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Yerima Rashida
Department of Biological Sciences, Gombe State University, Gombe, Nigeria

Auta Jehu
Department of Biology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Balarebe Sallau
Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

IMK Gadzama
Department of Biology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Corresponding Author:
Yerima Rashida
Department of Biological Sciences, Gombe State University, Gombe, Nigeria

Sublethal toxicity effects of glyphosate and propanil on some haematological parameters in juveniles of freshwater catfish, *Clarias gariepinus* (Teugels, 1986)

Yerima Rashida, Auta Jehu, Balarebe Sallau and IMK Gadzama

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Abstract
The use of agrochemicals in developing countries for the eradication of different pests/weeds has been on the increasing trend. This might be due to increasing human population, intensive agriculture, industrialization and urbanization. However, the objective of this work is to study the sub-lethal toxicity effects of glyphosate and propanil on some haematological changes in the freshwater fish *Clarias gariepinus* juveniles. The experiment was conducted in the laboratory for a period of 56 days. Juveniles fish of *Clarias gariepinus* of mixed sexs, males and females were randomly exposed to sub lethal concentrations of the two herbicides, 0.11, 0.14, 0.21 mg/L and control (0.00 mg/L) for glyphosate and 0.23, 0.31, 0.46 mg/L and control (0.00 mg/L) for propanil respectively. However, blood samples were collected for the determination of some haematological changes after 56 days. Assessment of haematological parameters revealed significant decreases in RBCC, HB and PCV. In contrast, a highly significant ($p \leq 0.05$) dose and duration dependent elevation was recorded in WBCC and MCH in all experimental groups compared with control exposures. There was also increased in lymphocytes and decreased in neutrophils counts in glyphosate and propanil exposure of *C. gariepinus* juveniles. The study has shown that the exposure of the fish *C. gariepinus* juveniles to the herbicides glyphosate and propanil can lead to alterations in the haematological parameters of both stages of the fish which could inflicts unfavourable physiological changes in the target organism, therefore leading to death of fish. Howerver, there is the need to protect fish with a view to sustaining our rich biodiversity in Nigerian. The study therefore, revealed that exposing *C. gariepinus* juveniles to sublethal concentrations of glyphosate and propanil causes an upset on the haematological parameters and implies poor environmental fitness for fish exposed to herbicides in natural environments.

Keywords: Haematological parameters, glyphosate, propanil, *Clarias gariepinus*, Sub lethal concentrations, herbicides

1. Introduction
Increased in concentration of different types of pollutants are connected with the industrial and communal waste waters and appearance of toxic materials in water ecosystem which enter water bodies. More than 60% of total pesticides used in agricultural sectors are herbicides, which are one of the main methods for controlling noxious weeds in agricultural and non-agricultural lands all over the world [1]. However, contamination of adjacent aquatic bodies as a result of wide- spread, continuous, and extensive use of pesticides particularly herbicides in agricultural systems has led to potential risk to a range of aquatic biota such as fish [2]. Sparling and Fellers [3], stated that more than 90% of pesticides sprayed to kill pests in agricultural lands never reach these target pests but it reached non-target organisms sharing these environments. Because of the agricultural drainage problems, herbicides and its degradations entered into water body resources include fish habitations [4, 5]. Pollution of the aquatic systems by these pesticide residues has become a major threat and a serious problem to living aquatic organisms mainly fish at all growth stages all over the world [6, 7]. Herbicides are chemicals are chemicals that are used for agricultural and industrial purposes for the eradication of different pests/weeds. They are detrimental against humans, plants and aquatic organisms such as fish because of their toxicity [8, 9]. Herbicides also accumulate largely in the soil, aquatic and biotic environments where they can easily exert their effect to the target organism such as fish. For example, several herbicides are recognized for causing renal and hepatic lesions in fish [10].

