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Acute Toxicity of Primextra (Atrazine) on a Freshwater Fish *Heterobranchus bidorsalis* (Geoffrey Saint-Hilaire)

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Abstract: The present study was undertaken to evaluate the acute toxicity of the herbicide primextra gold (atrazine) with emphasis on its effects on the behavioural responses, haematological, biochemical and histopathological alterations in the liver and kidney of a fresh water fish, *Heterobranchus bidorsalis* . Atotal of 300 juveniles (28.6g ± 30.4g) of the fish, were acclimatized for two weeks. After a range finding testthe acclimated fish were exposed in sets of ten (10) in triplicates to 0.00 mg/L (control), 5.28, 5.94, 6.60, 7.26 and 7.92 mg/L (labeled, A, B C, D and E) of the herbicide for 96 hours in semi-Static tanks. No death was recorded in the control but mortality was directly proportional to the concentrations of the herbicide in the lethal groups.The median lethal concentration (LC_{50}) of the herbicide was determined to be 12.59µg/L. Thereafter, the fish were exposed in sets of ten to three concentrations, *viz* 1(1/10th Lc_{50}), II(1/9th of LC_{50}) and III(1/8th of LC_{50}) in triplicates. The three concentrations, 1.26 µg/L, 1.40 µg/L, 1.57 µg/Lwere labeled, F, G and H respectively. A control group was set upsimultaneously. The fish in the control maintained normal behaviour while those exposed to the herbicide showed signs of stress. No mortality was recorded in the three concentrations (F, G and H).The values of RBC, PCV, Hb were reduced while significant increase ($p< 0.05$) in WBC was recordedin the treated groupscompared to the control. ALT, AST, ALP and glucosevalues were significantly increased in the treated groups while the serum protein was significantly reduced. Histopathological examination of the liver and kidney of the fish showed normal architecture of the organs in the control groups while the organs in the exposed groups exhibited different degrees of damage in correlation with the concentration of the herbicide. Results from this study showed that primextrais toxic to juveniles of *H. bidorsalis*, therefore, application of this herbicide close to riverine areas and fish farms should be avoided.

Key words: *Heterobranchus bidorsalis* · Primextra (Atrazine) · Mortality · Median Lethal Concentration · Behavioural · Haematological · Biochemical and Histopathological Alterations

INTRODUCTION

The best option to meeting the higher food demand on the African continent is not to raise overseas food imports but to increase domestic food production sufficiently through agriculture. Weeds have been one of the setbacks in increasing agricultural production. Herbicides have therefore been employed for the control of weeds in place of the traditional hoe-weeding culture which is both time and labour consuming.In the year, 2007, the estimated worldwide use of pesticides was at 2.4 billion kg and herbicides represent the largest proportion (40%) of pesticides use in agriculture [1]. Unfortunately, the disadvantages that arise from the use of pesticides are enormous as research has shown that herbicides have constituted serious pollutants in aquatic habitats and non-target organisms such as fish. Atrazine is one of the most effective and inexpensive herbicides in the world and is consequently used more frequently than any other herbicide [2]. When herbicides are applied in restricted areas, it is washed and carried away by rains and floods to nearby aquatic systems, thereby affecting aquatic biota, especially fish, which serves as a rich source of protein supplement for the teaming population.Primextra (Atrazine) is a common herbicide widely applied in farmlands in Nigeria [3]. It is readily available in the local market and used by both literate and illiterate farmers. Careless handling, wrong applications and run-offs from rains can cause this herbicide to be washed into water bodies where non- target organisms such as fish are adversely affected. Atrazine is mobile in the environment and is among the most detected pesticides in streams,

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rivers, ponds [4]. It has been reported that even the used container retains the product residue for a long time after application [5]. Chemicals and toxins affect humans directly or bio-accumulate in fish and other organisms consumed by humans causing developmental and neurological damages [6]. Many researchers have reported toxic effects of atrazine exposed to aquatic organism [1, 7-10].

The present study was undertaken to evaluate the acute toxicity of the herbicide primextra gold (Atrazine) with emphasis on behavioural responses, haematological, biochaemicaland the histopathological alterations in the liver and kidney of a fresh water fish, *Heterobranchus bidorsalis*.

MATERIALS AND METHODS

A total of 300 juveniles (28.6g ± 26.4g) of fresh water fishes *H.bidorsalis* were used in the assay. The specimen were weighed in grams (g) using electronic weighing balance (Model: Scout Pro SPU401) and the lengthin centimeter(cm) determined using meter rule.

Acclimatization of Fish Samples: The fishes were acclimatized for two weeks under laboratory conditions. The fish samples were fed on a commercial floating pellets diet (3% of the body weight per day). The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Some of the fish (≤ 3%) of the specimen died during the acclimation period while the rest survived and were used in the range finding test and the definitive test.

Source and Collection of Herbicide: Theherbicide, Primextra gold, a liquid herbicide (containing 360g of Propanil and 200g 2, 4. Dichlorophynoxyacetic acid 2, 4-D) for selective weed control were procured from the local market and used in the research.

Range Finding Tests: The range finding tests were carried out prior to the definitive test to determine the concentration of the test solutions to be used in the assay. The test water with the herbicide was changed after every 48 hours by replacing with fresh Primextra solutions in order to counterbalance decreasing herbicide concentrations.

Acute Toxicity Test: Based on the range finding test, the acclimated fish were exposed to five concentrations 5.28, 5.94, 6.60, 7.26 and 7.92 mg/l of the herbicide (Labeled, A, B, C, D and E) in sets of ten per tank in triplicates. A control experiment was set up simultaneously. The test media were changed every 48hours in an attempt to maintain a more constant concentration of the test media to prevent excessive accumulation of toxic metabolites. The exposure was continued up to 96 hours.

