20 ml of PP dissolved in 30 litres of water

### 69 2.4 Toxicity bioassay

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71  
72 According to established procedures, a static bioassay system in the laboratory was used for the final test and an acute  
73 toxicity experiment to obtain the 96-hour LC<sub>50</sub> values of Pretiolachorpyribenzoxim[11]. Before determining the  
74 concentrations of the test solution for the definitive test, a range finding test was conducted [12]. The experiment was  
75 carried out in 30 L of non-chlorinated and aerated water in rectangular plastic tanks (45cm x 30cm x 30cm)).

The behavioural actions of fish in each treatment was observed daily. In a definitive test, a group of 10 fish specimens (11.3-17.5cm length and 10.5-32.3g weight) were treated at random to nominal Pretiolachorpyribenzoxim doses of 0.0, 0.10, 0.125, 0.150, 0.175, and 0.2 ml being diluted in 30 litres of water. The experiment was conducted in triplicate, and fish mortality due to exposure to Pretiolachorpyribenzoxim was tracked for up to 96 hours at intervals of 24 hours to obtain LC<sub>50</sub> values of the test pesticides (Figure 1). The LC<sub>50</sub> of Pretiolachorpyribenzoxim was determined following the probit analysis method described by Finney [13]. Based on Hart *et al.* [14], National Academy of Sciences/National Academy of Engineering [15], Canadian Council of Resources and Environmental Ministry [16] and the International Joint Commission [17], the 96-hour LC<sub>50</sub> was multiplied with various application factors (AF) to estimate the safe level of the test pesticides. Standard techniques were used to analyse the test water's physicochemical characteristics, including temperature, dissolved oxygen, pH, total hardness, and conductivity [11]. The behavioural reactions (loss of reflex, erratic swimming, Fin deformation and air gulping and moulting) of the test fish in each experimental tank was observed at 24hr interval for 96hr.

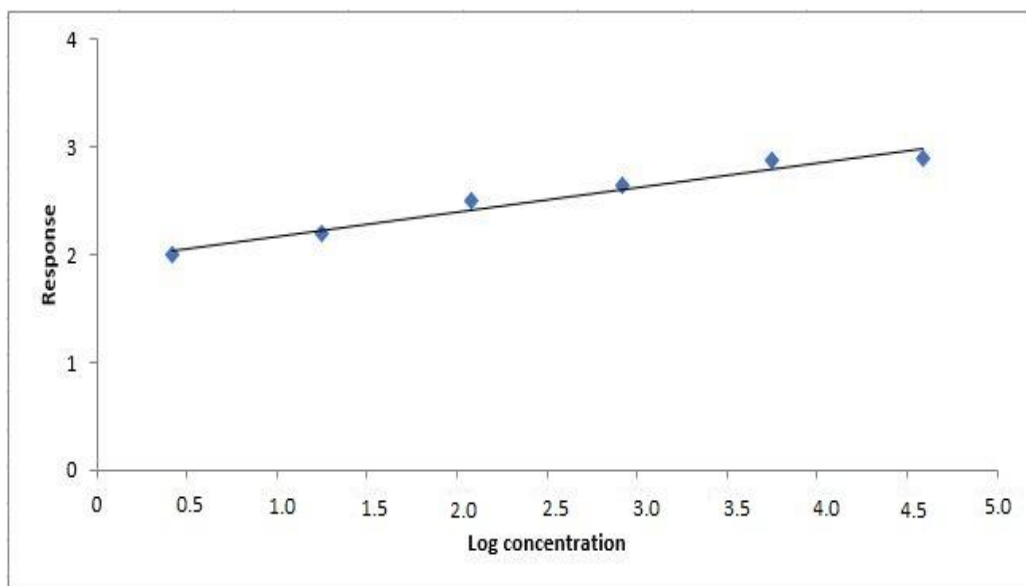


Fig. 1. Log of concentration of Pretiolachorpyribenzoxim herbicide and its probit value for juvenile *O. niloticus*. LC<sub>50</sub>=1.03