In Nigeria, glyphosate and propanil are among the common herbicides being continually applied on farmlands for high crop yield, the herbicide glyphosate (N-phosphonomethyl glycine), is a biocide with a broad-spectrum activity introduced for weed control in agricultural production fields in 1974 [11]. The most extensively used herbicide in Nigeria and worldwide is glyphosate because of its effectiveness in killing weeds without affecting the crop. The herbicide also influences plants, microorganisms, animals, and many components of the food chain [12]. In the formulation of glyphosate, some surfactants that are present are toxic to aquatic organisms and hence are not suitable for aquatic use [13]. Propanil (dichloropropionamide) is a known common herbicide, widely used in agriculture, agro-forestry and domestic homes to control unwanted plants [14]. It is mainly used in rice farms to control weeds. However, due to its accessibility in various forms, individuals including farmers indiscriminately apply this herbicide in farm lands and other facilities without recourse to its adverse impact on the immediate environment and aquatic health by extension. Presence of propanil in aquatic ecosystem and fish although in trace amount has been reported [15, 16]. According to Ensibi *et al*. [17], the presence of toxic substances in aquatic ecosystem can affect fish growth ultimately by reducing food accessibility, or by altering their metabolism. Toxic substances even at minute concentrations in aquatic environment can cause fish kill [18]. Close association between the circulatory system and external environment has made it easy for the determination of toxic effect of substances during clinical diagnosis of hematological parameter [19, 20, 21]. *Clarias gariapinus* has been the most popular choice as test organism because it is cheap and a rich source of animal protein, hardy, found in all fresh waters sources and widely cultured everywhere in Nigeria. Fish is considered as a model organism in conducting experimental studies such as toxicological and some pharmacological studies. Fish is a more attractive model organism in toxicology research because of it potentiality of application of the findings from these researches on humans and other environmental health issues [22]. However, based on the scientific based evaluation on animals, glyphosate and propanil are considered probably human carcinogen [23]. This prompts the need to investigate the toxicity of glyphosate and propanil herbicides on *C. gariepinus* juveniles through; the determination of haematological profile of glyphosate and propanil on *C. gariepinus* juveniles effect in this fish after its exposure for 56 days to different sub lethal doses of glyphosate and propanil herbicides respectively. Haematological indices have been recognized as valuable tools for evaluation of fish physiological status, the changes of which depend on fish species, age, cycle of sexual maturity and diseases [24, 25]. However, valuables data generated would give additional information to the potentials of glyphosate and propanil to cause adverse effects on vertebrates (humans inclusive). The present work is to study the haematological changes in the fresh waterfish *C. gariepinus* juveniles treated with sub-lethal doses of glyphosate and propanil.

2. Materials and methods

2.1 Experimental Fish

Juveniles of *C. gariepinus* of mixed sexes and fairly uniform sizes were obtained from a private fish farm in Zaria, Kaduna State, Nigeria. The Clarias species averaging 11.99±1.56 cm standard length and body weight of 4.25±1.17 g were used for the study. The fish were then transported in oxygenated polythene bags to the Fisheries Laboratory, Department of Biology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. They were fed on a commercial pellet diet (3% of body weight per day) and acclimatized for three weeks in 800 L rectangular tanks containing dechlorinated tap water (conductivity 2000 µs/cm; pH ≈ 7.5; Oxygen 90-95% saturation; temperature 25 °C; photoperiod 12:12 Light: Dark). The feeding stopped 24 hours prior to the commencement of the experiment.

2.2 Preparation of Herbicides Test Solutions

Two herbicides were used in the investigation. Both herbicides were purchased from a commercial outlet in Kaduna. Out of 360 g/L glyphosate, a stock solution (5 mg/L) of the toxicant was prepared by adding 1mL of the toxicant to 999 mls of water [26]. Similarly, out of 276 g/L propanil, stock solution of 1 mg/L was prepared in the same way as glyphosate. The stock solutions were used for preparing different concentrations of the test solutions by diluting measured volumes (i.e. 0.36 mL in 999.64 mL of dechlorinated tap water for 0.36 mg/L concentration). The dechlorinated tap water used had the same physical and chemical properties with the one used in acclimatizing the fish. The control solutions were made up of only dechlorinated tap water.

2.3 Experimental Design

From the result of the acute bioassay, fractions, 1/5, 1/10 and 1/20 of the 96h LC_{50} were used to determine sub-lethal concentrations for the herbicides as recommended by Oladimeji and Ologunmeta [27], using a static experiment where the water in the tanks were renewed every 24h. Appropriate volumes of stock solution were dispensed using syringe and measuring cylinder into a 25L tank containing 20L of dechlorinated tap water in each of the tanks except the control. The juveniles fish were exposed to nominal concentrations of toxicants for 56 days. The concentrations used for chronic study of the two toxicants were 0.11, 0.14, 21 mg/L and control (0.00 mg/L) for glyphosate and 0.23, 0.31, 0.46 mg/L and control (0.00 mg/L) for propanil. Each treatment was in triplicate. This means four treatments for each herbicide i.e. 12 experimental set-ups or tanks for each herbicide. The fishes were randomly assigned to give 10 fish per tank, given a total of 240 fish were used for both experiment with glyphosate and propanil. Fishes were fed 3% body weight with 35% crude protein level pelleted diet. The natural 12:12 day/night photoperiod was maintained. Test solution was renewed after every 24 hours.

2.4 Procedures for Haematological Studies of *Clarias gariepinus* Juveniles Exposed to Sub lethal Concentrations of Glyphosate and Propanil

2.4.1 Fish blood collection and analysis

For the sublethal study, five fish per replicate were sampled from the respective test mediums after 56 days for blood collection. Collection of blood from fish specimens was done following the procedure of Blaxhall and Daisely [28]. Fish specimens were anaesthetized using tricaine methanesulphonate (MS 222) to ensure easy collection of blood. Blood was collected by severance of caudal peduncle from the caudal artery. The caudal region was cut 2cm away and blood then collected with EDTA plastic tube [29]. Blood

specimens were transferred to the laboratory unit, Ahmadu Bello University (ABU) Teaching Hospital for heamatological analysis. Blood from the various treatments were analysed for the following haematological parameters: pack cell volume (PCV), haemoglobin (HB), red blood cell count (RBCC), White blood cell count (WBCC) and differentials using standard haematological procedures [28].