Determining the LC_{50} Values: The LC_{50} value of Primextra for *H. bidorsalis* was determined following the probit analysis method as described by Finney [9], Finney [10], OECD [11] and Okogwu *et al*. [12] for estimating Median Lethal Concentrations in toxicity Bioassays. The median lethal concentration was determined to be 6.34 mg/L. Thereafter, the fish were exposed in sets of ten to three sub lethal test concentrations, *viz* sublethal-1($1/10^{th}$ Lc_{50}), sublethal-II($1/9^{th}$ of LC_{50}) and sub lethal-III($1/8^{th}$of Lc_{50}) (0.63µg/L, 0.70µg/L, 0.79µg/L), labeled F, G and H. A control group was set up simultaneously and all in triplicates for 96 hours.

Behavioral Response: Some behavioral responses of the fish which include hyperactivity, equilibrium status, swimming rate, convulsions, somersaulting activity, fin movement and operculum movement were observed in exposed as well as the control group at 24, 48, 76 and 96 hours of exposure and recorded as suggested by OECD [11].

Mortality Rate: Variation in the mortality rate of the fish population based on lethal concentrations and duration of exposure were recorded.Dead fish were promptly removed to reduce further fowling of the water. A fish was considered dead if it makes no movement when prodded with a glass rod.

Analysis of the Physicochemical Properties of the Test Water: The physicochemical properties of the water namely, temperature, pH, conductivity, dissolved oxygen and total dissolved solutes were analyzed using the standard methods (APHA, AWWA, WPCE, 2005 [13]. Temperature reading was taken using a thermometer and the pH by the pH meter. The total Dissolved oxygen level of the test water, conductivity and total dissolved solutes of the water were also measured by the use of Hanna digital instruments.

Blood Collection and Excision of the Kidney: At 96 hour of the exposure, two fish from each tank (Six fish per group) were removed; the blood was collected by puncture of the caudal vein into tubes containing

anticoagulant potassium salt of ethylene diamine tetra-acetic acid (EDTA), Sodium fluoride tubes and plain tubes. Each chamber depth tube was labeled accordingly. The blood in the EDTA tubes was used for haematological analysis, while the blood samples in the plain tubes were used for biochamical analysis of the herbicides, the blood in the fluoride tubes was used for glucose analysis.

Assessing the Impact of the Herbicides on Some Haematological Parameters of the Fish Species: The blood parameters assessed in this study were determined using the method of Van Der Oost *et al*. [14].

Determination of the Red Blood Cells (RBC) Count: Whole blood (20 µL) was added to 3.98 mL of diluting fluid (10 % sodium citrate) and mixed thoroughly. After 5 minutes, the first few drops were discarded by holding the pipette vertically. The diluted blood was then introduced into the counting chamber and counted after three minutes with the aid of a compound microscope. The red blood cells in the four corner squares and one central square were counted. Therefore;

$$\text{Total RBC (mm/l)} = \frac{\text{Counted erythrocytes.}}{\text{Counted surface (mm2) x chamber depth x dilution}}$$

Determination of White Blood Cells (WBC) Count: Whole blood (20 µL) was added to 380 µL of diluting fluid (acetic acid, with gentian violet) and mixed. The glacial acetic acid lyses the red cells while the gentian violet slightly stains the nuclei of the leucocyte. The counting chamber was charged with the well mixed diluted blood (After discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 minutes. The four corners of the chamber were visualized under a low power (10X) objective microscope and the cells were counted in all the four marked corner squares.

$$\text{Total WBC (mm3)} = \frac{\text{Counted white blood cells}}{\text{Counted surface (mm2) x chamber depth x dilution}}$$

Determination of the Packed Cell Volume (PCV): Packed cell volume (PCV) was estimated as described by Van Der Oost *et al*. [14]. 1 mL blood sample was taken with EDTA. The filled tubes were placed in the microhaematocrit centrifuge and spun at 10, 000 rpm for 5 minutes. Spunned tubes were placed into a specially designed scale and the PCV was read as a percentage of the whole blood.

$$\text{PVC \%} = \frac{\text{Packed RBC column height}}{\text{Total blood volumn height}} \text{ x } 100$$

Calculation of Mean Corpuscular Volume (MCV): The mean corpuscular volume was calculated by using values of PCV % and the red blood cell counts and expressed in µm/3 [15].

Determination of the Haemoglobin (Hb) Concentration: Heamoglobin (Hb) concentration was determined using cyanomethaglobin technique as outlined by Van Der Oost *et al*. [14]. Whole blood (20 µl) was added to 4 ml of Drabkin's solution in a test tube in a 1:250 dilution. Drabkin's solution contains potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. This was well mixed, allowed to stand for 10 minutes at room temperature. The ferricyanide forms methaemoglobin which is converted to colored cyanmethaemogloin by the cyanide and the absorbance was read colorimetrically at 540 nm with Drabkin's solution as a blank.

$$\text{Heamoglobin (Hb)} = \frac{\text{Reading of test x conc standard}}{\text{Reading of standard}}$$

The wintrobe indices; (i) mean cell corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean cell haemoglobin content (MCH) were derived from these primary indices.

Assessing the Impact of the Herbicides on Some Biochemical Parameters of the Fish: Determination of aspartate aminotransferase (AST) activity: The activities of Aspartate Aminotransferase (AST) were assayed by the method of Ramesh *et al*. [16] as outlined in the Randox kit. Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazine with 2, 4-dinitrophenylhydrazine. The samples of the serum (0.1 ml) were pipetted into the sample test tubes only and 0.1 ml of distilled water was pipette into the blank test tube. Then, 0.5 ml of Reagent one (R1) containing phosphate buffer, L - aspartate and beta - oxoglutarate was pipetted into both the blank and serum sample test tubes respectively. The entire reaction medium was well mixed and incubated for 30 minutes in a water bath at 37°C. Immediately after incubation, 0.5 ml of Reagent two (R2) containing 2, 4-dinitrophenylhydrazine was added to the blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25°C. Finally, 5.0 ml of 0.4 sodium hydroxide solutions was added to both the blank and serum sample test tubes respectively and mixed thoroughly. The absorbance was read at a wavelength of 546 nm after 5 minutes.