## 2.5 Blood Collection and Analysis

After 96 hours of exposure, the fish's blood was collected. A disposable hypodermic syringe and needle were used to draw blood through the vertebral caudal blood artery. A 10 ml plain sample bottle was used to hold the blood sample and centrifuged. Thereafter the serum was decanted into another well-labelled plain blood sample bottle and kept in a freezer until used for laboratory analysis. Biochemical indices analysed i.e. total protein, creatinine, cholesterol, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were determined using a Reflotron ® Plus 8C79 (Roche Diagnostic, GmbH Mannheim, Germany), using commercial kits.

## 2.6 Data analysis

Data obtained for biochemical parameters were subjected to analysis of variance (ANOVA) making use of the Statistical Package for Social Science (SPSS) version 20.0; while the difference between treatments means was determined by Duncan's multiple range test of the same package [18].

## 3. RESULTS AND DISCUSSION

### 3.1 Results

The effects of Pretiolachorpyribenzoxim (PP) contamination on the physicochemical properties of water are shown in Table 1. The dissolved oxygen was significantly ( $P<0.05$ ) depleted in T5 and T6, compared to the T1 and the rest treatments. The water conductivity increases ( $P<0.05$ ) with increased concentration of PP contamination; specifically the conductivity in T4, T5 and T6 were significantly ( $P<0.05$ ) higher than T1, and T2. The water's total dissolved solids levels

111 increased progressively with an increase in PP concentration. The total dissolved solids in T5 and T6 were similar  
 112 (P>0.05) to T4 but significantly (P<0.05) lower than T1 and T2.  
 113  
 114

**Table 1.** Effects of Pretiolorchopyribenzoxim (PP) contamination on the physicochemical properties of water.

Treatments	Dissolved Oxygen(mg/l)	Temperature (°C)	Conductivity (µM/cm)	pH	Total Solids (mg/l)	Dissolved
T1	6.46 <sup>a</sup>	23.92	200.11 <sup>c</sup>	7.12	116.81 <sup>b</sup>	
T2	6.21 <sup>a</sup>	23.23	200.29 <sup>c</sup>	7.12	119.28 <sup>b</sup>	
T3	5.96 <sup>a</sup>	23.27	206.92 <sup>bc</sup>	7.07	119.65 <sup>b</sup>	
T4	5.21 <sup>a</sup>	23.51	215.29 <sup>b</sup>	7.06	121.20 <sup>ab</sup>	
T5	5.11 <sup>b</sup>	26.62	229.19 <sup>a</sup>	7.14	125.37 <sup>a</sup>	
T6	5.04 <sup>b</sup>	23.35	231.12 <sup>a</sup>	7.02	126.47 <sup>a</sup>	
SEM	0.14	0.55	3.28	0.02	1.06	
P-Value	0.01	0.45	0.01	0.44	0.01	

115 *T1: Control, no PP; T2: 0.10 ml of PP dissolved in 30 litres of water; T3: 0.125 ml of PP dissolved in 30 litres of water; T4:*  
 116 *0.150 ml of PP dissolved in 30 litres of water; T5: 0.175 ml of PP dissolved in 30 litres of water; T6: 0.20 ml of PP*  
 117 *dissolved in 30 litres of water; SEM: Standard error of the mean.*

118 *Mean values with the same superscript in the column are not significantly different from each other at (P>0.05).*  
 119

120 Effects of Pretiolorchopyribenzoxim (PP) contamination on the behavioural response of *Oreochromis niloticus* Juveniles  
 121 are presented in Table 2. The juveniles in the control group (T1) displayed normal behavioural response was observed.  
 122 However, among those juveniles in treatments 2, 3, 4, 5 and 6 behavioural responses such as loss of reflex, fin  
 123 deformation, erratic swimming, air gulping, and moulting were observed.