2.4.2 Total Erythrocyte Count (Red blood cell)
The total erythrocyte count was made with Neubaur's haemocytometer Hendricks solution was used for the red blood cell count. The blood was drawn up to 0.5mark in the RBC pipette and mixed the thoroughly taken in red pipette and diluted 1:200 with Hayem's fluid. The pipette was filled to 101 mark with the diluting fluid and shaken for 30 minutes to ensure mixing. The diluted suspension of cells then drawn into the chamber. The haemocyto meter was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer were counted with 4mm objectives and x 40 eyepiece microscope. The number of cells was multiplied by x 10 and this gave the total number of cells per cubic millimeter (mm^3) of blood [30].

2.4.3 Total Leucocytes Count (White blood cell)
Leucocytes were counted using Shaw's solutions A and B. The blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shaken the bulb of the pipette half way and then filled to 101
mark with solution B. A few drops were dispensed into the haemocytometer. The cells in the four large squares of the chamber were counted using a 4mm objective lens at 40 × magnification. The number of cells was multiplied 10 × to obtain the total number of leucocytes per cubic millimeter (mm^3) of blood [30].

2.4.4 Haematocrit (packed cells volume)
Packed cells volume was determination according to micro-westegren method as described by Blaxhall and Daisely [28]. The well mixed sampled blood from the severance of caudal peduncle was drawn into micro-haematocrit tube, 75 mm^3 long, and 1.1-1.2 mm^3 internal diameter. The tubes were then centrifuged for five minutes. The reading was taken with the aid of a micro- haematocrit reader and expressed as the volume of the erythrocytes per 100 cm^3.

2.4.5 Red Cells Indices
The absolute values made up of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the results of RBCC, haemoglobin and PCV/(Ht) [31].

$$\text{MCV } (\mu m^3) = \frac{\text{Ht\% } \times 10}{\text{RBCC (Cells mm}^3)}$$

$$\text{MCH (pg. Cell}^{-1}) = \frac{\text{Hb (g/100ml)} \times 10}{\text{RBCC (Cells mm}^3)}$$

$$\text{MCHC (g/100ml)} = \frac{\text{Hb (g/100ml)} \times 100}{\text{Ht\%}}$$

2.4.6 Leukocyte Differential Count
Two drops of blood were placed on a slide and made into a thin smear with another slide and left to dry. The smear was fixed with absolute methanol, then stained with giemsa's stain and rinsed in buffered distilled water. It was allowed to stand for 20-30 minutes after which the slide was washed again with buffered distilled water and allowed to air dried. Counting was made by the use of microscope.

2.5 Statistical Analysis
One-way Analysis of Variance (ANOVA) was used to test for significant difference between means using IBM Statics Version 20.0 for Windows 8, statistical analysis software and Duncan Multiple Range Test (DMRT) was used to test for significant difference between treatments when (p<0.05).

3. Results
The physicochemical parameters of the test water measured daily during sublethal toxicity bioassay with cadmium concentrations are presented in Table 1 and 2. The Temperature (T) (℃) range was between 20.10 to 26.70 ℃, Hydrogen ion concentration (pH) was between 7.70 to 8.20, Electrical Conductivity (EC) (µS/cm) was between 122 to 404 µS/cm, Total Dissolved Solids (TDS) (mg/L) was between 61 to 202 mg/L and Dissolved Oxygen (DO) (mg/L) was between 4.00 to 5.50 mg/L.

Table 1: Physico-chemical parameters of diluting water monitored during the sub lethal exposure of *Clarias gariepenus* juveniles to glyphosate for 56 days

Parameters	Range	Mean ± S.E
Temperature (T) (°C)	25.80– 26.70	26.23±0.08
Hydrogen ion Concentration (pH)	7.52–7.90	7.72±0.05
Electrical Conductivity (EC) (µS/cm)	142–236	188.42±10.41
Total Dissolved Solids (TDS) (mg/L)	71–118	93.33±5.22
Dissolved Oxygen (DO) (mg/L)	4.50–5.50	4.98±0.09

Table 2: Physico-chemical parameters of diluting water monitored during the sub lethal exposure of juveniles of *Clarias gariepenus* to propanil for 56 days

Parameters	Range	Mean ± S.E
Temperature (T) (°C)	20.10 – 25.70	22.78±0.58
Hydrogen ion Concentration (pH)	7.70 – 8.20	7.38±0.16
Electrical Conductivity (EC) (µS/cm)	122– 404	211.00±32.19
Total Dissolved Solids (TDS) (mg/L)	61 – 202	106.17±15.94
Dissolved Oxygen (DO) (mg/L)	4.00 – 5.20	4.46±0.12

