Original Research Article

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

ABSTRACT

Aims: The effects of Pretilachor pyribenzoxim (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 de-chlorinated and aerated water in rectangular plastic tanks (75x45x45 cm). 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 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. The LC_{50} of PP, test water's physicochemical characteristics and the juvenile's serum biochemical indices were determined.
Results: The dissolved oxygen was depleted (P<0.05) in T5 and T6, compared to the T1 and the rest 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 behavioural responses such as loss of reflex, fin deformation, erratic swimming, air gulping, and moulting were observed. The serum total 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: PP contamination affected the physicochemical properties of water. In addition, water contamination by PP produces behaviours such as loss of reflux, air gulping, erratic swimming, fin deformation and moulting in the juveniles. PP water contamination caused the reduction of the serum total protein and elevated creatinine, AST and ALT.

Keywords: O. niloticus; Pretilachor pyribenzoxim, toxicity, behavioural changes, serum biochemical indices; water quality

1. INTRODUCTION

One of the most popular chloroacetanilide herbicides for controlling annual grasses in rice fields and several broadleaf weeds is pretilachor pyribenzoxim [1]. For instance, Pretilachor pyribenzoxim is a significant herbicide used for rice weed management in Nigeria [2]. Pretilachor pyribenzoxim and other herbicides are also used in seed beds, seed transplant fields, and some crop fields, including wheat, barley, cotton, vegetables, and peanuts [3]. Because agricultural country sides are so close to bodies of water, herbicides can enter aquatic environments. 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 absence of adequate microbial degraders and under conditions of low temperature, low moisture, high alkalinity, and persistent biological activity, it may persist in soils for a very long time [3,4].

Recent research has shown the toxicity of Pretilachor pyribenzoxim and other herbicides on fish and other aquatic animals, drawing attention to the xenobiotic contamination of aquatic habitats [3, 5]. Fishes are the most notable of the non-target creatures and have been crucial in determining the possible risk of contamination in aquatic environments [6]. Since they were found to be likely carcinogens, the poisoning of aquatic environments by chloroacetamide herbicides like pretilachlor has drawn more attention [7]. To suppress weeds, pretilachlor (2-chloro-2′,6′-diethyl-N-[2-propoxyethyl] acetanilide) is a systemic herbicide that is widely used in rice fields. This herbicide prevents the synthesis of long-chain fatty acids, which slows cell division [8]. More sensitive early warnings are provided by behavioural changes brought on by chemical exposure than by traditional diagnostic procedures. Herbicide-induced behaviour changes in fish are still very rare [7]. Behavioural toxicity of fish most frequently uses measurements of swimming-oriented reactions, opercular movement, feeding attempts, jerk swimming, and schooling [7].

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

2. MATERIAL AND METHODS

2.1 Experimental Site

The experiment was carried out in the Central Laboratory of the Fisheries and Aquaculture Department, Faculty of Agriculture, Adekunle Ajasin University Akungba-Akoko, Nigeria; using a plastic tank of 50 litres capacity (75cm x 40cm x 40cm). Each tank was filled with 30 litres of unchlorinated water.

2.2 Fish Collection and Experiment

Two hundred and fifty (250) healthy Oreochromis niloticus juveniles were purchased from a reputable hatchery in Akure, Nigeria. The Adekunle Ajasin University's Animal Ethics Committee established guidelines for the care of laboratory animals, and these guidelines were followed when treating the fish. The health status of selected fishes was assessed based on the presence or absence of physical injuries and other morphological deformities. The juveniles were acclimatized under laboratory conditions for 14 days before the commencement of the experiment. During the acclimatization period, the fish were fed commercial trout pellets fed daily at 2% body weight (BW) and covered with netting materials to prevent jumping out of the water. The water was changed daily, uneaten feed and faecal matters were siphoned out and dead fish were also removed to minimize contamination of water.

