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TOXICOLOGICAL, HEMATOLOGICAL, AND BIOCHEMICAL RESPONSES OF CATFISH TO A NOVEL BRAND OF HERBICIDE

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Abstract: Herbicide activities in any aquatic system can disrupt the biological functions of the biota therein. This study investigated the toxicity, hematological, and biochemical responses of *Clarias. gariepinus* to a new brand of herbicide. Before the acclimatization of the fish, a range-finding test was done to determine the lethal dosage of the herbicide. The lethal dosage of the concentrations on the test fish was done using the endpoint (LC_{50}). Standard methods were used to assess and compute the hematological and biological parameters from the sub-lethal test. The Statistic Package for Social Science and Microsoft Excel version 2019 was used to compute the mean data collated and the Probit analysis. The analysis of the physical and chemical parameters of the borehole water used in this study showed $p<0.05$ and $p>0.05$ for the mean parameters analyzed. The water was considered suitable for the bioassay test. Findings from the lethal concentration (LC_{50}) short test at log 0.65 Probit, for 96 h, demonstrated that the herbicide was also toxic to the fish and provoked behavioral stress. The findings of the sub-lethal test using the biochemical and hematological biomarkers showed the following ranks; ALT (Alanine transaminase) > Urea > ALP (Alkaline phosphatase) > ALB (Albumin) > Creatinine and LYM (Lymnocytes) > WBC (White blood cell) > GRAN (Granulocytes) > RBC (Red blood cell) > HGB (Hemoglobin) > PLT (Platelet). There was no significant difference in the mean values for both biomarkers at p> 0.05 in the treatment and the control groups. There was a reduction in the hematological and biochemical indices which resulted in microcytic anemia in the fish after exposure to the herbicides at various concentrations exempting the control. This was due to oxidative stress as a result of the discharge of ROS (reactive oxygen species) in the blood cells and serum.

Keywords: endpoints, toxicity trials, herbicides, agriculture, biomarkers.

INTRODUCTION

Across the globe, sustainable food security is a vision of many international and governmental organizations to fight and prevent mortality, food shortages, starvation, and hunger, which are serious pointers that have been long identified as major difficulties that would encircle the globe around 2050, when the world population is due to reach 10 billion.

In Agriculture, there has been a rapid rise in the use of herbicides to boost the production of food to meet the demands of the evolving population rise (Lengai *et al.*, 2020; Oladokun *et al.*, 2020).

In line with this demand, different forms of herbicides used in targeting special plant weeds, are employed to increase the yield of crops. In Nigeria and many developing nations of the world, one of the most widely used herbicides is 1,1'-dimethyl-4-4'-bipyridinium, commonly called Paraquat (Mbuk *et al.*, 2009; Nwani *et al.*, 2014). Contrarily, this herbicide was outrightly banned in developed countries like the EU (European Union) back in the year 2007 (Dinis-Oliveira *et al.*, 2008).

It has been established that Paraquat is applied to desiccate and defoliate economic plants like sugarcane, sunflowers, potatoes, soybeans, beans, and cotton before harvesting (Bromilow, 2004; Gwathway and Craig, 2007). Based on the continuous utilization and high level of solubility of Paraquat in the areas of horticulture (non-agriculture) and agriculture, large quantities of it could have infiltrated the aquatic bodies via runoffs and surface water thus posing serious health and environmental threats to the biota living in there, even when the concentration is at sublethal (Marin-Morales *et al.*, 2013). Severe conditions like mortality, physiological influence, and cytogenetical damages might ensue when aquatic resource comes in contact with the residues of herbicides in water (Tortorelli *et al.*, 1990; Dinis-Oliveira *et al.*, 2008; Huang *et al.*, 2013; Ensibi *et al.*, 2013; Tsai, 2013). This mechanism of action can result in ecosystem instability, food web modification, and food chain disruption (Forget *et al.*, 1998; Burkepile *et al.*, 2000; Sande *et al.*, 2011).

However, to establish the hematological and physiological changes in aquatic resources like fish and the management and control of certain diseases, several biomarkers and indices have been proposed to ascertain what takes place in the biota. Parameters such as PCV, WBC, Hb RBC, MCHC, MCH, and MCV (packed cell volume, white blood cells, hemoglobin, red blood cells, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, and mean corpuscular volume), have been suggested by Dogan and Can (2011) to verify anomalies in the blood system of the fishes. Meanwhile, enzymes like Alanine transaminase (ALT), Alkaline phosphatase (ALP), glucose, and plasma proteins, have been suggested by El-sayed *et al.*, (2007) and Suvetha *et al.*, (2010) to ascertain the irregularities in the biochemical compositions of the fishes.

The African catfish is known as *C. gariepinus*; the family Clariidae is a sharp-tooted Pisces that is

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commonly used for ecological and toxicological assessment of chemicals. It is biologically relevant and cheap to purchase as an experimental model compared to other species because of its high adaptability to the environmental factors in Nigeria waters in-situ and ex-situ, and the juvenile stage responds variably to any induce biological settings (US EPA, 2000; Adeyemi *et al.*, 2014; Olaniyi and Omitogun, 2014; Olorunfemi *et al.*, 2015; Ndimele *et al.*, 2015; Olaniran *et al.*, 2019; Ibor *et al.*, 2020).

Few studies have elaborated on the influence of Paraquat on some biochemical and physiological parameters as well as on the toxicology of chemicals on the catfish ex-situ (Ogamba *et al.*, 2011, Ada *et al.*, 2012, Safahieh *et al.*, 2012; Ayanda *et al.*, 2015a and b; Nwani *et al.*, 2014; Olorunfemi *et al.*, 2015). Nonetheless, this study intends to expand on already existing works on the acute toxicity, biochemical, and hematological responses of *C. gariepinus* to varying concentrations of a novel brand of paraquat herbicide since the effects of paraquat on tropical fish species have not been exhausted. It will also stress and foster the importance of the toxicities when used for agricultural purposes and the likely impact on the aquatic environment in the long run across the food chain.

MATERIALS AND METHODS

Study location

This study was carried out in the toxicology laboratory of the University, of Benin City, Edo State, Nigeria.

Ethical approval

The ethical committee in charge of the regulation and use of animal species in laboratory studies of the University of Benin, Benin City, Edo State, Nigeria, sanctioned and approved this study. The experiment was done according to the standard set by Bennett *et al.*, (2016) and Sloman *et al.* (2019).

Procurement and maintenance of fish

The juvenile African catfish, *C. gariepinus* of average weight, and length of (18.24±2.22) g and (9.17±1.73) cm, respectively, were procured from a commercial fish farm on Ekehuan Road, Benin City. They were kept in glass tanks (aquaria) of dimensions 20 by 15 by 30 cm in the laboratory with a water capacity of about 6 liters. The water used was devoid of chlorine contents (de-chlorinated) which were later exposed to room temperature (28.5 ± 0.5 °C) as well in atmosphere air for about 24 hours. Dust and other air impurities were prevented by constantly changing the water every 12 hours. *C. gariepinus* diploid chromosome 2n = 58 (Lui *et al.*, 2010) was chosen for this study because of its sensitivity to pollutants (Bailey *et al.*, 1992).