Results of biochemical parameters of *C. gariepinus* juveniles exposed to sub-lethal nominal concentrations of glyphosate after 56 days are presented in Table 3. When the effects of sub-lethal concentrations of Glyphosate were considered, the toxicant elicited highly significant ($p \leq 0.05$) dose and duration dependent decreases in RBCC, HB and PCV. In contrast, highly significant ($p \leq 0.05$) dose and duration dependent elevations were recorded in WBCC and MCH. Control RBCC, HB and PCV were significantly ($p \leq 0.05$) higher than the exposed group. MCV and MCHC did not vary significantly between the control and the exposed fish. Fish exposed to the toxicant had significantly ($p \leq 0.05$) lower percentage neutrophils compared to the control group. For the exposed fish, neutrophil percentage was observed to decrease with increasing sub lethal concentrations of the toxicant. The percentage of lymphocytes in the exposed fish was observed to decrease with increasing concentrations of glyphosate. Lymphocyte level of the control group was significantly ($p \leq 0.05$) lower than those of the exposed fish.

Table 3: The effect of sub-lethal doses of glyphosate on some biochemical parameters of *C. gariepinus* juveniles after 56 days of exposure

Treatment (mg/L)	0.00	0.11	0.14	0.21
Parameters				
RBC ($x10^{12}$L	6.77 ± 022^{a}	4.12 ± 0.27^{b}	3.12 ± 027^{c}	2.55 ± 0.16^{c}
WBCC ($x10^{9}$L)	7.20 ± 0.41^{c}	9.90 ± 0.61^{b}	12.06 ± 0.66^{a}	13.34 ± 0.84^{a}
HB (g/100ml)	12.17 ± 0.30^{a}	7.44 ± 0.48^{b}	6.16 ± 0.39^{c}	5.73 ± 0.40^{c}
PVC (%)	34.29 ± 0.81^{a}	19.29 ± 1.49^{b}	15.83 ± 1.74^{b}	15.04 ± 1.72^{b}
MCV ($x10^{6}$Pgcell)	51.33 ± 2.09^{ab}	46.70 ± 1.58^{b}	50.18 ± 3.29^{ab}	57.83 ± 4.23^{a}
MCH ($x10^{6}$Pgcell)	18.13 ± 0.58^{c}	18.13 ± 0.30^{c}	20.18 ± 0.62^{b}	22.40 ± 0.73^{a}
MCHC (g/100ml)	35.58 ± 0.86^{a}	39.23 ± 1.50^{a}	42.36 ± 3.50^{a}	40.93 ± 3.01^{a}
Neutrophils (%)	17.75 ± 1.03^{a}	8.00 ± 2.92^{d}	11.13 ± 4.64^{c}	13.25 ± 5.49^{b}
Lymphocytes (%)	85.25 ± 1.03^{d}	95.00 ± 2.92^{a}	87.75 ± 4.77^{b}	86.50 ± 5.78^{c}

Means with the same superscript along rows are not significantly different ($p \leq 0.05$) (Mean values ± SE) n=3 Red blood cell = RBC, White blood cell = WBC, Haemoglobin = Hb, Pack cell volume = PCV, Mean cell volume = MCV, Mean cell hemoglobin = MCH, Mean cell haemoglobin concentration = MCHC.

Results of biochemical parameters of *C. gariepinus* juveniles exposed to sub-lethal nominal concentrations of propanil after 56 days are presented in Table 4. The results clearly showed highly significant ($p \leq 0.05$) dose dependent decreased in RBCC, HB and PCV. In addition, the control values for RBC, HB, PCV, MCH and MCHC were significantly ($p \leq 0.05$) higher than the exposed group. However, the control value for WBCC was significantly lower compared to the exposed groups at the higher propanil concentrations. It was significantly ($p \leq 0.05$) elevated in 0.31 and 0.46 mg/L respectively, but the elevation recorded no significant ($p \leq 0.05$) difference at the lowest concentration 0.23 mg/L. MCV did not vary significant ($p \leq 0.05$) between the control and the exposed fish as well as between concentrations. Fish exposed to the toxicant had significantly ($p \leq 0.05$) lower. The percentage neutrophils of the control fish were higher than the exposed group, but only significantly ($p \leq 0.05$) at the highest concentration of propanil. For the exposed fish, neutrophil percentage was observed to decrease with increasing sub lethal concentrations of the toxicant. The percentage of lymphocytes (87%) in the exposed fish to the lowest propanil concentration (0.23 mg/L) was observed to decreased significantly ($p \leq 0.05$) to 81% and 82% with increasing concentrations 0.31 and 0.46 mg/L respectively.