2.3 Source and Processing of Pretiolachort Pyribenzoxim

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

2.4 Toxicity bioassay

According to established procedures, a static bioassay system in the laboratory was used for the final test and an acute toxicity experiment to obtain the 96-hour LC_{50} values of Pretiolachor pyribenzoxim [11]. Before determining the concentrations of the test solution for the definitive test, the range finding test was conducted [12]. The experiment was carried out in 30 L of de-chlorinated and aerated water in rectangular plastic tanks (75 x 45 x 45 cm). The treatment period saw daily observations of fish behaviour. 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 Pretiolachor pyribenzoxim 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 Pretiolachor pyribenzoxim was tracked for up to 96 hours at intervals of 24 hours to obtain LC_{50} values of the test pesticides (Figure 1). The LC_{50} of Pretiolachor pyribenzoxim 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_{50} 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].

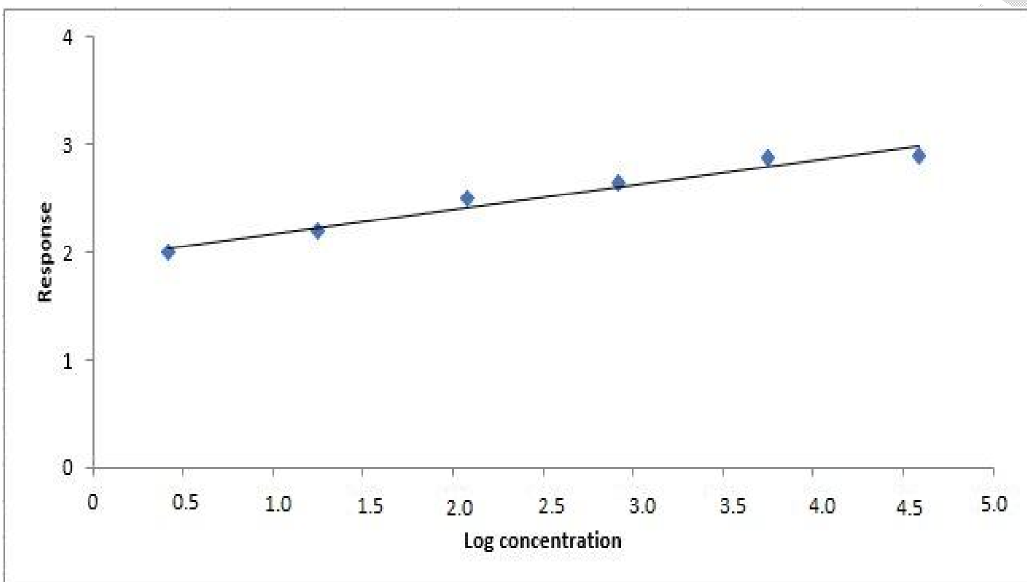

Fig. 1. Log of concentration of Pretiolachor pyribenzoxim herbicide and its probit value for juvenile *O. niloticus.*
LC_{50}**=1.03**

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

Table 1. Effects of Pretiolachor pyribenzoxim (PP) contamination on the physicochemical properties of water.

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

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).

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

Table 2. Effects of Pretiolachor pyribenzoxim (PP) contamination on the Behavioural Response of *Oreochromis niloticus* Juveniles