Acclimatization

The fish were acclimated for one week (7 days) under controlled room temperature (28.7-29.4 ºC), pH (4.81 ± 0.32), biological dissolved oxygen (BOD5) 6.8 ± 0.42 mg l-1, and DO 6.6 ± 0.1 mg l-1. During the period of acclimatization, the test organisms were fed to satisfaction two times daily with a fish meal consisting of pellets and crude protein, 42 %, and 3,400 Kcal kg-1 DE respectively, at 3 % body weight. Before the controlled biological experiment, feeding was altered a day to the experiment under established standards by UNEP, (2000). Also, during this period, the test organisms were scrutinized for diseases and pathogens. The organisms were scrutinized for disease by the observation of their breathing rate, white patches or spots on their fins and body, and darting or twitching around the body of the test tanks. Fishes observed are immediately isolated and quarantined in a separate test tank and treated accordingly depending on the symptoms observed. The water used for the bioassay test was changed every 24 h to avoid the accumulation of wastes of metabolic origin (Olorunfemi *et al.*, 2015). A total of 400 juvenile fishes were acclimatized before the main experiment.

Toxicant selected

The total contact herbicide product of The Candel company limited, Plot 40, block 4, Jokyemi Street of Christ Avenue, Lekki Scheme 1, Lagos, manufactured by Hubei Sandonda Co. Limited 93, East Beijing Road Jingzhou, Hubei 434001 China, was procured from the Venco Nigeria Limited, Textile Mill Road branch, Benin City, Nigeria. The active ingredients therein were 1,1'-dimethyl-4-4'-bipyridinium – 24 % WW with emetic, dye, and stench formulations. NAFDAC Reg. No.: A5-0966, Batch No.: 20150425, Manufactured April 25, 2015, and Expiry date on April 24, 2017.

Acute toxicity testing

The static renewal acute toxicity test was carried out under the guidelines set by the OECD (1992) as modified by Olorunfemi *et al.*, (2015). A serial dilution was carried out to determine the range of concentrations to be used for the experimental setup. Ten (10) fishes each in triplicates were exposed to 0, 2.2, 4.3, 6.4, and 8.5 ml/L of the test concentrations, making it a total of 150 used to determine the 96 h LC_{50} of the herbicide. The survival and death rates were documented sequentially at every 24 h for 4 days (96 h) in each of the concentrations used. Any deceased fish observed in the test tank was removed to avoid build-up or the addition of organic wastes. Spasmodic movements, appendage movement, spinning rate, equipoise status, and hyperactivity, were the most observed behaviors in the experimental setup.

In vivo sub-lethal testing

The concentrations used in the sub-lethal testing were 0, 0.1, 0.2, 0.3, and 0.4 ml/L in triplicates. Every 24 h, the test water was changed and the same concentrations were re-introduced into the test tanks. The experimental exposure period lasted for 21 days. On day 21, blood samples were collected from the fish through the caudal vein by pricking it with a 5 mL needle syringe (heparinized). About 100 mL of blood was extracted from the fish from each test tank.

The blood samples were stored in treated EDTA (ethylene-diamine tetra-acetic acid) vials. The collected samples were used for the valuation of the hematological analysis for WBC, RBC, and Hb count. Some samples of the collected samples were centrifuged at 10 000 r/min for five minutes, to determine the plasma and serum counts for the valuation of Creatinine, ALB, ALP, and ALT.

Hematologic analysis

The method of Rusia and Sood (1992) as modified by Nwani *et al.*, (2014) and Lengai *et al.*, (2020), was used to determine the WBC and RBC counts with an enhanced Neubauer hemocytometer. The blood content was assessed at 540 nm wavelength by employing the cyanmethemoglobin technique of Blaxhall and Daisley, (2006). According to the technique of Nelson and Morris (1986), as modified by Nwani *et al.*, (2014) and Oladokun *et al.*, (2020), the PCV counts were estimated at 11 000 r/min for six minutes with the aid of an enhanced centrifuge (Hawksley hematocrit).

The technique of Dacie and Lewis (1984) as modified by Nwani *et al.*, (2014) and Lengai *et al.*, (2020), was used to assess the following Red blood cell indices, such as MCV (Mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), LYM (Lymnocytes), WBC (White blood cell), GRAN (Granulocytes), RBC (Red blood cell), HGB (Haemoglobin), RDW-SD (Red cell distribution width-standard deviation), PLT (Platelet), RDW-CV (Red cell distribution width-coefficient of variation), PDW (Platelet distribution width), MPV (Mean platelet volume), PCT (Plateletcrit) and P-LCR (Platelet larger cell ratio).

Biochemical estimation

The o-toluidine plasma glucose technique by Lowry *et al.*, (1951), Reitman and Frankel (1957), and Cooper and Daniel (1970) as modified by Lengai *et al.*, (2020) and Oladokun *et al.*, (2020) was used to determine the ALB, ALT, and ALP levels.

Physical and chemical parameters

The water used in the experimental setup was characterized and determined using the APHA (2005) and ASTM (2013) methodologies. Concerned parameters assessed were pH, EC (Electrical conductivity), water temperature, TDS (total dissolved oxygen), DO (dissolved oxygen), BOD5 (biological dissolved oxygen), COD (chemical dissolve oxygen demand), Cl (chlorine), P (phosphate), NO2 (nitrite), NO3 (nitrate), Fe (iron), Zn (zinc), Mn (manganese), Cu (copper) and TCC (total coliform counts).

Statistical Analysis

The mean values of the water parameters used in this study were subjected to ANOVA; one-way analysis was set at $P < 0.05$. The lethal dosage of the concentrations on the test fish was done using the method of Finney (1971) to determine the endpoint (LC_{50}). The SPSS (Statistic Package for Social Science) version 21.0 Inc. Chicago, Illinois, USA, and Microsoft Excel version 2019 were used to compute the mean data collated in this study.

RESULTS AND DISCUSSION

The results of the behavior, percentage of mortality, and LC_{50} of the novel brand of paraquat in *C. gariepinus* after exposure for 96 h

The results of the percentage of mortality of the novel brand of herbicide in *C. gariepinus* after exposure to different concentrations for 96 h are presented in Tables 1-4. The behavioral observations when the herbicide was exposed to *C. gariepinus* were wheezing for air, irregular movement, and restlessness, Though, standard behavior was detected in the control groups. The mean percentage of mortality was noticed to be between 4 and 5 out of the 10 test organisms used.

Table 1.

Mortality rates of C. *gariepinus* juveniles exposed to varying concentrations of the novel herbicide (N=10)

	Mortality					
Concentration (mg/l) /L	24 hours	48 hours	72 hours	96 hours	No of Mortality	Percentage Mortality
Control	0	0	0	0	0/10	0
2	0	2	0	2	04/10	50
4	2	0	1	2	05/10	50
6	2	1	2	0	05/10	50
8	4	1	0	0	05/10	50

Table 2.