124 **Table 2.** Effects of Pretiolorchopyribenzoxim (PP) contamination on the Behavioural Response of *Oreochromis niloticus*  
 125 Juveniles

Behavioural response	Treatments	Duration of Exposure (hours)			
		24	48	72	96
Loss of Reflex	T1: 0.00 ml PP in 30L of H <sub>2</sub> O	-	-	-	-
	T2: 0.10 ml PP in 30L of H <sub>2</sub> O	-	-	+	+
	T3: 0.125 ml PP in 30L of H <sub>2</sub> O	-	+	+	+
	T4: 0.15 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T5: 0.175 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T6: 0.12 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
Air Gulping	T1: 0.00 ml PP in 30L of H <sub>2</sub> O	-	-	-	-
	T2: 0.10 ml PP in 30L of H <sub>2</sub> O	-	+	+	+
	T3: 0.125 ml PP in 30L of H <sub>2</sub> O	-	+	+	+
	T4: 0.15 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T5: 0.175 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T6: 0.12 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
Erratic Swimming	T1: 0.00 ml PP in 30L of H <sub>2</sub> O	-	-	-	-
	T2: 0.10 ml PP in 30L of H <sub>2</sub> O	-	-	+	+
	T3: 0.125 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T4: 0.15 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T5: 0.175 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T6: 0.12 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
Fin deformation	T1: 0.00 ml PP in 30L of H <sub>2</sub> O	-	-	-	-
	T2: 0.10 ml PP in 30L of H <sub>2</sub> O	-	-	+	+
	T3: 0.125 ml PP in 30L of H <sub>2</sub> O	-	+	+	+
	T4: 0.15 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T5: 0.175 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
	T6: 0.12 ml PP in 30L of H <sub>2</sub> O	+	+	+	+

	T5: 0.175 ml PP in 30L of H <sub>2</sub> O	+	+	+	+
Moulting	T6: 0.12 ml PP in 30L of H <sub>2</sub> O	+	+	+	+

Note: +: Observed; -: Not-observed

Table 3 shows the effects of Pretiolachorpyribenzoxim (PP) contamination on the serum biochemical indices of *Oreochromis niloticus* juveniles. The serum total of the juveniles in treatments 2, 3, 4, 5 and 6 was significantly ( $P < 0.05$ ) low when compared to T1. The serum creatinine of the juveniles was significantly ( $P < 0.05$ ) affected by the PP exposure, such that serum creatinine level increased with increased PP contamination from treatments 1 to 6. The serum aspartate aminotransferase concentration of the juvenile in T3, T4, T5 and T6 were significantly ( $P < 0.05$ ) higher than T1. The serum Alanine aminotransferase concentration of the juvenile in T2, T3, T4, T5 and T6 were significantly ( $P < 0.05$ ) higher than T1. The serum cholesterol concentration was not significantly ( $P > 0.05$ ) affected by the treatments.

**Table 3.** Effects of Pretiolachorpyribenzoxim (PP) contamination on the Serum biochemical indices of *Oreochromis niloticus* Juveniles

Treatments	Total protein(g/dl)	Creatinine(mg/dl)	AST (U/L)	ALT(U/L)	Cholesterol(mg/dl)
T1	9.95 <sup>a</sup>	0.67 <sup>b</sup>	61.78 <sup>c</sup>	21.16 <sup>b</sup>	81.30
T2	7.76 <sup>b</sup>	0.71 <sup>b</sup>	66.26 <sup>bc</sup>	27.96 <sup>a</sup>	82.80
T3	7.53 <sup>b</sup>	0.76 <sup>ab</sup>	73.31 <sup>ab</sup>	27.24 <sup>a</sup>	80.64
T4	7.94 <sup>b</sup>	0.80 <sup>ab</sup>	72.97 <sup>ab</sup>	26.16 <sup>a</sup>	82.35
T5	7.36 <sup>b</sup>	0.86 <sup>a</sup>	77.01 <sup>a</sup>	27.94 <sup>a</sup>	91.50
T6	7.21 <sup>b</sup>	0.89 <sup>a</sup>	74.97 <sup>a</sup>	28.66 <sup>a</sup>	81.03
<b>STD</b>	<b>1.25</b>	<b>0.00</b>	<b>6.31</b>	<b>2.91</b>	<b>8.33</b>
P-Value	0.04	0.02	0.04	0.01	0.66