Behavioural response	Treatments	Duration of Exposure (hours) 24	48	72	96
	T1: 0.00 ml PP in 30L of H_2O	-	-	-	-
	T2: 0.10 ml PP in 30L of H_2O	-	-	+	+
	T3: 0.125 ml PP in 30L of H_2O	-	+	+	+
	T4: 0.15 ml PP in 30L of H_2O	+	+	+	+
	T5: 0.175 ml PP in 30L of H_2O	+	+	+	+
Loss of Reflex	T6: 0.12 ml PP in 30L of H_2O	+	+	+	+
	T1: 0.00 ml PP in 30L of H_2O	-	-	-	-
	T2: 0.10 ml PP in 30L of H_2O	-	+	+	+
	T3: 0.125 ml PP in 30L of H_2O	-	+	+	+
	T4: 0.15 ml PP in 30L of H_2O	+	+	+	+
	T5: 0.175 ml PP in 30L of H_2O	+	+	+	+
Air Gulping	T6: 0.12 ml PP in 30L of H_2O	+	+	+	+
	T1: 0.00 ml PP in 30L of H_2O	-	-	-	-
	T2: 0.10 ml PP in 30L of H_2O	-	-	+	+
	T3: 0.125 ml PP in 30L of H_2O	+	+	+	+
	T4: 0.15 ml PP in 30L of H_2O	+	+	+	+
	T5: 0.175 ml PP in 30L of H_2O	+	+	+	+
Erratic Swimming	T6: 0.12 ml PP in 30L of H_2O	+	+	+	+
	T1: 0.00 ml PP in 30L of H_2O	-	-	-	-
	T2: 0.10 ml PP in 30L of H_2O	-	+	+	+
	T3: 0.125 ml PP in 30L of H_2O	-	+	+	+
	T4: 0.15 ml PP in 30L of H_2O	+	+	+	+
	T5: 0.175 ml PP in 30L of H_2O	+	+	+	+
Fin deformation	T6: 0.12 ml PP in 30L of H_2O	+	+	+	+
	T1: 0.00 ml PP in 30L of H_2O	-	-	-	-
	T2: 0.10 ml PP in 30L of H_2O	-	-	+	+
	T3: 0.125 ml PP in 30L of H_2O	-	+	+	+

	T4: 0.15 ml PP in 30L of H_2O	+	+	+	+
	T5: 0.175 ml PP in 30L of H_2O	+	+	+	+
Moulting	T6: 0.12 ml PP in 30L of H_2O	+	+	+	+

Note: +: Observed; -: Not-observed

Table 3 shows the effects of Pretiolachor pyribenzoxim (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 Pretiolachor pyribenzoxim (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^{a}	0.67^{b}	61.78^{c}	21.16^{b}	81.30
T2	7.76^{b}	0.71^{b}	66.26^{bc}	27.96^{a}	82.80
T3	7.53^{b}	0.76^{ab}	73.31^{ab}	27.24^{a}	80.64
T4	7.94^{b}	0.80^{ab}	72.97^{ab}	26.16^{a}	82.35
T5	7.36^{b}	0.86^{a}	77.01^{a}	27.94^{a}	91.50
T6	7.21^{b}	0.89^{a}	74.97^{a}	28.66^{a}	81.03
SEM	0.29	0.02	1.48	0.68	1.96
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 T5 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- Balawi et al. [24]. The fishes in treatment groups that were exposed to PP in this study displayed some abnormal behaviour, including air gulping, erratic swimming, deformed fins, and moulting, which are caused by inhibition of acetylcholinesterase activity leading to accumulation of acetylcholine in cholinergic synapses that causes hyperstimulation of the toxicant and exhaustion due to respiratory difficulty [25, 26]. According to reports, stressed fish exhibit hyperactivity, and to escape the stressful environment, they need more oxygen to meet their energy needs [23].

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

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

4. CONCLUSION

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

ETHICAL APPROVAL

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

REFERENCES

1. Chauhan BS, Abeysekara ASK, Kulatunga SD, Wickrama UB. 2013. Performance of Different Herbicides in a Dry-Seeded Rice System in Sri Lanka. Weed Technol.; 27(3), 459–462. http://www.jstor.org/stable/43702622.

2. Ibrahim U, Rahman SA. B.A. Babaji BA. 2011. Cost –benefit analysis of rice production under weed control methods in Lafiagi area of Kwara. NSUK J Sci Technol; 1(1&2): 40-47.