Percentage mortality rates of *C. gariepinus* juveniles exposed to varying concentrations of the novel herbicide (N=10)

No of deaths at 96 hours in Triplicates						
Concentration (mg/l)/L				Mortality	Percentage Mortality (%)	Probit
	1	2	3			
Control	0	0	0	0/10	0	2.5
2	0	2	2	04/10	40	4.75
4	2	0	3	05/10	50	5
6	2	3	0	05/10	50	5
8	4	1	0	05/10	50	5

Table 3.
Mortality records of *C. gariepinus* juveniles exposed to the novel herbicide concentrations for 96 h

Concentration (mg/L)/L	Log Conc.	Total No. of Fish	No. Dead 96 hours	Mean Mortality	% Mortality	Probit
0	0	10	0	0	0	2.5
2	0.3	10	4	1.33	40	4.75
4	0.6	10	5	1.66	50	5
6	0.77	10	5	1.66	50	5
8	0.9	10	5	1.66	50	5

Table 4.
Mean mortality rates of *C. gariepinus* juveniles exposed to acute concentrations of the novel herbicide for 96 hours

Concentration (mg/L)	Log Conc.	% Mean Total Mortality	Mean Valve	Probit
0	**0**	**0**	**0**	**2.5**
2	0.3	40	1.33	4.75
4	0.6	50	1.66	5
6	0.77	50	1.66	5
8	0.9	50	1.66	5

The results from the behavioral activities of the fish when exposed to the novel brand of herbicide in this study, showed a level of toxicosis of the herbicide. The symptoms observed in this study conformed to what was obtained and noted by Ayuba and Ofojekwu (2002), Onusiriuka (2002), Auta and Ogueji (2007), Okomoda and Ataguba (2011), Aderolu *et al.*, (2010), Olorunfemi *et al.*, (2015), Nwani *et al.*, (2014), Olaniran *et al.*, (2019) and Ibor *et al.*, (2020) such as the air gulping, abnormal restlessness, erratic and stressful symptoms. These symptoms could have been a result of the physiological influences and mechanism of action of the herbicides on the gills and blood (respiratory, biochemical, and hematological) systems of the fish.

The probit of death against the log concentration of *C. gariepinus* at 96 h and LC_{50} was also determined (Figure 1). The LC_{50} at 96 h was 0.65 mg/L (4.47 L) with lower and upper confidence limits of 0.678 mg/L (4.76 L) and 0.735 mg/L (5.43 L) respectively. The computed regression equation was found to be Y= 20*x + -13 (R = 4.447 (log 0.65) Y = probit kill) Figure 1. The Person goodness of fit using Chi-square showed no significant difference (P > 0.05) among the concentration of the chemical used and the Z score mean estimate (3.736) revealed that there was a significant difference (P < 0.05) amongst the concentrations with the standard error of 3.025.

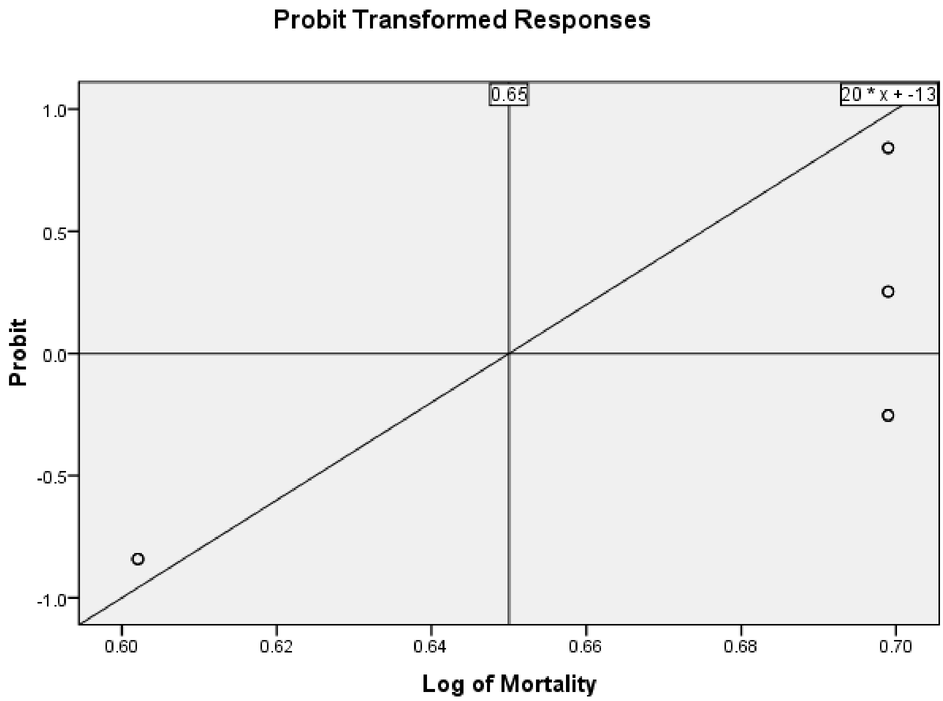

Fig. 1. Linear relationship between the mean probit death and log concentration of *C. gariepinus* Juveniles exposed to the novel herbicide for 96 h.

The findings from the short toxicity evaluation for 96 h, revealed that the herbicide is highly noxious. The 96 h LC_{50} obtained in this study, showed that when the herbicide is released into the natural environment, there is a possibility that about 50% of the population of fish in the region at which the concentration is median, will be exterminated. The trend of mortality observed in this study demonstrated that the death of the test organism was both concentration and time-reliant. A previous study also established similar trends in different species of fish (Pandey *et al.,* 2005; Ada *et al.*, 2012; Safahieh *et al.,* 2012; Nwani *et al.,* 2013 and 2014; Olaniran *et al.*, 2019; Ibor *et al.*, 2020). It can be asserted here, that *C. gariepinus* showed a positive response to the toxicant likely because of the age of the species used, and the positive environmental conditions affecting them at the time the study was conducted.

The results of the hematological and biochemical parameters

The results of the changes in the various hematological parameters are presented in Table 5. It was observed that the values obtained showed no significant increase at $P > 0.05$ in the mean concentration as compared to the control groups. However, there was a decrease in the MCV, MCH, and MCHC count in the exposed fish at the end of the biological test. The values are MCV: control (140.43 fl). 0.1 ml (141.70 fl), 0.2 ml (97.70 fl), 0.3 ml (86.33 fl) and 0.4 ml (36.83 fl), MCH: control (46.33 pg). 0.1 ml (46.83 pg), 0.2 ml (32.60 pg), 0.3 ml (28.73 pg) and 0.4 ml (12.27 pg) and MCHC: control (32.67 g/dl). 0.1 ml (32.83 g/dl), 0.2 ml (22.10 g/dl), 0.3 ml (22.00 g/dl) and 0.4 ml (11.00 g/dl).

Table 5.