Table 4: The effect of sub-lethal doses of propanol on some biochemical parameters of juveniles in *C. gariepinus* after 56 days of exposure

Treatment (mg/L)	0.00	0.23	0.31	0.46
Parameters				
RBC ($x10^{12}$L	183.58 ± 9.33^{a}	147.17 ± 10.29^{b}	122.67 ± 3.78^{c}	130.33 ± 3.62^{bc}
WBCC ($x10^{9}$L)	1825.17 ± 131.42^{c}	2057.83 ± 171.95^{bc}	2331.17 ± 150.52^{ab}	2611.42 ± 95.03^{a}
HB (g/100ml)	10.167 ± 0.30^{a}	5.44 ± 0.48^{b}	4.16 ± 0.39^{c}	3.73 ± 0.41^{c}
PVC (%)	37.08 ± 1.14^{a}	27.25 ± 1.16^{b}	24.00 ± 1.47^{bc}	21.50 ± 1.20^{c}
MCV ($x10^{6}$Pgcell)	2.06 ± 0.08^{a}	1.97 ± 0.20^{a}	1.98 ± 0.14^{a}	1.66 ± 0.10^{a}
MCH ($x10^{6}$Pgcell)	0.57 ± 0.03^{a}	0.40 ± 0.05^{b}	0.34 ± 0.03^{b}	0.29 ± 0.03^{b}
MCHC (g/100ml)	27.46 ± 0.41^{a}	19.44 ± 0.71^{b}	16.95 ± 0.69^{c}	16.75 ± 1.01^{c}
Neutrophils (%)	22.75 ± 1.35^{a}	21.45 ± 2.94^{ab}	18.74 ± 0.66^{ab}	16.20±1.54b
Lymphocytes (%)	84.67 ± 0.56^{ab}	87.50 ± 1.13^{a}	81.58 ± 0.70^{b}	82.92 ± 2.36^{b}

Means with the same superscript along rows are not significantly different ($P \leq 0.05$) (Mean values±SE) n=3 Red blood cell = RBC, White blood cell = WBC, Haemoglobin = Hb, Pack cell volume = PCV, Mean cell volume = MCV, Mean cell haemoglobin = MCH, Mean cell haemoglobin concentration = MCHC.

4. Discussion

After exposure to sublethal concentrations of Glyphosate and Propanil, the result for haematological indices showed significant differences ($p<0.05$) in Hb, RBC, WBC, PCV, MCV, MCH and MCHC between the control and the various treatments. Blood is considered to be the only liquid tissue thus; contained different types of cells which help in homeostasis [32]. However, haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different pollutants, such as herbicides [32]. These often leads death of an organism including fish. Reduction in Hb concentration with increased concentration of Glyphosate and Propanil observed conforms with report of Yaji *et al.* [16], in which *O. niloticus* was exposed to sublethal concentrations of propanil. Reduction in Hb in the juveniles