$$AST = \frac{\text{Abs of sample} - \text{abs of} - \text{blk}}{\text{Slope}}$$

Determination of the Alanine Aminotransferase (ALT) Activity: The activities of alanine aminotransferase (ALT) were assayed by the method of Ramesh *et al*. [16] as outlined in Randox Kit. The serum sample (0.1 ml) was pipetted into the sample test tube and 0.1 ml of distilled water pipette into blank test tube, 500 µl of the ALT substrate buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate (R1) were added. The entire reaction media were well mixed and incubated for 30 minutes in a water bath at 37°C and pH 7.4. Immediately after incubation, 0.5 ml of Reagent two (R2) containing 2, 4-dinitrophenylhydrazine was added to the blank and sample test tubes. These were thoroughly mixed and allowed to stand for exactly 20 minutes at 25°C. Finally, 0.5 ml of sodium hydroxide solution was added to both the blank and serum sample test tubes respectively and mixed thoroughly. The absorbance were read at wavelength of 546 nm after 5 minutes.

$$ALT = \frac{\text{Abs of sample} - \text{abs of blk}}{\text{Slope}}$$

Determination of the Alkaline Phosphatase (ALP) Activity Principle: The activities of alkaline phosphatase were assayed by the method of Blahova *et al*. [17] as outlined in Randox kit, used. The alkaline phosphatase act upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure: Into three test tubes labeled test, serum, standard and blank were added 50 µl of serum, 50 µl of standard and 50 µl distilled water respectively. Then, 0.05 ml (50 µl) of alkaline phsophatase substrate was added into the labeled test tubes, mixed gently for 3 minutes and incubated for exactly 10 minutes at 37°C. After that, alkaline phosphatase color developer (2.5 ml) was addad at timed intervals of 1 minute and mixed well. The absorbance was measure at 630 nm.

$$\text{Alkaline phosphatase} = \frac{\text{Absorbance of sample x value of standard}}{\text{Absorbance of standard}}$$

Determination of Total Protein: The total protein content was determined using the method of Tayeb *et al*. [18]. This was based on the principle that cupric ions in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. Into three test tubes labeled serum, standard and blank were added 20 µl of serum, 10 µl standard and 10 µl distilled water respectively. Thereafter, 0.5 ml of reagent R1(Biuret reagent composed of NaOH, Na-K- tartrate, potassium iodide and cupric sulphate) was added to all the test tubes and incubateed for 3 minutes at 25°C. The absorbance was measured at 500 nm.

$$\text{Protein concentration (g/dl)} = \frac{\text{Absorbance of sample x standard conc}}{\text{Absorbance of standard}}$$

Determination of Glucose: Glucose determination using a kit Tench plasma glucose concentration was analysed using a commercial enzyme kit (Glu L 1000, PLIVA-Lachema, Czech Republic). Samples were added to a solution of glucose oxidase, peroxidase and 4-aminoantipyrine; then after 10 min, incubation samples were moved to 96-well microtiter plate (200 µL) and their absorbance (500 nm) was measured by the plate reader (Tecan Sunrise, USA). The glucose concentration was consequently determined using the absorbance of glucose standard (10mmol/L). The quantification limit of this method was 0.021 mmol/L; the working range was 0.065-45 mmol/L, repeatability was 1.05% and the working volume was 10 µL.

Determination of Bilirubin: The total bilirubin content was determined using the method described by Khoshnood [19]. This was based on the pricinciple that conjugated bilirubin reacts with diazonitizedsulphonic acid to form a blue coloured complex and total bilirubin is determined in the presence of caffeine which releases albumin-bound bilirubin in the reaction with diazonitizedsulphonic acid.

The kit used contains Sulphanilic (29mmol/L), Hydrochloric acid (0.17N), Sodium Nitriate (25mmol/L), Caffeine (0.26mol/L), Sodium benzoate (0.52 mol/L), Tartrate (0.93 mol/L), Sodium Hydroxide (1.9N).

Procedure: Two test tubes were labelled sample bank and and sample test and were set up in duplicates. Then, O.2ml of reagent 1, 2ml sodium chloride (9g/I) and O.2ml sample was added. into the sample blank test tubes. Simultaneusly, O.2ml reagent 1, O.05ml reagent 2, 2ml sodium chloride (9g/L) and O.2ml sample was added. The content of the test tubes were mixed thoroughly, allowed to stand for five minutes at 25°C.

The absorbance of the sample against the sample blank was read at 546nm.

CALCULATION

Conjugated bilirubin (μ mol/L) = 246 x ACB (546 nm)

Histopathological Assessment of the Liver and Kidney: At96 hours of the exposure, twofish from the sub lethal concentrations of the herbicide were dissected and kidney and liver, excised and processed for histopathologial studies following the procedure adopted by Tayeb *et al*. [20] with some minor modifications as follows; the tissues were fixed in 10% normal-saline for I week to prevent autolysis, improve staining quality and to aid optical differentiation of cells, then dehydrated to remove water and wax using different grades of alcohol ranging from 50% to absolute alcohol for 30 minutes each. The dehydrated tissues were cleared by removing the alcohol from the tissue by immersing them through three (3) changes of xylene for 30 minutes each. The cleared tissues were impregnated and infiltrated to remove the clearing agent (Xylene) in a hot oven at temperature 60°c by passing it through three (3) changes of molten paraffin in a hot air oven.The infiltrated tissues were buried or embedded with molten paraffin wax in an embeddingmold and allowed to solidify. The block of the tissues were sectioned using rotary microtome and then trimmed to obtain the cutting surface of the tissue at 5 micron. The sectioned tissues were dewaxed in xylene for 10 minutes and xylene removed by rinsing in descending order of alcohol ranging from absolute, 90%, 70%, 50% alcohol for 2 seconds each. It was then washed in 2 changes of water and stained in haematoxylin for five (5) minutes, washed in 2 changes of water again and differentiated in 1% acid, then rewashed in tap water, counter stained in Eosin for 2 minutes, cleaned in xylene and finally mounted in D.P.X., then, dried for histopathological examination under the microscope.