Summary of the hematological parameters of *C. gariepinus* exposed to varying concentrations of the novel herbicide for 21 days (three weeks)

		Control	**0.1 ml**	**0.2 ml**	**0.3 ml**	**0.4 ml**	**P-value**
Analytes	**Units**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	
WBC	(×10^9/µl)	0.87±0.50 (0.2-2)	11.33±6.5 (0.00-32.7)	26.53±15.32 (0.1-59.1)	3.47±2.00 (0.00-5.5)	0.97±0.56 (0.1-2.6)	P>0.05
LYM	%	32.83±18.96 (0-98.5)	60.83±35.1 (0.00-99.0)	65.47±37.80 (0.00-99.3)	65.83±38.01 (0.00-99.0)	33.00±19.05 (0.00-99.0)	P>0.05
MID	%	0.13±0.08 (0-0.4)	1.13±0.7 (0-2.8)	0.83±0.48 (0.00-2.2)	0.23±0.13 (0.00-0.40)	0.10±0.06 (0.00-0.30)	P>0.05
GRAN	%	0.37±0.21 (0-1.1)	1.13±0.7 (0.00-2.8)	0.37±0.21 (0.00-0.7)	0.60±0.35 (0.00-1.10)	0.23±0.13 (0.00-0.7)	P>0.05
LYM	(×10^9/µl)	0.67±0.38 (0-2)	11.17±6.4 (0.00-32.4)	25.90±14.95 (0.00-57.4)	3.40±1.96 (0.00-5.40)	0.87±0.50 (0.00-2.60)	P>0.05
MID	(×10^9/µl)	0.00±0.00 (0.00-0.00)	0.07±0.00 (0.00-0.20)	0.47±0.27 (0.00-1.3)	0.00±0.00 (0.00-0.00)	0.00±0.00 (0.00-0.00)	P>0.05
GRAN	(×10^9/µl)	0.00±0.00 (0.00-0.00)	0.10±0.10 (0.00-0.20)	0.13±0.08 (0.00-0.40)	0.07±0.04 (0.00-0.10)	0.00±0.00 (0.00-0.00)	P>0.05
RBC	(×10^12/µl)	0.09±0.05 (0.02-0.21)	0.34±0.20 (0.03-0.9)	0.70±0.40 (0.00-1.44)	0.20±0.12 (0.00-0.31)	0.06±0.04 (0.00-0.19)	P>0.05
HGB	(g/dl)	0.40±0.23 (0.1-0.96)	1.71±1.0 (0.13-4.4)	3.68±2.12 (0.4-7.7)	0.88±0.51 (0.00-1.33)	0.30±0.17 (0.10-0.70)	P>0.05
HCT	%	1.20±0.69 (0.3-2.9)	5.13±3.0 (0.40-13.2)	10.63±6.14 (0.00-23.1)	2.63±1.52 (0.00-4.00)	0.70±0.40 (0.00-2.10)	P>0.05
MCV	(fl)	140.43±81.08 (133.3-150)	141.70±81.8 (133.3-153)	97.90±56.52 (0.00-160.4)	86.33±49.85 (0.00-130)	36.83±21.27 (0.00-110.5)	P>0.05
MCH	(pg)	46.33±26.75 (43.3-50)	46.83±27.0 (43.3-51.1)	32.60±18.82 (0.00-53.5)	28.73±16.59 (0.00-43.3)	12.27±7.08 (0.00-36.8)	P>0.05
MCHC	(g/dl)	32.67±18.86 (32-33)	32.83±19.0 (32.5-33.0)	22.10±12.76 (0.00-33.3)	22.00±12.70 (0.00-33.0)	11.00±6.35 (0.00-33.0)	P>0.05
RDW-CV	%	3.63±2.10 (0-10.9)	5.53±3.2 (0.00-8.90)	9.30±5.37 (0.00-15.5)	11.30±6.52 (0.00-23.9)	5.00±2.89 (0.00-15.0)	P>0.05
RDW-SD	(g/dl)	21.30±12.30 (0-63.9)	23.30±13.5 (0.00-69.9)	60.00±34.64 (0.00-99.5)	53.30±30.77 (0.00-81.7)	24.47±14.13 (0.00-73.4)	P>0.05
PLT	(×10^3/µl)	14.33±8.28 (2-33)	12.67±7.3 (0.00-30.0)	28.00±16.17 (8.00-50.0)	16.33±9.43 (0.00-38.0)	4.67±2.69 (1.00-10.00)	P>0.05
MPV		3.87±2.23 (0-6)	4.00±2.3 (0.00-6.1)	9.27±5.35 (5.8-12.6)	5.60±3.23 (0.00-10.8)	1.97±1.14 (0.00-5.90)	P>0.05
PDW	(fl)	4.63±2.68 (0-7.5)	0.00±0.00 (0.00-0.00)	6.53±3.77 (6.4-6.8)	3.47±2.00 (0.00-10.4)	0.00±0.00 (0.00-0.00)	P>0.05
PCT	%	0.00±0.00 (0-0.01)	0.00±0.00 (0.00-0.00)	0.03±0.02 (0.00-0.06)	0.01±0.01 (0.00-0.04)	0.00±0.00 (0.00-0.00)	P>0.05
P-LCR	%	0.00±0.00 (0.00-0.00)	0.00±0.00 (0.00-0.00)	15.53±8.97 (0.00-31.7)	7.67±4.43 (0.00-23)	0.00±0.00 (0.00-0.00)	P>0.05

Note: MID means the combined worth of other leukocytes not categorized as granulocytes or lymphocytes

Similarly, the variations in the chief hematological parameters (MCHC, MCH, and MCV), indicated that there were no significant differences in their mean when likened to their respective control at P>0.05. More so, it was observed that the lymphocytes dominated the leukocytes in the *C. gariepinus* peripheral body fluid when the novel brand of herbicide was exposed to it (Table 5). The ranks of the other hematological parameters in terms of abundance were in this form: LYM>WBC>GRAN>RBC>HGB and RDW-SD>PLT>RDW-S>PDW>MPV>PCT>P-LCR.

The diagnostic influence of the herbicide on the fish was determined by using hematological and biochemical assays. From this study, it was observed that the PCV, Hb, and RBC blood parameters of the fish decreased when exposed to the novel brand of herbicide. This could be linked to swift hemolysis and deformed erythropoiesis of the red blood cells. Erythropoiesis takes place at the head of the renal organ with about 10% of the immature blood cells specifically red blood cells, containing tetrameric iron and globin (hemoglobin) materials low in oxygen. This might lead to hypoxia condition of the fishes thus changing their physiological and metabolically reactions. Possible anomalies include cell deformation, swelling, vacuolation of the cytoplasm, and nuclear deformities of the blood cells (Zaahkook *et al.,* 2016; Elias *et al.*, 2018; Amaeze *et al.,* 2020; Oladokun *et al.,* 2020). Similarly, there was also a reduction in the level of the MCHC, MCH, and MCV in the fish. This agrees with what was obtained by Haux *et al.,* (1985), Nussey *et al.,* (1995), Nwani *et al.,* (2014), and Amaeze *et al.,* (2020). The reduction in the hematological parameters could result in microcytic anemia and an imbalance in the osmoregulatory system. This denoted that the novel brand of herbicide has inhibitory potentials that can impede hematological functions that can also affect the O2 carrying volume of the erythrocytes.