of *C. gariepinus* could be due to an increase in the rate of erythrocyte destruction or decrease in the release of erythorocytes in to circulation. Ada *et al.* [32], states that Hb reduction contributes to the anaemic state of organisms which leads to alteration in respiration, metabolism and triggers morbidity and death of an organism. Hence, Hb decrease in exposed fish might have altered the available of oxygen in the body tissues, resulting to the production of low energy and reduced metabolic rate, these explains the changes in their biochemical, histological and low food consumption and further leads to loss of body weight. The decreased in RBC with concentrations increased of Glyphosate and Propanol observed implies impairment in erythrocyte production caused by the toxicant. The result agrees with the report of George *et al.* [48], that the alterations were attributed to direct or feedback response of structural impairment to RBC membranes resulting in haemolysis and impairment in haemoglobin producyion stimulated by exposure to atrazine and metolachlor. showed significant difference (p<0.05), it increased with increase in concentration of both glyphosate and propanil. The increase in WBC counts suggests the incidence of leucocytosis, a condition where more WBC is released in the blood system for adaptive immune response to glyphosate and Propanil. Leucocytosis in *O. niloticus* was reported after 96 h propanil exposure [35]. Nwani *et al.* [36] reported significant increase in WBC count in *C. gariepinus* exposed to sub lethal concentrations of pesticide Fenthion and attributed it to adaptive immune response to Fenthion toxicity. Similarly, the findings of Mukherjee *et al.* [37], suggested that when the pollutant entered the animal tissues, it could have combined with biochemical constituents of the cells and formed xenobiotics. Due to these reactions, the production of WBCs might be increased thereby increasing the process of elimination of toxic substances from the tissues. The trend in WBC with increased concentration of glyphosate and propanil is also similar to that observed in *C. gariepinus* exposed to paraquat dichloride by Seiyaboh *et al.* [38]. The WBC showed a marked increase in the least treated concentration (0.11 mg/L glyphosate, 0.23 mg/L propanil) followed by the highest concentration 0.21 mg/L glyphosate, 0.46 mg/L propanil; the high elevation of WBC count might be as a result of an attempts made by the fish to fight against stress and trauma cause by herbicides. Ada *et al.* [32], reported that increase in WBC count is regarded as an adaptation of organisms to fight foreign bodies from cells. Baker *et al.* [39], also opined that sharp increase in exposed blood cells above that of the control group could be resistance to prevalent adaptability and unwanted change to the environment. The study. The low values of PCV in fish exposed to the herbicides glyphosate and propanil could have arisen from the reduction in RBC volume caused ionic exchange as a result of hypoxic conditions, osmotic concentration and changes that occurs due to ion losses from the blood plasma [33]. Red cell parameters MCV, MCH and MCHC are important indicators of anaemic conditions [29]. The analysis of red cell indices (the values of MCH, MCHC and MCV are the lowest statistically significant) suggests that these specimens exhibit anemic changes of a microcytic and hypochromic type. This is further authenticated by the fact that Hb and RBC were observed to be statistically lower in the exposed fish than in the control over the exposure period, however, this is in tandem with the report of Ada *et al.* [32], that herbicides may trigger the multiplication of the blood cells to make up for the low load of haemoglobin per cell, Zubair [40] and Edori *et al.* [41], reported similar trends in fish exposed to different toxicants. Insignificant changes observed in MCV values exposed to sublethal concentrations of propanil and MCHC values exposed to sublethal concentrations of glyphosate, when compared with the control, suggested normochromic anaemia, while the significant elevation in MCV, MCH and MCHC values of fish exposed the herbicides in sublethal concentrations, might be due to the swelling of RBC as a result of hypoxia or Macrocytic anaemia in the fishes exposed to glyphosate and propanil as also suggested by Jastrzebska and Protasowickiz [42]. The increased in MCV, MCH and MCHC in the present study might also be due to defensive mechanism against the herbicides toxicity. Leucocytes are important biomarkers of stress thus give information on health condition of animals with regards to their immune status [43]. Leukocyte differentials have been reported to be sensitive markers of stress and provide information on the immune status of organisms [42]. Leukocyte differentials are also known as cells of innate immune responses which are myeloid in nature and comprised of mononuclear (monocytes) and polymorpho- nuclear (neutrophils, Eosinophils, Basophils and lymphocytes) phagocytes [43]. Increase in lymphocyte and decreased in neutrophils counts in *C. gariepinus* juveniles exposed to sublethal concentrations of Glyphosate and Propanil compare with the control suggested possible immune defence against the stress induced by the herbicides Glyphosate and Propanil. Micah *et al.*[44], reported similar observations in neutrophils and lymphocytes of fingerlings of heteroclarias exposed to acute concentrations of glyphosate. Yaji *et al.* [11], revealed leukocytosis, neutrophilia and lymphopenia in *Oreochromis niloticus* in a Static Bioassay exposed to sublethal concentrations of propanil for 56 days when compared with the control group and they attributed the differences to stress induced by propanil. Basophils, monocytes and eosinophils were not observed in fish expose to Glyphosate and Propanil. This suggested reduction in concentration of cell types. Difficulty in the preservation of basophils might be the reason why basophils are difficult to identify in fish blood [45, 46]. Absence of basophils, Eosinophils and granulocytes were reported for *Hopliias malabarcus* captured from the wild [47].

5. Conclusion

The haematological parameters are considered as most sensitive in monitoring toxicity of glyphosate and propanil with other herbicides especially at sublethal doses. Further, the fish *C. gariepinus* may be considered as a suitable model to study the toxicity of herbicides in the aquatic ecosystems. The present study records that glyphosate and propanil severely impairs various haematological parameters, hence, would serve as a useful tool for further monitoring and ecological assessment of these aquatic organisms, which is considered to be a rich source of animal protein including and also important food source for human beings.

6. Recommendation

Therefore, the effects of glyphosate, propanil and other herbicides should be investigated in the field hence, the use of herbicides should also be restricted or at least be minimized for the betterment of the ecosystem and conservation of aquatic habitat.