Data Presentation and Statistical Analysis: The dose mortality response analysis were performed by probit analysis with the SPSS 21.1 Computer program (SPPS INC. Chicago IL, USA) to determine the LC_{10}-LC_{90}. The patterns of variations due to the exposure time and treatments were estimated by a one way analysis of variance (ANOVA), p- values less than 0.05 ($p<0.05$) were considered to be statistically significant.

RESULTS

Mortality Rate

Mortality Rate of *H. bidorsalis* During the 96 hour Exposure to Primextra: No mortality was recorded in the control group during the 96 hour exposure. However, 63.33, 70.00 and 83.33 % motality was recorded in Group A, B and C respectively. In Group D, 96.67 % mortality was recorded while 100.00 % mortality was recorded in Group E.

Median Lethal Concentration LC_{50}: Median Lethal Concentration of Primextra exposed to *H. bidorsalis*: The median Lethal concentration which is the concentration of the toxicant that will kill 50% of the test organism, in this bioassay, *H. bidorsalis* exposed to primextra was determined by Probit method (Graphical method) to be LC50 = 12.59μg/L as shown in Figure 2.

Impact of the Herbicide O the Physico-Chemical Variables of the Treatments at 96 Hours: The physico-Chemical Variables of the Treatments at 96 hours are presented in Tabele 1.

The temperature value was 27.40 ± 0.06 in the control. The value increased to 27.60 ± 0.06 in group A, later to 27.77 ± 0.09 in group B and 28.03±0.19 in group C.The pH value for *H. bidorsalis* exposed to primextra was 7.57 ± 0.03 in the control. The value was reduced to 7.40 ± 0.00 in group A, 7.30 ± 0.06 in group B and 6.93 ± 0.09 in group C.The value of DO in the treatment of *H. bidorsalis*with primextra was 5.13 ±0.03 in the control. It decreased to 4.67 ±0.09 in group A, 4.37b ±0.15 in group B and 3.20 ±0.26 in group C. The value of conductivity in the exposure of *H. bidorsalis*to primextra was 749.33 ± 0.88in the control. The value increased to 753.67 ± 2.96 in group A, 789.00 ± 5.20 in group B and 805.33±2.03 in group C. In the exposure of *H. bidorsalis* to primextra, the value of the TDS was 328.00 ± 1.53 in the control. The value increased to 369.00 ± 0.08 in group A, 386.00 ± 6.66 in group B and 422.67 ± .78 in group C.

Behavioral Responses: The fish in the control group maintained normal behavior throughout the assay. However, the fish subjected to different concentrations of primextra displayed uncoordinated behaviors at different durations as presented in Table 2. The fish exhibited from mild, moderate to strong hyperactivity, equilibrium status, swimming rate, surfacing to gulp for air, convulsion, somersaulting, fin and opercula movement activities in correlation with the concentration and duration of exposure.

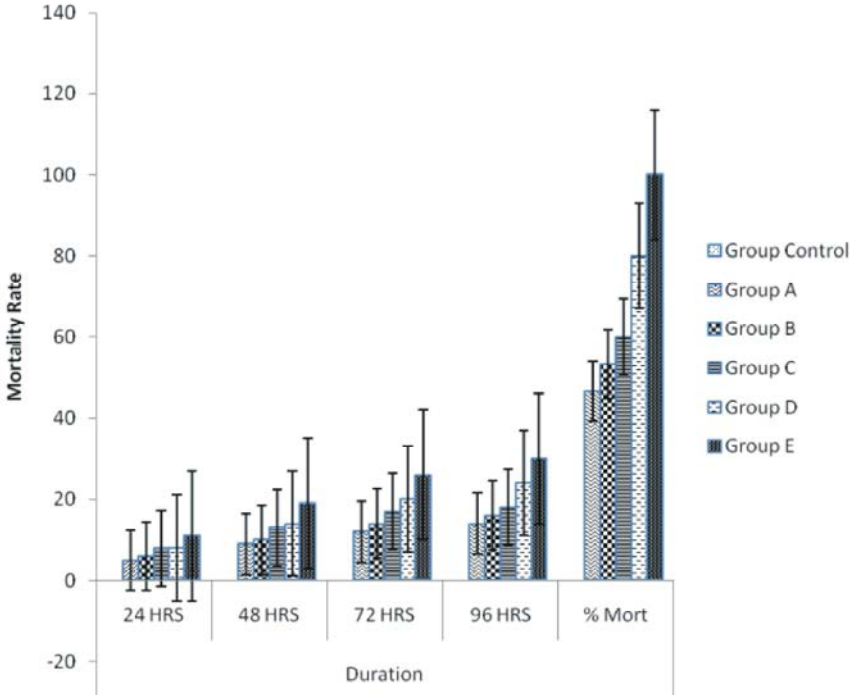

Fig. 1: Mortality rate of *H. bidorsalis* during 96 hour exposure to different lethal concentrations of primextra at different durations. The vertical lines represent standard error.