The presence of abundant leukocytes in the peripheral cells of the blood is an indication of leucocytosis stress to the herbicide (Nwani *et al.,* 2014). The implication is that the fish's immune system was trying to adjust to the herbicide's toxicants thus releasing enough defense lymphocytes from the leukocyte tissue; lymphomyeloid.

Generally, there is a local link between the levels of glucocorticoid and leukocytes in their stimulation of the lymphocyte percentages in living things in turn decreasing the levels of lymphocytes. This study revealed that the level of lymphocytes exceeded that of the leucocytes because the test fishes were trying to develop immunity to stress when exposed to the sublethal concentrations of the herbicide to survive. That is the reason no death was recorded. With this information, the lymphocytes can be identified as an immuno- capable cell. Similar defense mechanisms in fish ecotoxicological response have been established by El-Sayed *et al.,* (2007), Suvetha *et al.,* (2010), and Nwani *et al.,* (2014).

The results of the biochemical parameters showed no significant increase at P>0.05 in the treated group when likened to the control group at the end of the biological test (Table 6). The differences in the enzyme parameters showed that the parameters were in the following ranks: ALT>Urea>ALP>ALB>Creatinine Table 6. The results of the biomarkers for the liver enzymes of the fish, when exposed to the novel brand of herbicide, provoked an increase in the activities of the ALT and AST. This increased in the biochemical activities responded as the concentration increased. Thus, the action of the herbicide on the test organism was concentration-dependent. Philip *et al.,* (1995), John *et al.,* (2007), Suvetha *et al.,* (2010), Li *et al.,* (2011), Ayanda *et al.,* (2015 and 2015b), Adesina *et al.,* (2017), and Michael (2018), have also established similar concentration-dependent activities in related fish species exposed to varying chemical concentrations. This elevated serum activity of the biochemical parameters could elicit transamination, which is a process to meet the metabolic energy demand of the fish when the toxicant impacts cell damage. Besides, this process elevated the levels of glucose in the fish. The concentration of the protein was increased as a result of the fish responding to the high demand for metabolic functions caused by the herbicide. Overproduction of protein enzymes like alkaline and acid phosphates due to the physiological response in the fish to the herbicide might cause kidney and liver damage. This is due to the production of ROS (reactive oxygen species) which could lead to the inhibitory defense of the antioxidant, proteins, and lipids because of oxidative stress (Nwani *et al.,* 2014; Kaur and Jindal, 2017). Variations in percentage composition and distributions of varied hematological and biochemical parameters in the whole blood, the tissue of catfish exposed to varying concentrations of novel herbicides.

Table 6.

Summary of the biochemical parameters of *C. gariepinus* exposed to varying concentrations of the novel herbicide for 21 days (three weeks)

		Control	**0.1 ml**	**0.2 ml**	**0.3 ml**	**0.4 ml**	**P-value**
Analytes	**Units**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	**Mean± SE (Min-Max)**	
ALT	(u/l)	36.33±20.98 (21-48)	44.00±25.4 (32-60.0)	52.33±30.22 (30-77)	36.67±21.17 (0.00-62)	36.33±20.98 (25.0-45.0)	P>0.05
ALP	(u/l)	7.70±4.45 (6-9)	11.03±6.4 (8.2-16.5)	15.13±8.74 (8-29.4)	10.03±5.79 (0.00-20.7)	8.33±4.81 (7.40-8.90)	P>0.05
ALB	(g/l)	4.90±2.83 (2.6-8.5)	16.27±9.4 (9.7-25.0)	11.53±6.66 (3.6-27)	18.37±10.60 (0.00-34.6)	6.80±3.93 (5.20-8.10)	P>0.05
Urea	(mg/dl)	30.90±17.84 (25.1-39.6)	35.03±20.2 (28.4-41.0)	35.63±20.57 (27.5-44.7)	32.57±18.80 (0.00-49.4)	27.33±15.78 (16.5-38.5)	P>0.05
Creatinine	(mg/dl)	0.47±0.27 (0.2-0.9)	0.23±0.1 (0.2-0.3)	0.40±0.23 (0.2-0.7)	0.33±0.19 (0.00-0.60)	0.23±0.13 (0.20-0.30	P>0.05

The percentage composition and distributions of varied hematological parameters in the whole blood tissue of catfish. It was noticed that MCV had the highest percentage (45 %) of peripheral hemoglobin. This was followed by MCH (15 %), MCHC (11 %), LYM (11%), RDW (7 %), PLT (5 %), PDW (2 %), RDW-CV (1 %), MPV (1 %) and others (0 %) (Figure 2).

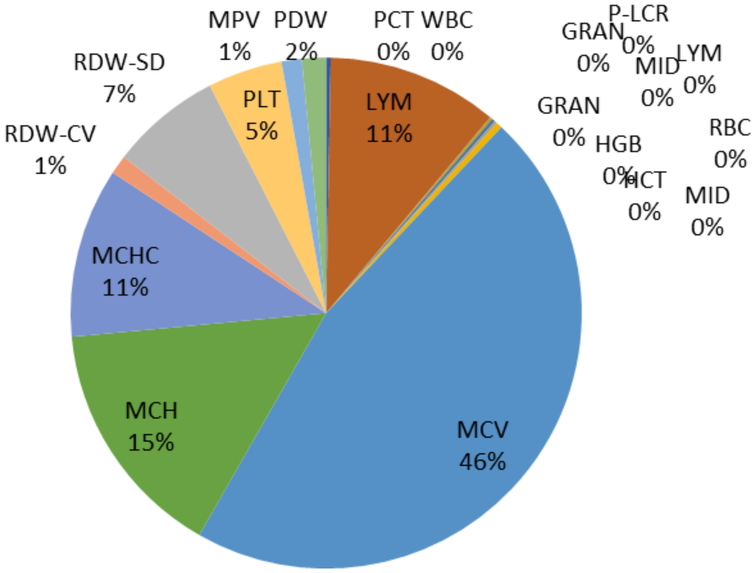

Fig. 2. Percentage composition and distributions of varied hematological parameters in the whole blood tissue of catfish.

Moreover, it was noticed that the percentage composition and distribution were as follows: ALT (45 %), Urea (38 %), ALP (10 %), ALB (6 %), and Creatinine (1 %) in the peripheral hemoglobin (Figure 3). It can be inferred that both the hematological and biochemical parameters of the fish, were influenced by the activities of the novel herbicides. This could be seen from the fluctuation of the percentage composition and distribution of the parameters with MCV and ALT dominating. Evidence of these indicates oxidative stress due to the formation of ROS (Nwani *et al.*, 2014; Adesina *et al.*, 2017; Kaur and Jindal, 2017).