7. References

1. Elalfy MM, Aboumosalam MS, Ali FR. Biochemical, hematological and pathological effects of bispyribac sodium in female albino rats. Journal of Veterinary Science Technology. 2017;8:467.
2. Guo Y, Meng XZ, Tang HL, Zeng EY. Tissue distribution of organochlorine pesticides in fish collected from the Pearl River Delta, China: Implications for fishery input source and bioaccumulation. Environmental Pollution. 2008;155:150-156.
3. Sparling D, Fellers G, McConnell LL. Pesticides and amphibian population declines in California. USA. 2001;20:1591-1595.
4. Rao KRS. Pesticide Impact on Fish Metabolism (Discovery Publishing House, Delhi); c1999.
5. Sana U, Zorriehzahra MJ. Ecotoxicology: a review of pesticides induced toxicity in fish. Advance Animal Veterinary Science. 2015;3:40-57.
6. Firat O, Cogun HY, Yuzereroglu TA, Gok G, Kargin F. Kotemen Y. A comparative study on the effects of a pesticide (Cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. Fish Physiology Biochemistry. 2011;37:657-666.
7. Iwama GK, Vijayan MM, Morgan JD. The stress response in fish. Icthyology, Recent research advances Oxford and IBH publishing Co, Pvt. Ltd, Delhi; c2000.
8. Adedeji OB, Okocha RO. Overview of pesticide toxicity in fish. Advances in Environmental Biology; c2012. p. 2344-2352.
9. Fishel F, Ferrell J, MacDonald G, Sellers B. Herbicides: How toxic are they. Pesticide Information Office, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Disponible; c2013.
10. Hogan CMS. Draggan (Ed.), Herbicide, The Encyclopedia of Earth; c2014.
11. Benbrook CM. Trends in glyphosate herbicide use in the United States and globally. Environmental Sciences Europe. 2016;28(1):1-15.
12. Krüger M, Schrödl W, Neuhaus J, Shehata AA. Field investigations of glyphosate in urine of Danish dairy cows. Journal of Environment and Toxicology. 2013;3(5):100186.
13. Sani A, Idris MK. Acute toxicity of herbicide (glyphosate) in *Clarias gariepinus* juveniles. Toxicology Reports. 2016;3:513-515.
14. Nwani CD, Ekwueme HI, Ejere VC, Onyeke CC, Chukwuka CO, Peace OS, *et al*. Physiological effects of paraquat in juvenile African catfish *Clarias gariepinus* (Burchfiel 1822). Journal of Coastal Life Medicine. 2015;3(1):35-43.
15. Call DJ, Brooke LT, Kent RJ, Knuth ML, Anderson C, Moriarity C. Toxicity, bioconcentration, and metabolism of the herbicide propanil (3′, 4′-dichloropropionanilide) in freshwater fish. Archives of Environmental Contamination and Toxicology. 1983;12(2):175-182.
16. Yaji AJ, Iheanacho SC, Ogueji EO. Haematology and biochemical responses in *Oreochromis niloticus* exposed to subacute doses of Aronil in a flow through bioassay. Egyptian Journal of Aquatic Biology and Fisheries. 2018;22(3):89-98.
17. Ensibi C, Pérez-López M, Rodríguez FS, Míguez-Santiyán MP, Yahya MD, Hernández-Moreno D. Effects of deltamethrin on biometric parameters and liver biomarkers in common carp (*Cyprinus carpio* L.). Environmental toxicology and pharmacology. 2013;36(2):384-391.
18. Dinis-Oliveira R, Duarte JA, Sánchez-Navarro A, Remiao F, Bastos ML, Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Critical reviews in toxicology. 2008;38(1):13-71.
19. Witeska M, Kondera E, Szymanska M, Ostrysz M. Hematological changes in common carp (*Cyprinus carpio* L.) after short-term lead (Pb) exposure. Polish Journal of Environmental Studies. 2010;19(4):825-831.
20. Raina S, Sachar A. Effect of heavy metal, Zinc and Carbamate pesticide, Sevin on haematological parameters of fish, *Labeo boga*. The International Journal of Innovative Research in Science, Engineering and Technology. 2014;3(5):12636-12644.
21. Sharma J, Langer S. Effect of manganese on haematological parameters of fish, *Garra gotyla gotyla*. Journal of Entomology and Zoologica Studies. 2014;2(3):77-81.
22. Govind P. A review of fish model in experimental pharmacology. International Reserch Journal of Pharmacology. 2011;2(9):33-36.
23. Portier CJ, Armstrong BK, Baguley BC, Baur X, Belyaev I, *et al*. Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA). Journal of Epidemiology Community Health. 2016;70(8):741-745.
24. Soundararajan M, Veeraiyan G, Samipillai SS. Effect of heavy metal arsenic on haematological parameters of fresh water fish, *Tilapia mossambica*. International Journal of Modern Research and Reviews 2014;2(3):132-135.
25. Mallesh B, Pandey PK, Kumar K, Vennila A, Kumar S. Bioconcentration of hexavalent chromium in *Cirrhinus mrigala* (Ham 1822): effect on haematological parameters. Journal of Biology Earth Science. 2015;5(1):59-67.
26. Reish DL, Oshida OS. Manual of Methods in aquatic environment research. Part 10, Short-term Static bioassays. FAO Fisheries Technical Paper Rome. 1987;247:62.
27. Oladimeji AA, Ologunmeta RT. Toxicity of water borne lead to *Tilapia niloticus*. Nigerian Journal of Applied Fisheries and Hydrobiology. 1987;2:19-24.
28. Blaxhall P, Daisley K. Some blood parameters of the *Rainbow trout* I. The Kamloops variety. Journal of Fish Biology 1973;5:1-8.
29. Iheanacho SC, Ikwo TN, Igweze N, Chukwuidha C, Ogueji EO, Onyeneke R. Effect of different dietary inclusion levels of melon seed (*Citrullus lanatus*) peel on growth, haematology and histology of *Oroechromis niloticus* juvenile. Turkish Journal of Fisheries and Aquatic Sciences. 2018;18(3):377-384.
30. Hesser EF. Methods for routine fish hematology. The progressive fish-culturist. 1960;22(4):164-171.
31. Dacie JV, Lewis SM. Practical hematology. 11th edition, New York: Churchill Livingstone; c2011. p. 41.