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; T1: Control, no PP; T2: 0.10 ml of PP dissolved in 30 litres of water; T3: 0.125 ml of PP dissolved in 30 litres of water; T4: 0.150 ml of PP dissolved in 30 litres of water; T5: 0.175 ml of PP dissolved in 30 litres of water; T6: 0.20 ml of PP dissolved in 30 litres of water; SEM: Standard error of the mean.

Mean values with the same superscript in the column are not significantly different from each other at ( $P > 0.05$ ).

### 3.2 Discussion

The physicochemical and biological properties of water have a major role in determining a water sheet's productivity [19]. The observed decrease in dissolved oxygen in T5 and T6 in this study implies that the concentration of PP in T5 and T6 was sufficient to change the water's chemical makeup, which in turn adversely affected the level of dissolved oxygen in those treatment groups [20]. Inferentially, PP water contamination at concentrations of 0.20 ml per 30 litres of water and 0.17 ml per litre of water could lower the amount of dissolved oxygen. According to earlier research, low dissolved oxygen has an impact on fish metabolism, growth, development, and motility [19].

As high or low conductivity ranges are utilised to detect environmental changes and contamination, conductivity tests are of utmost relevance in assessing water quality [21]. The rise in water conductivity seen in this study together with an increase in PP concentration, particularly from concentrations of 0.015 ml per 30 litres of water suggests that PP contamination may harm the aquatic environment because an increase in conductivity causes osmotic stress in aquatic life, which ultimately changes biological communities [21].

Because the density of total dissolved solids controls how much water flows into and out of an organism's cells, changes in the concentration of dissolved solids can be hazardous to fish. Too high or too low of a concentration can stunt the fish's growth or even kill it [22]. Therefore, based on this fact, the relatively elevated total dissolved solid reported from concentrations of 0.015 ml PP per 30 litres of water further supports the ability of the contaminant to significantly reduce the water quality.

Poor water quality will have an impact on fish and other aquatic species, which will cause death and behavioural changes [23]. The behavioural response seen when *O. niloticus* was exposed to PP is comparable to that seen when *Cyprinus carpio* was exposed to various doses of fenthion by Alkahemal- Balawiet *al.* [24]. The fishes in treatment groups that were exposed to PP in this study displayed some abnormal behaviour, such as air gulping, erratic swimming, deformed fins, and moulting, which are caused by inhibition of acetylcholinesterase activity. Hence, leading to accumulation of acetylcholine in cholinergic synapses that causes hyperstimulation of the toxicant and exhaustion due to

167 **respiratory difficulty**[25, 26]. According to reports, stressed fish exhibit hyperactivity, and to escape the stressful  
168 environment, they need more oxygen to meet their energy needs [23].

169 Fish and other aquatic life are negatively impacted by toxins or pollutants, and tissues overloaded with toxins or pollutants  
170 may also have their body's oxidative metabolism, including protein synthesis, glycolysis, and lipid synthesis, affected [27].  
171 In addition, changes in the biochemical blood profile reveal changes in the organism's metabolism and biochemical  
172 functions brought on by the effects of various pollutants, and they enable researchers to better understand how these  
173 pollutants work [28].