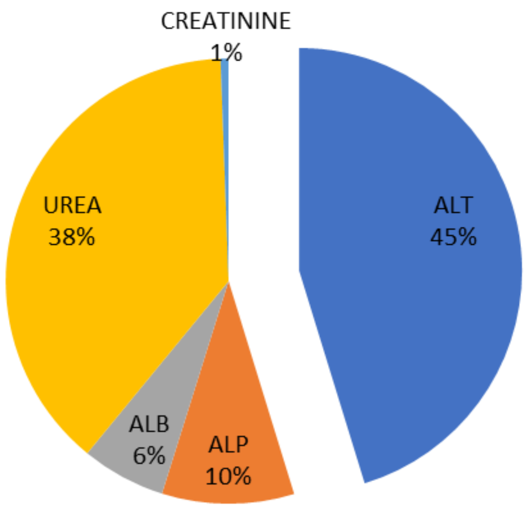

Fig. 3. Percentage composition and distributions of varied biochemical parameters in the whole blood tissue of catfish.

Quantification and assessment of the physical and Chemical parameters of the borehole water

The outcome of the physical and chemical analysis of the Borehole water used in this study is presented in Table 7. Some of the elements were present above permissible limits and showed significant and non-significant values ($P < 0.05$ and $P > 0.05$) respectively. The minimum and maximum limits of the parameters analyzed were. The minimum and maximum limits of the parameters analyzed were pH (6.42-7.48), EC (184.5-224 µS/cm), water temperature (28.7-29.4 ºC), DO (5.8-7.1 mg/l), BOD5 (0.00-0.40 mg/l), COD (2.2-3.4 mg/l), Cl (18.6-25.3 mg/l), P (0.10-0.25 mg/l), NO2 (0.01-0.04 mg/l) NO3 (0.38-0.57 mg/l), Fe (0.24-0.53 mg/l), Mn (0.01-0.034 mg/kg), Zn (0.07-0.11 mg/l), Cu (0.00-0.005 mg/l) and TCC (0.00-1.00 mg/l).

The findings from the quantification of the borehole water parameters showed that the range of values is within the slated limits recommended for toxicity trials as recommended by OECD (1992). These values agree with what was obtained from related studies by Okomoda and Ataguba (2011), and Olorunfemi *et al.*, (2015). Hence, the water quality is deemed to be fit for the use of toxicity examination, without causing any deleterious harm to the fish.

Table 7.
Summary of the physical and chemical characteristics of the Borehole water used for the acute and chronic toxicity of dilution

Code	Units	Mean± SE (Min-Max)	DPR/FMEnv 1997	P-values
pH		6.46±0.57 (6.42-7.48)	6.5-9.2	P<0.05
EC	µS/cm	199.4±21.14 (184.5-224)	NS	P<0.05
H_2O Temp.	ºC	29.07±0.35 (28.7-29.4)	30 ºC	P<0.05
TDS	mg/l	97.93±10.84 (90.1-110.3)	2000	P<0.05
DO	mg/l	6.5±0.66 (5.8-7.1)	5	P<0.05
BOD_5	mg/l	0.13±0.23 (0.00-0.4)	NS	P>0.05
COD	mg/l	2.83±0.60 (2.2-3.4)	40	P<0.05
Cl	mg/l	21.1±3.66 (18.6-25.3)	200-600	P<0.05
P	mg/l	0.16±0.08 (0.10-0.25)	5	P>0.05
NO_2	mg/l	0.023±0.01 (0.01-0.04)	NS	P>0.05
NO_3	mg/l	0.46±0.10 (0.38-0.57)	20	P<0.05
Fe	mg/l	0.36±0.15 (0.24-0.53)	20(0.1-1.0)	P>0.05
Mn	mg/l	0.017±0.02 (0.01-0.034)	5(0.05-0.5)	P>0.05
Zn	mg/l	0.09±0.02 (0.07-0.11)	1(5-15)	P<0.05
Cu	mg/l	0.002±0.003 (0.00-0.005)	0.05-1.5	P>0.05
TCC	MPN/100m	0.33±0.57 (0.00-1.00)	0.00	P>0.05

NS: Not specified. All metals and non-metals are expressed in mg/l except conductivity (µS/cm) and pH (no units). DPR = Department of Petroleum Resources (1997), FMEnv = Federal Ministry of Environment (1997) maximum permissible limits for domestic water pollutants. The $P<0.05$ is significant; $P>0.05$ is non-significant.

CONCLUSIONS

The findings of this study have shown and established that a novel brand of herbicides is noxious and has inhibitory effects on *C. gariepinus*. If this herbicide finds its way via runoffs from the point and non-point sources to river bodies, it could induce severe toxicity, severe behavioral changes, oxidative stress and alter the biochemical and hematological activities of fishes in the water. Therefore, we recommend the outright ban of herbicides in agronomic activities and suggest an eco-friendly approach like bioherbicides for the management of farm weeds.

Findings

The findings of the sub-lethal test using the biochemical and hematological biomarkers showed the following ranks; ALT (Alanine transaminase) > Urea > ALP (Alkaline phosphatase) > ALB (Albumin) > Creatinine and LYM (Lymnocytes) > WBC (White blood cell) > GRAN (Granulocytes) > RBC (Red blood cell) > HGB (Hemoglobin) > PLT (Platelet). It was observed that the PCV, Hb, and RBC blood parameters of the fish decreased when exposed to the novel brand of herbicide. This could be linked to swift hemolysis and deformed erythropoiesis of the red blood cells. There was a reduction in the hematological and biochemical indices which resulted in microcytic anemia in the fish after exposure to the herbicides at various concentrations exempting the control. This was due to oxidative stress as a result of the discharge of ROS (reactive oxygen species) in the blood cells and serum.

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AUTHORS CONTRIBUTIONS

Osayande Ernest Ebun-Igbeare and Osikemekha Anthony Anani contributed equally to the

Conceptualization, methodology, data collection, data validation, data processing, writing, original draft preparation, writing, review, and editing.

CONFLICT OF INTEREST

The authors have not any competing financial, professional, or personal interests from other parties.

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REFERENCES

Ada FB, Ekpenyong E, Ayotunde EO, Haematological, biological and behavioral changes in *Oreochromis niloticus* (Linne 1757) juveniles exposed to paraquat herbicide. Journal of Environmental Chemistry and Ecotoxicology, 4, 64-74, 2012.

Aderolu A. Ayoola SO, Otitoloju AA, Effects of Acute and sub-lethal concentrations of Actellic on Weight changes and Haematology parameters of *C. gariepinus*. World Journal of Biological Research, 3, 30-39, 2010.

Adesina SA, Falaye AE, Ajani EK, Evaluation of hematological and serum biochemical changes in *C. gariepinus* juveniles fed graded dietary levels of boiled sunflower (*Helianthus annuus*) seed meal replacing soybean meal. Ife Journal of Science, 19 (1), 51-68, 2017. https://dx.doi.org/10.4314/ijs.v19i1.7.