3. Nwani CD, Ama UI, Okoh F, Oji UO, Ogbonyealu RC, Ibiam AA, and Udu-Ibiam O. 2013. Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*. African J Biotechn. 2013; 12(5), 499-503. http://www.academicjournals.org/AJB. DOI: 10.5897/AJB12.2433
4. Huang Y, Xiao L, Li F, Xiao M, Lin D, Long X, Wu Z. 2018. Microbial Degradation of Pesticide Residues and an Emphasis on the Degradation of Cypermethrin and 3-phenoxy Benzoic Acid: A Review. Molecules (Basel, Switzerland). 2018; 23(9), 2313. https://doi.org/10.3390/molecules23092313

5. Chang J, Liu S, Zhou S, Wang M, Zhu G. 2011. Effects of butachlor on reproduction and hormone levels in adult zebrafish (*Danio rerio*). Exper Toxicolog Pathol; 65(1-2), 205–209. https://doi.org/10.1016/j.etp.2011.08.007

6. Lakra WS, Nagpure NS. Genotoxicological studies in fish. 2009;: a review. Indian J. Anim. Sci. 79:93–98.

7. Soni R, Verma SK . 2018. Acute toxicity and behavioural responses in *Clarias batrachus* (Linnaeus) exposed to herbicide pretilachlor. Heliyon 2018; 4(12), e01090. https://doi.org/10.1016/j.heliyon.2018.e01090

8. Kaushik S, Inderjit Steribig JC, Cedergreen N. 2006. Activities of mixtures of soil-applied herbicides with different molecular targets. Pest Manage Sci;; 62: 1092–1097.

9. Araoye PA. 2008.Physical factors and their influence on fish species composition in Asa Lake, Ilorin, Nigeria. Rev. Trop. (Int. J Trop Biol.; 57(1-2):167-175

10. Yem IY, Bankole NO, Umar R, Ibrahim A, Ewutanure SJ. 2020. Food habit and growth pattern of Nile Tilapia Oreochromis niloticus in Wase Dam, Nigeria. Int J Fisher Aquatic StUD. 2019; 2020; 8(4): 257-260

11. APHA, AWWA, WPCF. 2005. Standard methods for the examination of water and waste water, 21st 401 ed. American Public Health Association, Washington, DC.

12. Nwana CD, Ama UI, Okoh B, Oji UO, Ogbonyealu RC, Ibiam AA, Udu-Ibiam O. 2013 Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zillii*. African J Biotech: 12(5), 499-503.

13. Finney DT. Probit Analysis. Cambridge University Press, Cambridge, p.333.1971.

14. Hart WB, Weston RF, Dermann JG. 1948. An apparatus for oxygenating test solution which fish are used as test animals for evaluating toxicity. Trans. Am. Fish Soc.; 75:288.

15. NAS/NAE. Water Quality Criteria, EPA-R3–033; US, Government Printing Office: Washington, DC, USA. 1973.

16. CCREM. Canadian Water Quality Guidelines; Canadian Council of Resources and Environmental Ministry, Inland Waters Directorate, Environment Canada: Ottawa, ON, Canada.1991.

17. IJC. New and Revised Great Lakes Water Quality Objectives. Great Lake Basin, Windsor; IJC: Ottawa, ON, Canada. 1977.

18. Oloruntola OD, Agbede JO, Onibi GE, Igbasan FA .2016. Replacement value of rumen liquor fermented cassava peels for maize in growing rabbit diet. Archivos de Zootecnia. 2016; 65 (249): 89-97.

19. De AK. Environmental Chemistry. 6th edition. New Age Int. (P) Ltd., New Delhi. 169-170 pp.2007.

20. Nimesh N, Jain S. 2016. Effect of dissolved oxygen on physiology and behaviour of freshwater fishes. Voyager. 2016. 7, Special Issue 2016, 1-7. e): 2455-054X

21. Zhang Y, Zhao Q, Ding S. 2019. The responses of stream fish to the gradient of conductivity: A case study from the Taizi River, China, Aquatic Ecosystem Health & Management. 2019; 22:2, 171-182, DOI: 10.1080/14634988.1622994.

22. Weber-Scannel P, Duffy L.K. 2007. Effects of Total Dissolved Solids on Aquatic Organisms: A Review of Literature and Recommendation for Salmonid Species. American J Envir Sci. 2020; 3 (1): 1-6.