174 Blood serum contains blood proteins, commonly known as serum proteins. They perform a variety of tasks, such as  
175 transporting lipids, hormones, vitamins, and minerals to support immune system activity [29]. The decreased serum total  
176 protein concentration associated with PP exposure or pollution in this study may suggest that the pollutant accelerated the  
177 process of liver or kidney deterioration or interfered with normal/proper protein digestion or absorption [30]. This result  
178 agreed with previous reports by [31] and [29], who observed decreased protein concentration in the various concentrations  
179 of pretilachlor exposure. The fish's altered energy metabolism and protein structure may be the cause of the lower total  
180 protein generated after PP exposure [29]. Enzymes in fish are thought to be sensitive biochemical toxicity markers [32],  
181 and the probability of kidney, heart, and other muscle damage in the fish as a result of their exposure to PP is thus  
182 indicated by the rise in serum creatinine, AST, and ALT in juveniles exposed to PP in this study [33]. The elevated AST  
183 and ALT recorded in juveniles due to PP exposure in this study are in tandem with [34], who recorded elevated AST and  
184 ALT following their exposure to some herbicides. The adverse effects of pollutants or toxic chemicals on the liver and  
185 kidney were blamed for the increase in the activity of serum ALT, AST, and creatinine in **O.niloticus** [35].  
186  
187

#### 188 **4. CONCLUSION**

189  
190 PP contamination has negative effects on the physicochemical properties such as dissolved oxygen, conductivity and total  
191 dissolved solids of water. In addition, water contamination by PP produces behaviours such as loss of reflex, air gulping,  
192 erratic swimming, fin deformation and moulting in the juveniles. Finally, PP water contamination caused the reduction of  
193 the serum total protein and elevated creatinine, AST and ALT. Therefore to reduce the severe risk related to the use of  
194 agrochemicals, Agricultural usage of PP in the environment, especially near water bodies, must be controlled.  
195

#### 196 **COMPETING INTERESTS**

197  
198 The author has declared that no competing interests exist.  
199

#### 200 **AUTHORS' CONTRIBUTIONS**

201  
202 Author ICA designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the  
203 manuscript, managed the analyses of the study, managed the literature searches, and approved the final manuscript.  
204  
205

#### 206 **ETHICAL APPROVAL**

207  
208 The Adekunle Ajasin University's Animal Ethics Committee established guidelines for the care of laboratory animals, and  
209 these guidelines were followed when treating the fish.  
210  
211

#### 212 **REFERENCES**

- 213  
214 1. Chauhan BS, Abeysekera ASK, Kulatunga SD, Wickrama UB. 2013. Performance of Different Herbicides in a Dry-  
215 Seeded Rice System in Sri Lanka. *Weed Technol.*; 27(3), 459–462. <http://www.jstor.org/stable/43702622>.
- 216  
217 2. Ibrahim U, Rahman SA. B.A. Babaji BA. 2011. Cost –benefit analysis of rice production under weed control  
218 methods in Lafiagi area of Kwara. *NSUK JSciTechnol*; 1(1&2): 40-47.
- 219  
220 3. Nwani CD, Ama UI, Okoh F, Oji UO, Ogbonyealu RC, Ibiama AA, and Udu-Ibiama O. 2013. Acute toxicity of the  
221 chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*. *African J*  
222 *Biotechn.* 2013; 12(5), 499-503. <http://www.academicjournals.org/AJB>. DOI: 10.5897/AJB12.2433