Adeyemi JA, Adewale OO, Oguma AY, Mortality, oxidative stress and hepatotoxicity in juvenile African catfish, *C. gariepinus* Burchell, exposed to lead and cypermethrin. Bulletin Environmental Contamination and Toxicology, 92 (5), 529-533, 2014.

Amaeze NH, Komolafe BO, Salako AF, Akagha KK, Briggs, T-MD, Olatinwo OO, Femi MA, Comparative assessment of the acute toxicity, hematological and genotoxic. Heliyon, 6 (8), e04768, 2020.

APHA, *Standard Methods for the Examination of Water and Wastewater*. 21st ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA. 2005.

ASTM, *Standard Test Method for Determination of Polychlorinated Biphenyls (PCBs) in Waste Materials by Gas Chromatography Active Standard ASTM D6160* | Developed by Subcommittee: D02.04.0L. 2013.

Auta J, Ogueji EO, *Acute Toxicity and behavioral effects of chlorpyrifos-Ethyl pesticide to juvenile of C. gariepinus Teugels.* Proceeding, 22nd Annual Conference of Fisheries Society of Nigeria (FISON) held at School of Nursing, Birnin Kebbi. pp. 264-272, 2007.

Ayanda OI, Oniye SJ, Auta JA, Ajibola VO, Acute toxicity of glyphosate and paraquat to the African catfish (*C. gariepinus*, *Teugels* 1986) using some biochemical indicators. Tropical Zoology, 28 (4), 152-162, 2015a. https://dx.doi.org/ 10.1080/03946975.2015.1076661.

Ayanda OI, Oniye SJ, Auta JA, Ajibola VO, Bello OA, Responses of the African catfish *C. gariepinus* to long-term exposure to glyphosate-and paraquat-based herbicides. African Journal of Aquatic Science, 40 (3), 261-267, 2015b.

Ayuba VO, Ofojekwu PC, Acute toxicity of the root extract of Jimson's Weed: *Datura innoxia* to the African catfish (*C. gariepinus*) fingerlings. Journal of Aquatic Science, 17 (2), 131-133, 2002.

Bailey MJ, Biely P, Poutanen K, "Interlaboratory Testing of Methods for Assay of Xylanase Activity". Journal of Biotechnology, 23, 257–270, 1992.

Bennett RH, Ellender BR, Mäkinen T, Miya T, Pattrick P, Wasserman RJ, … Weyl OLF, Ethical considerations for field research on fishes. Koedoe, 58 (1), 2016. https://dx.doi.org/10.4102/koedoe.v58i1.1353.

Blaxhall PC, Daisley K, Routine haematological methods for use with fish blood. Journal of Fish Biology, 5 771-781, 2006.

Bromilow RH. Paraquat and sustainable agriculture. Pesticides Management Science, 60 (4), 340-349, 2004.

Burkepile DE, Moore MT, Holland M M, Susceptibility of five non-target organisms to aqueous diazinon exposure. Bulletin of Environmental Contamination and Toxicology, 64 (1), 114-121, 2000.

Cooper GR, McDaniel V, The determination of glucose by the orthotoluidine method. *Standard Methods and. Clinical Chemistry*, 6, 159-170, 1970.

Dacie J, Lewis SM, *Practical hematology*. 6th ed. New York, London: Churchill Livingstone. pp. 148-149, 1984.

Department of Petroleum Resources (DPR) and Federal Ministry of Environment Nigeria (FMEnvN), *Maximum permissible limits for domestic water pollutants*. 1997.

Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML, Carvalho F, Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Critical Review in Toxicology, 38, 13-71, 2008.

Dogan D, Can C, Hematological, biochemical, and behavioral responses of *Oncorhynchus mykiss* to dimethoate. Fish Physiology and Biochemistry, 37, 951-958, 2011.

Elias NS, Abouelghar GE, Sobhy HM, El Miniawy, HM, Elsaiedy EG, Sublethal effects of the herbicide thiobencarb on fecundity, histopathological and biochemical changes in the African catfish (*C. gariepinus*). Iranian Journal of Fisheries Science, 19 (3), 1589-1614, 2018. https://dx.doi.org/10.22092/ijfs.2018.119669.

El-Sayed YS., Saad TT, El-Bahr SM, Acute intoxication of deltamethrin in monosex Nile tilapia, *Oreochromis niloticus* with special reference to the clinical, biochemical and hematological effects. Environmental Toxicology and Pharmacology, 24, 212-217, 2007.

Ensibi C, Pérez-López M, Soler Rodríguez F, Míguez-Santiyán MP, Yahya MN, Hernández-Moreno D, Effects of deltamethrin on biometric parameters and liver biomarkers in common carp (*Cyprinus carpio* L.). Environmental Toxicology and Pharmacology, 36, 384-391, 2013.

Finney DJ, *Probit Analysis*. 3rd edition Cambridge University Press, London, pp. 333, 1971.

Forget J, Pavillon JF, Menasria MR, Bocquene G, Mortality and LC50 values for several stages of the marine copepod, *Trigriopus brevicornis* (Muller) exposed to the metals arsenic and cadmium and the pesticides atrazine, carbofuran, dichlorvos, and malathion. Ecotoxicology and Environmental Safety, 40 (3), 239-244, 1998.

Gwathway CO, Craig Jr C, *Defoliants for cotton*. In: Pimentel D, editor. Encyclopedia of pest management. Boca Raton: CRC Press, pp. 135-137, 2007.

Haux C, Sjöbeck ML, Larsson Å, Physiological stress responses in a wild fish population of perch (*Perca fluviatilis*) after capture and during subsequent recovery. Marine Environmental Research, 15, 77-95, 1985.

Huang C, Zhang X, Jiang Y, Li G, Wang H, Tang X, Wang Q, Paraquat induced convulsion and death: a report of five cases. Toxicology and Industrial Health, 29, 722-727, 2013.

Ibor OR, Andem AB, Eni G, Arong GA, Adeogun AO, Arukwe A, Contaminant levels and endocrine disruptive effects in *C. gariepinus* exposed to simulated leachate from a solid waste dumpsite in Calabar, Nigeria. Aquatic Toxicology, 219, 105375, 2020.

Kaur, M., Jindal. R. (2017). Oxidative stress response in liver, kidney, and gills of *Ctenopharyngodon idellus* (cuvier and valenciennes) exposed to chlorpyrifos. *MOJ Biology and Medicine*. 1(4),103–112. DOI: 10.15406/mojbm.2017.01.00021.

Lengai GMW, Muthomi JW, Mbega ER, Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientia Africana, 7, e00239, 2020.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, Protein measurement with the Folin phenol reagent. Journal of Biology and Chemistry, 193, 265-275, 1951.

Lui RL, Blanco DR, Margarido VP, Filho OM, Chromosome characterization and biogeographic relations among three populations of the driftwood catfish *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae) in Brazil. Biological Journal of Linnae Society, 99 (3), 648–656, 2010. https://doi.org/10.1111/j.1095-8312.2009.01389x.