23. Akinsorotan AM. 2014.Toxicity of Dizensate (glyphosate herbicide) on *Clarias gariepinus* fingerlings. Advanced Research in Biological Sciences; 2 (1), 1-5.

24. Alkahemal-Balawi HF, Ahmad Z, Al-Akel AS, Al-Misned F, Suliman EAM, Al Ghanim KA. 2011.Toxicity bioassay of lead acetate and effects of its sub-lethal exposure on growth, haematological parameters and reproduction in Clarias gariepinus. African J Biotechnol. 2011:10 (53), 11039-11047.

25. Vindas MA, Gorissen M, Höglund E, Flik G, Tronci V, Damsgård B, Thornqvist P, Nilsen TO, Winberg S,O Overli O, Ebbesson OE. 2017. How do individuals cope with stress? Behavioural, physiological and neuronal differences between proactive and reactive coping styles in fish. J. Exp. Biol.; 220, 1524–1532. doi: 10.1242/jeb.153213

26. Adewumi B, Michael OA, Damilohun SM. 2018. Histological and Behavioural Changes of *Clarias gariepinus* Juveniles Exposed to Chlorpyrifos and force. International J Biol Sci Applic.; 5(1), 1-12.

27. SiaSu GL, Ramos Gliceria B, SiaSu MLL. .2013. Bioaccumulation and histopathological alteration of total lead in selected fishes from Manila Bay, Philippines. Saudi J. Biol. Sci. 2013; 20:353–355. doi: 10.1016/j.sjbs.03.003.

28. Luskova V, Svoboda M, Kolarova J. The effects of diazinon on blood plasma biochemistry in carp (Cyprinus carpio L.) Acta Vet Brno.; 71:117–123. doi: 10.2754/avb200271010117.

29. Thiripurasundari S, Asokan, Thangapandiyan S. 2002. Acute Toxicity Impact of Herbicide Pretilachlor on Biochemical, Haematological and Ionic Changes in Gill, Liver and Kidney of a Cultivable Fish *Labeo rohita* (Hamilton). Inter J Pharm Biolog Sci; 2019; 9 (2): 990-999

30. Javed M, Ahmad MI, Usmani N, Ahmad M. 2017. Multiple biomarker responses (serum biochemistry, oxidative stress, genotoxicity and histopathology) in Channa punctatus exposed to heavy metal loaded waste water. Scientific reports; 7(1), 1675. https://doi.org/10.1038/s41598-017-01749-6

31. Davinder PS, Jasvirinder SK, Alka GK, Yadvinder S. 2016.Toxicological effect of pretilachlor on some physiological processes of cyanobacterium Synechocystis sp. strain PUPCCC 64. J Appl Biol Biotechnol.; 4 (01):12-19.

32. Ugbomeh, A.P., Bob-manuel, K.N.O., Green, A., and Taylorharry, O .2019. Biochemical toxicity of Corexit 9500 dispersant on the gills, liver and kidney of juvenile *Clarias gariepinus*. Fish Aquatic Sci 22, 15 (2019). https://doi.org/10.1186/s41240-019-0131-6.

33. Sparling DW. 2016. Ecotoxicological essentials: environmental contaminants and their biological effects on animals and plants. UK: Academic Press - Elsevier;. ISBN-978-0-12-801947-4. Pp 490.

34. Fathy M, Mohamed IA, Farghal AIA, Temerak SAH, Sayed AEH. 2019. Hemotoxic effects of some herbicides on juvenile of Nile tilapia *Oreochromis niloticus*. Environl Sci Pollut Res Inter; 26(30), 30857–30865. https://doi.org/10.1007/s11356-019-06280-

35. Gaafar A, Elmanakhly E, Soliman MK, Soufy H, Zaki MS, Mohamed SG, Hassan SM. 2010. Some pathological, biochemical and hematological investigations on Nile tilapia (*Oreochromis niloticus*) following chronic exposure to edifenphos pesticide. J Am Sci.; 6: 542–55.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

PP: Pretiolachor pyribenzoxim
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline phosphatase
DO: Dissolved oxygen
TDS: Total dissolved solids