- 223 4. Huang Y, Xiao L, Li F, Xiao M, Lin D, Long X, Wu Z. 2018. Microbial Degradation of Pesticide Residues and an  
224 Emphasis on the Degradation of Cypermethrin and 3-phenoxy Benzoic Acid: A Review. *Molecules* (Basel,  
225 Switzerland). 2018; 23(9), 2313. <https://doi.org/10.3390/molecules23092313>
- 226 5. Chang J, Liu S, Zhou S, Wang M, Zhu G. 2011. Effects of butachlor on reproduction and hormone levels in adult  
227 zebrafish (*Danio rerio*). *ExperToxicologPathol*; 65(1-2), 205–209. <https://doi.org/10.1016/j.etp.2011.08.007>
- 228 6. Lakra WS, Nagpure NS. Genotoxicological studies in fish. 2009;: a review. *Indian J. Anim. Sci.* 79:93–98.
- 229 7. Soni R, Verma SK . 2018. Acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to  
230 herbicide pretilachlor. *Heliyon* 2018; 4(12), e01090. <https://doi.org/10.1016/j.heliyon.2018.e01090>
- 231 8. Kaushik S, Inderjit Steribig JC, Cedergreen N. 2006. Activities of mixtures of soil-applied herbicides with different  
232 molecular targets. *Pest Manage Sci*; 62:1092–1097.
- 233 9. Araoye PA. 2008. Physical factors and their influence on fish species composition in Asa Lake, Ilorin, Nigeria. *Rev.*  
234 *Trop. (Int. J Trop Biol.*; 57(1-2):167-175
- 235 10. Yem IY, Bankole NO, Umar R, Ibrahim A, Ewutanure SJ. 2020. Food habit and growth pattern of Nile Tilapia  
236 *Oreochromis niloticus* in Wase Dam, Nigeria. *IntJFisher Aquatic Stud.*; 8(4): 257-260
- 237 11. APHA, AWWA, WPCF. 2005. Standard methods for the examination of water and waste water, 21st 401 ed.  
238 American Public Health Association, Washington, DC.
- 239 12. Nwana CD, Ama UI, Okoh B, Oji UO, Ogbonyealu RC, Ibiam AA, Udu-Ibiam O. 2013 Acute toxicity of the  
240 chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*. *African J*  
241 *Biotech*: 12(5), 499-503.
- 242 13. Finney DT. *Probit Analysis*. Cambridge University Press, Cambridge, p.333. 1971.
- 243 14. Hart WB, Weston RF, Dermann JG. 1948. An apparatus for oxygenating test solution which fish are used as test  
244 animals for evaluating toxicity. *Trans. Am. Fish Soc.*; 75:288.
- 245 15. NAS/NAE. *Water Quality Criteria*, EPA-R3-033; US, Government Printing Office: Washington, DC, USA. 1973.
- 246 16. CCREM. *Canadian Water Quality Guidelines*; Canadian Council of Resources and Environmental Ministry, Inland  
247 Waters Directorate, Environment Canada: Ottawa, ON, Canada. 1991.
- 248 17. IJC. *New and Revised Great Lakes Water Quality Objectives*. Great Lake Basin, Windsor; IJC: Ottawa, ON,  
249 Canada. 1977.
- 250 18. Oloruntola OD, Agbede JO, Onibi GE, Igbasan FA . 2016. Replacement value of rumen liquor fermented cassava  
251 peels for maize in growing rabbit diet. *Arch. de Zootec.* 2016; 65 (249): 89-97.
- 252 19. De AK. *Environmental Chemistry*. 6th edition. New Age Int. (P) Ltd., New Delhi. 169-170 pp. 2007.
- 254 20. Nimesh N, Jain S. 2016. Effect of dissolved oxygen on physiology and behaviour of freshwater fishes. *Voyager*.  
255 2016. 7, Special Issue 2016, 1-7. e): 2455-054X
- 256 21. Zhang Y, Zhao Q, Ding S. 2019. The responses of stream fish to the gradient of conductivity: A case study from  
257 the Taizi River, China, *Aquatic Ecosystem Health & Management*. 2019; 22:2, 171-182, DOI:  
258 10.1080/14634988.1622994.
- 259 22. Weber-Scannel P, Duffy L.K. 2007. Effects of Total Dissolved Solids on Aquatic Organisms: A Review of  
260 Literature and Recommendation for Salmonid Species. *American J Envir Sci.* 2020; 3 (1): 1-6.
- 261 23. Akinsorotan AM. 2014. Toxicity of Dizensate (glyphosate herbicide) on *Clarias gariepinus* fingerlings. *Adv. Rsch in*  
262 *Biol. Sci*; 2 (1), 1-5.