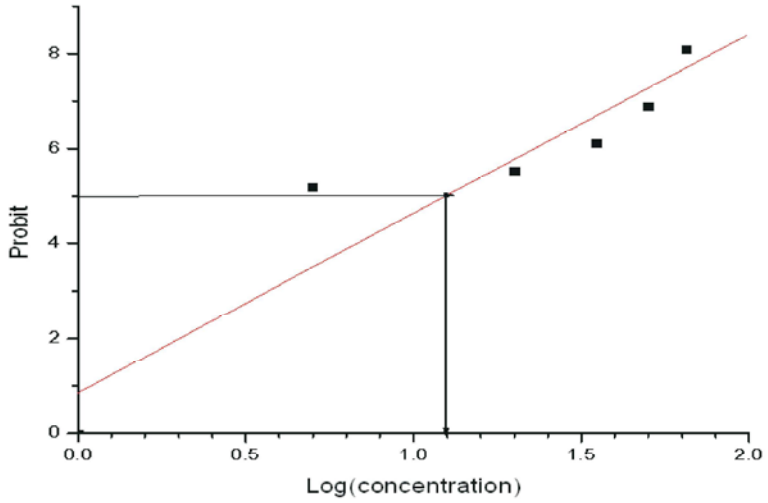

Fig. 2: Probit Method of Estimating Median Lethal Concentration (LC50) of Primextra in *H. bidorsalis*. (LC50 = 12.59 µg/L)

Table 1: Physicho-chemical parameters of the treatments on *H. bidorsalis* at 96hours

Conc.	Temperature (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	TDS (mg/L)
Control	27.40±0.06a	7.57±0.03a	5.13±0.03a	749.33±0.88a	328.00±1.53a
F	27.60 ±0.07b	7.40±0.00b	4.67±0.09b	753.67±2.96b	369.00±2.08b
G	27.77±0.09b	7.30±0.06b	4.37±0.15b	789.00±5.20b	386.00 ±6.66b
H	28.03±0.19b	6.93±0.09b	3.20±0.26b	805.33±2.03b	422.67±4.78b

Conc.-Concentration, DO- dissolved oxygen, TDS- Total dissolved solutes. Values with different alphabetic superscripts differ significantly ($p < 0.05$) between concentrations within the same column.

Table 2: Impact of Primextra on some Behavioral parameters of *Heterobranchus bidorsalis* at Various Concentrations and Durations

Conc. (µg/L)	Time(h)	Hyperactivity	Equilibrium status	Swimming rate	Gulping for air	Convulsion	Somersaulting activity	Fin movement	Opercula movement
Control	24h	-	+++	++	-	-	-	++	++
	48h	-	+++	+	-	-	-	++	++
	72h	-	+++	++	-	-	-	++	++
	96h	-	+++	++	-	-	-	++	++
A	24h	+	++	++	-	-	-	++	++
	48h	+	++	++	-	-	-	++	++
	72h	+	++	++	-	-	-	++	++
	96h	+	+	++	+	-	-	++	++
B	24h	++	+	++	+	-	-	++	++
	48h	++	+	++	+	-	-	++	++
	72h	+	++	++	+	-	-	++	++
	96h	+	++	++	+	-	-	++	++
C	24h	+++	+	++	+	+	+	+++	+++
	48h	++	+	++	+	+	+	++	++
	72h	++	+	++	+	+	+	++	++
	96h	++	+	++	+	-	-	++	++

Keys: - = None, + = Mild; ++ = Moderate; +++ = Strong, TC = Toxicant concentration

Heamatological Effects of the Herbicide on the Peripheral Blood of *H. bidorsalis*: The haematological parameters such as RBC, WBC, Hb, PCV, MCH, MCV and MCHC in *H. bidorsalis* exposed to primextra, oryzoplus and dragon are presented in Table 2. The value of RBC in the control for was 9.67 ± 0.80. The value decreased to 8.42 ± 0.72 in group F and 8.38 ± 0.68 in group G. In all, the values of RBC generally decreased though the decrease was not significantly different ($p > 0.05$) compared to the control. The WBC value in the control was 87500 ± 6.90. The value significantly increased to 10400 ± 7.40 in group F, 10800 ± 8.50 in group G and 10600 ± 8.00 in group H. In all, there was concentration dependent significant increase ($p < 0.05$) in the values of WBC in all concentrations compared to the control. The Hb value in the control was 6.90 ±0.55. The value decreased to 5.50 ±0.40, 5.70 ±0.44 and 5.70 ±0.38 in groups F, G and H respectively. The PCV value in the control was 32.01 ± 1.00. The value decreased to 32.00 ± 1.2 in group F, 30.00 ± 1.04 in group G and 31.05 ± 1.08 in group H. The MCH value in the control was 7.14 ± 0.75. The value decreased to 6.53 ± 0.45 in group F, 6.80 ± 0.46 in group G and finally to 6.58 ± 0.55 in group H. The MCV value was 33.09 ± 1.04 in the control. The value significantly increased to 38.01 ± 1.22 in group F, 35.80 ± 1.65 in group G and finally to 35.80 ± 1.45 in group H. In all, there were increases in MCV in fish exposed to all concentrations but significant differences ($p < 0.05$) were observed only in fish exposed to group F.

The value of MCHCwas 21.56 ± 0.35 in the control. The value decreased to 17.19 ± 0.32 in group F, 19.00 ± 0.24 in group G and 18.39 ± 0.20 in group H. However, no significant differences ($p > 0.05$) were observed between the treated groups and the control.

Biochemical Effects of the Herbicide on the Peripheral Blood of *H. bidorsalis*: The biochemical parameters which include, AST, ALP, ALT, glucose, Protein and bilirubin of *H. bidorsalis* exposed primextra are presented in Table 4.

The value of AST was 48.45 ± 0.50 in the control. The value significantly increased to 68.01 ± 0.40 in group F, 70.20 ± 2.50 in group G and 74.20 ± 1.95 in group Hcompared to the control. The value of ALP was 60.10 ± 0.85 in the control. The value increased to 90.05 ± 0.76 in group F, 94.20 ± 1.1in group G and 98.40 ± 1.84 in group H. In all, there was significant increase ($p < 0.05$) in the values of ALP in all concentrations compared to the control. The value was 43.50 ± 0.43 in the control. The value decreased to 75.50 ± 1.70 in group A, 70.30 ± 1.45 and finally to 70.50 ± 1.64 in group C.

The glucose value was 50.40 ± 0.85 in the control. A significant increase ($p< 0.05$) was recorded in all the groups treated with primextra compared to the control. The value of protein was 6.55 ± 0.15 in the control group. The value of protein was 6.55 ± 0.15 in the control group. A significant difference ($p<0.05$) was recorded in all the treatments compared to the control. The value of bilirubin was 5.04 ± 0.14 in the control. The value increased to 5.50 ± 0.14 in group A, 6.40 ± 0.21 in group B and 5.86 ± 0.19 in group C. However, they were not significantly different ($p > 0.05$) compared to the control.