Marin-Morales MA, Ventura-Camargo BC, Hoshina MM, Toxicity of herbicides: impact on aquatic and soil biota and human health. In Tech, 2013. https://doi.org/10.5772/55851.

Mbuk RO, Ato RS, Nkpa NN, The role of paraquat (1,1, -dimethyl-4,4-bipyridinium chloride) and glyphosate (N-phosphonomethyl glycine) in translocation of metal ions to subsurface soils. Pakistan Journal of Analytical Environmental Chemistry, 10 (1and2),19-24, 2009.

Michael PO, Toxicity effect of atrazine on histology, hematology and biochemical indices of *C. gariepinus*. International Journal of Fisheries and Aquatic Studies, 6 (3), 87-92, 2018.

Ndimele PE, Jenyo-Oni A, Kumolu-Johnson CA, Chukwuka KS, Onuoha S, Effects of acute exposure to endosulfan (organochlorine pesticides) on hematology of African Mud Catfish, *C. gariepinus* (Burchell, 1822). Bulletin of Environmental Contamination and Toxicology, 95 (2),164-170, 2015.

Nelson DA, Morris MW, *Basic methodology. Chap. 27. Hematology and coagulation*, part IV. In: Nelson D.A. Henry, J.B. *Clinical diagnosis and management by laboratory methods*. 17th ed. Philadelphia. Editors: W.B. Saunder Company, pp. 578-625, 1989.

Nussey G, Van Vuren JHJ, du Preez H H, Effect of copper on the hematology and osmoregulation of the Mozambique tilapia *Oreochromis mossambicus* (Cichlidae). *Comput. Biochem., Physiology, Pharmacology, Toxicology, and Endocrinology,* 111, 369-380, 1995.

Nwani CD, Ama UI, Okoh F, Oji UO, Ogbonyealu RC, Agha AA, Udu-Ibiam O, Acute toxicity of the chloroacetanilide herbicide butachlor and its effects on the behavior of the freshwater fish *Tilapia zilli*. Africa Journal of Biotechnology, 12, 499-503, 2013.

Nwani CD, Ekwueme HI, Ejere VC, Onyeke CC, Chukwuka CO, Peace OS, Nwadinigwe AO, Physiological effects of paraquat in juvenile African catfish, *C. gariepinus* (Burchell 1822). Journal of Coastal Life Medicine, 3 (1), 35-43, 2014. https://doi.org/10.12980/JCLM.3.2015JCLM-2014-0113.

Ogamba EN, Inyang IR, Azuma IK, Effect of paraquat dichloride on some metabolic and enzyme parameters of *Clarias gariepinus*. Current Research Journal of Biological Science, 3, 186-190, 2011.

Okomoda VT, Ataguba GA Blood glucose response of *C. gariepinus* exposed to acute concentrations of glyphosate-isopropyl ammonium (Sunsate®). Journal of Agricultural and Veterinary Science, 3 (6), 69-75, 2011.

Oladokun EI, Sogbanmu TO, Anikwe JC, Sublethal concentrations of dichlorvos and paraquat induce genotoxic and histological effects in the *C. gariepinus*. Environmental Analysis and Health Toxicology, 35 (3), e2020013, 2020. https://doi.org/10.5620/eaht.2020013.

Olaniran EI, Sogbanmu TO, Saliu JK, Biomonitoring, Physico-Chemical and Biomarker Evaluations of Abattoir Effluent discharges into the Ogun River from Kara Market, Ogun State, Nigeria using *C. gariepinus*. Environmental Monitoring and Assessment, 191 (1), 44, 2019.

Olaniyi, WA, Omitogun OG, Embryonic and larval developmental stages of African giant catfish *Heterobranchus bidorsalis* (Geoffroy Saint

Hilaire, 1809) (Teleostei, Clariidae). *SpringerPlus*, 3 (1), 677, 2014.
Olorunfemi DI, Olomukoro JO, Anani OA, Evaluation of Toxicity Potential of Process water by using Fish Toxicity and Micronucleus Tests. *Studia Universitatis "VasileGoldiş"Seria Ştiinţele Vieţii*, 25, 1, 5-10, 2015.
Onusiriuka BC, Effect of sub-lethal concentration of formalin on weight gain in the African cat fish, *Clarias garipinus* (tougals). Journal of Aquatic Science, 17, 66 – 68, 2002.
Organization for Economic Cooperation and Development (OECD), *Test No. 203: fish, acute toxicity test. [OECD Guidelines for the testing of chemicals. Section 2: effects on biotic systems]*. Paris: Organization for Economic Cooperation and Development; French. 1992.
Pandey S, Kumar R, Sharma S, Nagpure NS, Srivastava SK, Verma MS, Acute toxicity bioassays of mercuric chloride and malathion on airbreathing fish *Channa punctatus* (Bloch). Ecotoxicology and Environmental Safety, 61, 114-120, 2005.
Reitman S, Frankel SA, Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. America Journal Clinical Pathology, 28, 56-63, 1957.
Russia U, Sood SK, *Routine Haematological tests*. In: Kanai, Mukherjee, L. (Ed) Medical Laboratory Technology, Tata McGrow Hill Publishing Company Limited, New Delhi, pp. 252-258, 1992.
Safahieh A, Jaddi Y, Yavari V, Zadeh RS, *Sub-lethal effects of herbicide paraquat on hematological parameters of benny fish Mesopotamichthys sharpeyi*. In: 2nd International Conference on Biotechnology and Environment Management. Singapore: IACSIT Press, pp. 141-145, 2012.
Sande D, Mullen J, Wetzstein M, Houston J, Environmental impacts from pesticide use: a case study of soil fumigation in Florida tomato production. International Journal of Environmental Research and Public Health, 8 (12), 4649-4661, 2011.
Sloman KA, Bouyoucos JA, Brooks EJ, Sneddon LU, Ethical considerations in fish research. Journal of Fish Biology, 94, 556–577, 2019. https://doi.org/10.1111/jfb.13946.
Suvetha L, Ramesh M, Saravanan M, Influence of cypermethrin toxicity on ionic regulation and gill Na+/K+-ATPase activity of a freshwater teleost fish *Cyprinus carpio*, Environmental Toxicology and Pharmacology, 29, 44-49, 2010.
Tortorelli MC, Hernández DA, Re Vázquez G, Salibián A, Effects of paraquat on mortality and cardiorespiratory function of catfish fry *Plecostomus commersoni*. Archives of Environmental Contamination and Toxicology, 19, 523-529, 1990.
Tsai WT, A review on environmental exposure and health risks of herbicide paraquat. Toxicological and Environmental Chemistry, 95, 197-206, 2013.
United States Environmental Protection Agency, *Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates*. 2nd ed. Washington, D.C.: United States Environmental Protection Agency. 2000.
Zaahkook SAM, Abd El-Rasheid HG, Ghanem MH, AL-Sharkawy SM, Physiological and oxidative stress biomarkers in the freshwater catfish (*C. gariepinus*) exposed to pendimethalin-based herbicide and recovery with EDTA. International Journal of Advance Research, 4 (10), 243-264, 2016.