- 263 24. Alkahemal-Balawi HF, Ahmad Z, Al-Akel AS, Al-Misned F, Suliman EAM, Al Ghanim KA.2011.Toxicity bioassay of  
264 lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in  
265 *C.gariepinus*. African J Biotechnol. 2011;10 (53), 11039-11047.
- 266 25. Vindas MA, Gorissen M, Höglund E, Flik G, Tronci V, Damsgård B, Thornqvist P, Nilsen TO, Winberg S,O Overli  
267 O, Ebbesson OE. 2017. How do individuals cope with stress? Behavioural, physiological and neuronal differences  
268 between proactive and reactive coping styles in fish. J. Exp. Biol.; 220, 1524–1532. doi: 10.1242/jeb.153213
- 269 26. Adewumi B, Michael OA, Damilohun SM.2018. Histological and Behavioural Changes of *Clariasgariepinus*  
270 Juveniles Exposed to Chlorpyrifos and force. International J BiolSciApplic.; 5(1), 1-12.
- 271 27. SiaSu GL, Ramos Gliceria B, SiaSu MLL..2013. Bioaccumulation and histopathological alteration of total lead in  
272 selected fishes from Manila Bay, Philippines. Saudi J. Biol. Sci. 2013;20:353–355. doi: 10.1016/j.sjbs.03.003.
- 273 28. Luskova V, Svoboda M, Kolarova J. The effects of diazinon on blood plasma biochemistry in carp (*Cyprinus carpio*  
274 L.) Acta Vet Brno.;71:117–123. doi: 10.2754/avb200271010117.
- 275 29. ThiripurasundariS, Asokan, Thangapandiyan S. 2002. Acute Toxicity Impact of Herbicide Pretilachlor on  
276 Biochemical, Haematological and Ionic Changes in Gill, Liver and Kidney of a Cultivable Fish *Labeorohita*  
277 (Hamilton). Inter JPharm BiologSci; 2019; 9 (2): 990-999
- 278 30. Javed M, Ahmad MI, Usmani N, Ahmad M.2017. Multiple biomarker responses (serum biochemistry, oxidative  
279 stress, genotoxicity and histopathology) in *Channapunctatus* exposed to heavy metal loaded waste water.  
280 Scientific reports; 7(1), 1675. <https://doi.org/10.1038/s41598-017-01749-6>
- 281 31. Davinder PS, JasvirinderSK, AlkaGK, YadvinderS.2016.Toxicological effect of pretilachlor on some physiological  
282 processes of cyanobacterium *Synechocystis* sp. strain PUPCCC 64. J ApplBiolBiotechnol.; 4 (01):12-19.
- 283 32. Ugbomeh, A.P., Bob-manuel, K.N.O., Green, A., and Taylorharry, O .2019. Biochemical toxicity of Corexit 9500  
284 dispersant on the gills, liver and kidney of juvenile *Clariasgariepinus*. Fish Aquatic Sci 22, 15 (2019).  
285 <https://doi.org/10.1186/s41240-019-0131-6>.
- 286 33. Sparling DW. 2016. Ecotoxicological essentials: environmental contaminants and their biological effects on  
287 animals and plants. UK: Academic Press - Elsevier;. ISBN-978-0-12-801947-4. Pp 490.
- 288 34. Fathy M, Mohamed IA, Farghal AIA, Temerak SAH, Sayed AEH. 2019. Hemotoxic effects of some herbicides on  
289 juvenile of Nile tilapia *Oreochromis niloticus*. EnvironSciPollut Res Inter; 26(30), 30857–30865.  
290 <https://doi.org/10.1007/s11356-019-06280->
- 291 35. Gaafar A, Elmanakhly E, Soliman MK, Soufy H, ZakiMS, Mohamed SG, Hassan SM.2010. Some pathological,  
292 biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to  
293 edifenphos pesticide. J Am Sci.; 6: 542–55.

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## 296 DEFINITIONS, ACRONYMS, ABBREVIATIONS

297 PP: Pretilachlorpyribenzoxim

298 ALT: Alanine aminotransferase

299 AST: Aspartate aminotransferase

300 ALP: Alkaline phosphatase

301 DO: Dissolved oxygen

302 TDS: Total dissolved solids

303