Histopathological Studies: The result of histopathological examination of the kidney from *H. bidorsalis* exposed to primextra is presented on Plate 1. The liver from the control group showed normal hepatic architecture with normal hepatocyte (NH) and central vain (CV).

Table 3: Haematological parameters (mean ± SE) of juvenile *H. bidorsalis*after 96 h exposure to Primextra

		Haematological parameters						
Pesticide	Concentration (µg/L)	RBC (×106)	WBC (×103)	Hb (%)	PVC (%)	MCH (pgcell/L)	MCV (flcell/L)	MCHC (g dl/L)
Primextra	Control	9.67 ± 0.80a	8750 ± 6.90a	6.90 ±0.55a	32.01 ± 1.00a	7.14 ± 0.75a	33.09 ± 1.04a	21.56 ± 0.35a
	F	8.42 ± 0.72a	10400 ± 7.40b	5.50 ±0.40a	32.00 ± 1.23a	6.53 ± 0.45a	38.01 ± 1.22b	17.19 ± 0.32a
	G	8.38 ± 0.68a	10800 ± 8.50b	5.70 ±0.44a	30.00 ± 1.04a	6.80 ± 0.46a	35.80 ± 1.65a	19.00 ± 0.24a
	H	8.66 ± 0.67a	10600 ± 8.00b	5.70 ±0.38a	31.05 ± 1.08a	6.58 ± 0.55a	35.80 ± 1.45a	18.39 ± 0.20a

Table 4: Biochemical parameters (mean ± SE) of juvenile *H. bidorsalis*at 96 h exposure to and Primextra. Values with different alphabetic superscripts differ significantly (p< 0.05) between concentrations within the same column

		Biochemical parameters					
Pesticide	Concentration (µg/l)	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	Glucose (mg/dL)	Protein (mg/dL)	Bilirubin (mg/dL)
Primextra	Control	48.45 ± 0.50a	60.10 ± 0.85a	43.50 ± 0.43a	50.40 ± 0.85a	6.55 ± 0.15a	5.04 ± 0.14a
	A	68.01 ± 0.40b	90.05 ± 0.76b	75.50 ± 1.70b	60.20 ± 0.86b	3.00 ± 0.08b	5.50 ± 0.14a
	B	70.20 ± 2.50b	94.20 ± 1.12b	70.30 ± 1.45b	55.00 ± 0.45b	3.50 ± 0.08b	6.40 ± 0.21a
	C	74.20 ± 1.95b	98.40 ± 1.84b	70.50 ± 1.64b	55.50 ± 0.35b	3.30 ± 0.45b	5.86 ± 0.19a

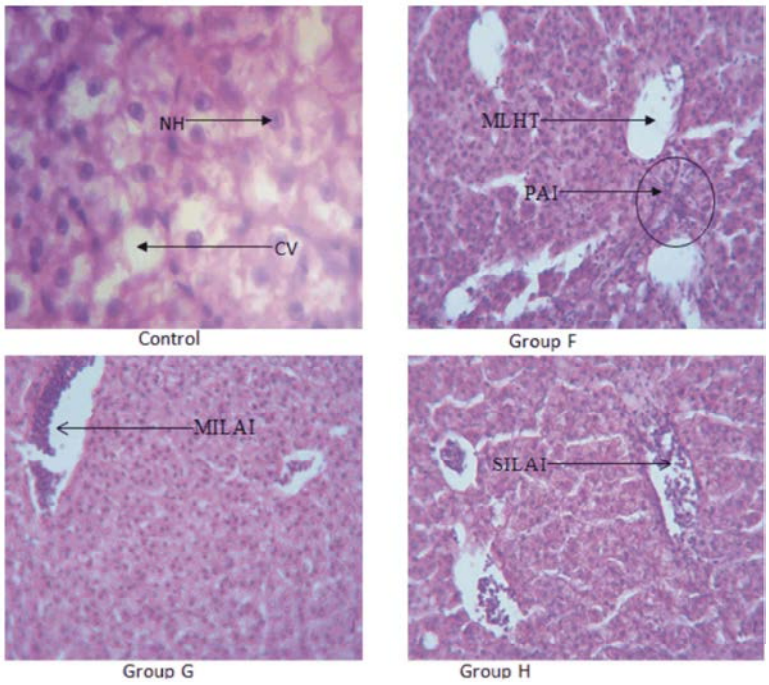

Plate 1: Photomicrographs of the liver of *H. bidorsalis*exposed to primextra (x 600). Control showing normal hepatocyte (H) and central vain (CV). Group F showing portal aggregate of inflammation(PAI) and moderate loss of hepatic tissue (MLHT), Group G showingmoderate intra lobular aggregate of inflammation(MILAI) and Group H showing severe intra lobular aggregate of inflammation(SILAI).

However, group Fshowed moderate damage on the hepatic tissuewith portal aggregate of inflammation(PAI) and moderate loss of hepatic tissue (MLHT) while those in group G showed moderate damage on the hepatic tissue withmoderate intralobular aggregate of inflammation (MILAI).In group H, there was severe damage on the hepatic tissue with severe intra lobular aggregate of inflammation (SILAI).