32. Ada FB, Ekpenyong E, Ayotunde EO. Haematological, biological and behavioural changes in *Oreochromis niloticus* (Linne 1757) juveniles exposed to Paraquat herbicide. Journal of Environmental Chemistry and Ecotoxicology. 2012;4(3):64-74.
33. Alwan SF, Hadi AA, Shokr AE. Alterations in haematological parameter of fresh water fish. *Tilapia Zilli* exposed to Aluminium. Journal of Science and Its Applications. 2009;3(1):12-19. 32
34. Babatunde RO, Kareem AT, Olayemi OO, Adelusi FT, Ugege BH, Elesho RO, *et al*. Effect of use of agrochemical among arable crop farmers in Osun state. Int. J Agric. Food Sci. 2020;2(1):01-04. DOI: 10.33545/2664844X.2020.v2.i1a.27
35. Mallum SS, Sogbesan OA, Haruna AB. Haematological assessment of *Oreochromis niloticus* (Linn, 1787) exposed to acute toxicity of the herbicide Propanil. International Journal of Research and Life Science. 2015;2(10):49-56.
36. Nwani CD, Somdare PO, Ukonze JA, Ejere VC, Nwadinigwe AO, Nwani JC, *et al*. Subchronic exposure to fenthion induces hematological changes in liver tissue of African catfish *Clarias gariepinus*. Journal of Aquatic Animal Health. 2016;28(4):229-234. 35
37. Mukherjee S, Ray D, Adhikari D, Ghosh T. Impairment of haematological profile of *Channa punctatus* exposed to Sodium arsenite. International Journal of Applied Biology and Pharmaceutical Technology. 2015;6:223-229.
38. Seiyaboh EI, Inyang IR, Gijo AH, Adobeni GD. Acute toxicity of paraquat dichloride on blood plasma indices of *Clarias gariepinus*. Journal of Environmental Science, Toxicology and Food Technology. 2013;**7**(6):15-17.
39. Baker FJ, Silverton RE. Introduction to medical laboratory technology. Butterworth-Heinemann. 7th edn. Bounty Press Limited, Ibadan, Nigeria; c2001. p. 448.
40. Zubair A. Toxicity bioassay and effects of sublethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. African Journal of Biotechnology. 2012;11(34):8578-8585.
41. Edori OS, Ekpete OA, Edori ES. Effect of Paraquat on organ indices and haematology in *Clarias gariepinus* after Chronic exposure. British Journal of Pharmaceutical Research. 2013;3(4):1106-1114.
42. Jastrzebska E, Protasowicki M. Effects of cadmiun and nickel exposure on hematological parameters of common carp, *Cyprinus carpio* L. Acta Ichthyologica et Piscatoria, 2005, 1(35).
43. Jeney G. (Ed.). Fish diseases; Prevention and Control Strategie's. Academic press, London wall; c2017. p. 8.
44. Micah AD, Adakole JA, Yusuf A, Mohammed NA. Acute effects of glyphosate on the behavioural and hematologal characteristics of Heteroclarias (hybrid) fingerlings. Journal of Aquaculture Engineering and Fisheries Research. 2017;3(1):13-18.
45. Romão S, Donatti L, Freitas MO, Teixeira J, Kusma J. Blood parameter analysis and morphological alterations as biomarkers on the health of *Hoplias malabaricus* and *Geophagus brasiliensis*. Brazilian Archives of Biology and Technology. 2006;49(3):441-448.
46. Tabrez S, Zughaibi TA, Javed M. Bioaccumulation of heavy metals and their toxicity assessment in Mystus species. Saudi Journal of Biological Sciences. 2021;28(2):1459-1464.
47. Ranzani-Paiva MJT. Celulas do sangue periferico e contagem diferencial de leucocitos de tainha *Mugil platanus*, Gunther, 1880 (*Osteichtyes, Mugilidae*) da regiao estuarinalagunar de Cananeia SP (Lat. 25 00'S-Long 47 55W), Boletin Instituto de Pesca. 2018;22(1):A 23-40.
48. George ADI, Akinrotimi OA, Nwokoma UK. Haematological changes in African catfish (*Clarias gariepinus*) exposed to mixture of atrazine and metolachlor in the laboratory. Journal of Fisheries Science and communication. 2017;11:048-054.