The impact of primextra on the Histology of the kidney of *H. bidorsalis* at 96 Hour exposure is presented on Plate 2. The control shows normal renal architecture with glomeruli (G) and renal tubule (RT). Group F showed moderate effect on the renal tissue with moderate focal loss of renal tissue (FLRT) and mild intra renal hemorrhage (MIRH). Group Gshowed severe effect on the renal tissue with loss of glomeruli and severe focal loss of

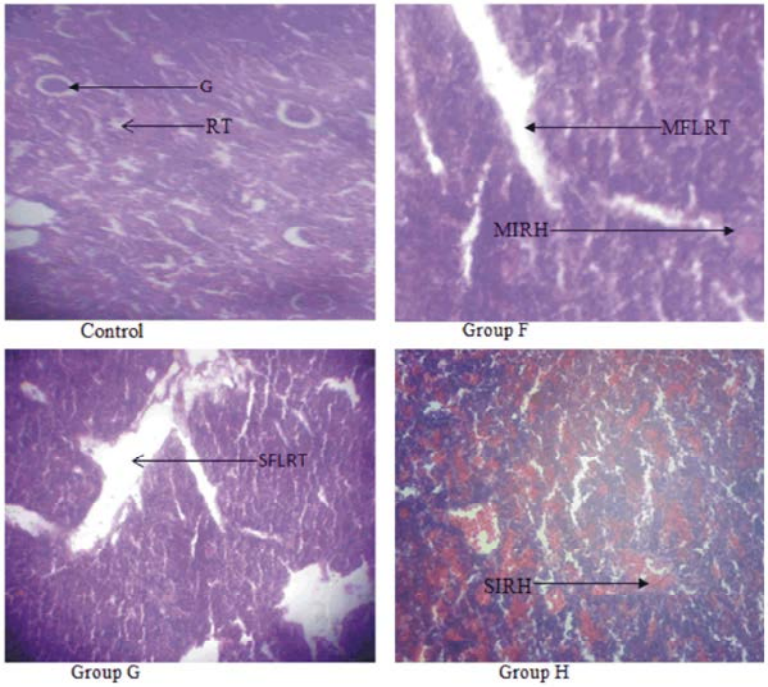

Plate 2: Histopathological impact of primextra on the kidney of *H. bidorsalis*at 96 hour exposure. The control group showing normal glomeruli (NG) and renal tubules (RT), group F showing moderate focal loss of renal tissue (MFLRT) and mild intra renal hemorrhage (MIRH), group G showing severe focal loss of renal tissue (SFLRT), Group H showing severe effect on the renal tissue with loss of glomeruli and sever intra renal hemorrhage (SIRH).

renal tissue (SFLRT). Group H showed severe effect on the renal tissue withloss of glomeruli and severeintra renal haemorrhage (SIRH).

DISCUSSION

Fish lives and carries out its activities in water, consequently its performance depends directly or indirectly on the quality of the water it lives in. The dose dependent increase in temperature recorded in the present work is in agreement with the report of Ayoola [21], Nwani *et al*. [22], Nwani *et al*. [23] and Olafedehan *et al*. [24]. The primary interest in the temperature of surface waters is the inverse relationship it has with oxygen solubility. Unlike the temperature, the DO in the present study showed an indirect proportional relationship with the concentration of the herbicide which means that as the concentration of the herbicide increased, the DO decreased. The 100% mortality recorded in the highest lethal concentration in the present study could be attributed to oxygen depletion in the group. Dissolved oxygen is absolutely essential for the survival of all aquatic organisms (Not only fish but also invertebrates such as crabs, clams and zooplankton). Hypoxia or oxygen depletion is a phenomenon that occurs in aquatic environments as DO becomes reduced in concentration to a point detrimental to aquatic organisms living in the system [28]. Moreover, oxygen affects a vast number of other water indicators, not only biochemical but esthetic ones like the odour, clarity and taste.

Behavioral responses are the most sensitive indication of potential toxic effects [31, 32]. In the present study, the fish in the control exhibited normal behaviour throughout the 96 hour exposure while the fish subjected to different concentrations of primextra (F, G and H) displayed uncoordinated behaviours at different times of exposure. Many researchers [21, 22, 24, 33] have reported similar behavioural responses such as strong hyperactivity, loss of equilibrium, strong, swimming rate, moderate convulsion and somersaulting, strong fin and opercula movement, erratic swimming, sudden quick movement, gasping for air.

The decrease in RBC, PCV, Hb and significant increase in WBC recorded in the present study is in consonance with the report of some researchers. Ramesh *et al*. [34] reported a significant decrease in RBC

and Hb and enhanced WBC in their exposure of *Cyprinuscarpio* to atrazine. Olafedehan *et al*. [24] reported that the exposure of *C. gariepinus* to 2, 4-D caused significant decrease in PVC, RBC and Hb, but significant increase in WBC compared to the control and attributed the rapid destruction of the red blood cells to the toxicant and the increase in WBC as probable defense mechanism deployed by the fish to protect itself from the assault of 2, 4-D. Blood is one of the important pathophysiological reflectors of the whole body of an organism. Blood acts and reflects the pathological status of animals exposed to xenobiotics [14]. Many authors have noted that haematological studies are of ecological and physiological interest in helping to understand the relationship between blood parameters and the environment [35-38] Any deviation from normal in the blood components of an animal is an indication of danger in the health status of that animal [13] and it helps to determine stresses due to environmental, nutritional and or pathological factors [39].

Alterations in some biochemical parameters such as ALT, AST, ALP and glucose, proteinrecorded in the present study are in agreement with the report of some authors [40] reported significant ($P< 0.05$) alterations in the concentrations of glucose, total protein, albumin, lactate, phosphorus and calcium as well as in the activities of ALT, AST, ALP compared to the control group in the acute Exposure of Common Carp (*Cyprinuscarpio*) to atrazine Herbicide. Concomitant increase in the activities of ALT and AST in the serum of treated organisms indicates acute hepatocellular injury [41-43] reported significant increases in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) in *C. gariepinus* exposed glyphosate and paraquat based herbicide.

Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish [44]. In the present study, the two organs (Liver and kidney) examined for histopathological impactof the herbicide from the control groups showed normal architectural features of the organs while those exposed to varying concentrations of the herbicide showed various degree of damage of the organs in correlation with the concentrations of the herbicides. The histopathological changes in the liver in the present study are in agreement with the work of Van Der Oost *et al*. [14] and Olufayo and Alade [45] who, observed the typical darkly stained specks of necrotic nuclei and normal hepatocytes in the liver of their control fish but reported a focal loss of hepatic tissue with clumping in some areas, giving rise to mild hepatic architectural distortion in the group exposed to 0.43 mg/l of 2, 4-Dichlorophenoxyacetic Acid. They further reported an infiltration of inflammatory cells with hepatic architectural distortion and focal loss of hepatic tissue with a mild architectural distortion in the 0.57 mg/l group and finally in the 0.72 mg/l group presented focal areas of aggregation of inflammatory cells with architectural distortion, vacuolization, focal loss and common lesion.

United Nations [5] noted that the exposure to atrazine in fish is most often associated with the degenerative changes in the kidney and gills and also with the alteration in the liver tissues.Alterations of different components of renal corpuscles and renal tubules in rainbow trout (*Oncorhynchusmykiss)* after exposure to atrazine at the range of 5-40 µg/L for 28 days have been reported [46]. Histological changes in the kidney could lead to malfunctions in the organ and eventualdeath. Olafedehan *et al*. [38] studied the effects of cypermethrin on the kidney of *H. bidorsalis* and observed that the kidney cells were massively destroyed. They discovered that the renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions. Yang *et al*. [47] revealed lesions in kidney tissues including an extensive expansion in the lumen, degenerative and necrotic changes of tubular epithelia and shrinkage of the glomeruli after acute 96 h exposure to atrazine in rare minnow (*Gobiocyprisrarus*)

CONCLUSION

The results of this study showed that primextra (Atrazine) reduced the quality of the test water, adversely affected thebehaviour of the test organisms (Fish), altered some haematological and biochemical parameters of fish as well asdamaged the liver and kidney of the fish. These results indicate that primextrais toxic to aquatic organisms and its use should be minimized especially near aquatic environment.

REFERENCES

1. Solomon, K.R., K. Dalholfe, D. Volz and G. Van der Kraak, 2013. Effects of Herbicides on Fish, pp: 409.
2. Graymore, M., Stagnitti and G. Allison, 2001. Impacts of atrazne on aquatic ecosystem. Environ. Int., 26(8): 483-95.

3. Olua, N.S., Obiezue, R. Nduka, Nwanyoluaru, A.J. Ochulor and E. Onuoha, 2010. Toxicity and Histopathological Effect of Atrazine (Herbicide) on the earthworm *Nsukkadrilusmbae* under laboratory conditions. Animal Research International, 7(3): 1287-1293.
4. Ubachukwu, P.O., N.S. Oluah, C.U. Omeje and C.B. Ikele, 2012. Effect of the Herbicide Primextra on Tissue Cholesterol Level in Clariasgariepinus. Animal Research International, 9(1): 1524-1528.
5. United Nations, 2011. Policy Brief: Water Quality Challenges and Impacts, pp: 22.
6. Bttagline, W.A., E.M. Thurman, S.J. Kalkhoff and S.D. Porter, 2003. Herbicides andtransformation products in surface waters of the Midwestern United States. Journal of the American Water Resources Association, 39(4): 743-756.
7. Hayes, T.B., L.L. Anderson, V.R. Beasley, R.M. Shane Shane, T. De Solla, Iguchi, H. Ingranham, Z. Kestemont, Kniewald and V.S. Langlois, 2011. Demasculinization and feminization of male gonads by atrazine: Consistent effects of across vertebrate classes. The Journal of Steroid Biochemistry and Molecular Biology, 127(2): 64-73.
8. Syngenta, 2014. Material Safety Data Sheet. Syngenta Crop Protection Incorporated, 140 Research Lane, Park Guelph, Canada, pp: 6.
9. Finney, D.J., 1971. Probitanalysis. Univ, Press, Cambridge, pp: 333.
10. Finney, D.J., 1978. Statistical Nethod in Biological Assay. London Griffin., pp: 508.
11. Organization for Economic Cooperation and Development (OECD), 2014. Guide line for the testing of chemicals: Fish, acute Toxicity test. OECD: Paris, France, pp: 21.
12. Okogwu, O.I., Q. Anionwo, D.C. Anoke and P.O. Ugwuezi, 2015. Behavioural, Haematological and Histopathological changes in the African Catfish, *Clariasgariepinus* Exposed to 2, 4-Dichlorophenoxyacetic Acid (2, 4-D). Nig. J. Biotech., 30: 26-35.
13. Fondriest, 2016. Fundamentals of Environmental Measurements. Fondriest Environmental Inc., PP: 73.
14. Van Der Oost, R., J. Beber and N.P.F. Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Phamacology, 13: 57-149.
15. Alimba, C.G., A.A. Bakare and O.O. Aina, 2012. Liver and kidney dysfunction in wistar rats exposed to municipal landfill leachate. Resources and Environment, 2: 150-163.
16. Ramesh, M., R. Rinivasan and M. Saravanan, 2009. Effect of atrazine (Herbicide) on blood parameters of common carp *Cyprinuscarpio* (Actinopterygii: Cypriniformes). African Journal of Environmental Science and Technology, 3(12): 453-458.
17. Blahova, J., H. Modra, M. Sevcikova, P. Marselek, L. Zelnikova and M. Svobodova, 2014. Evaluation of Biochemical, haematological and histopathological responses and recovery ability of common carp (*Cyprinuscarpio*) after acute exposure to atrazine herbicide. Biomed Research International, 14: 1-8.
18. Tayeb, W., A. Nakbi, M. Trabelsi, N. Attia, A. Milled, and M. Hammami, 2010. Hepatoxicity induced by sub-acute exposure, of rats to 2, 4-Dichlorophenoxyacetic acid based herbicide 'Desmournedlourd'. J. Hazard. Mater., 180: 225-233.
19. Khoshnood, Z., 2017. Effects of environmental pollution on f ish: a short review Transylv. Rev. Syst. Ecol. Res., 19(1): 49-60, 50.
20. Tayeb, W., A. Nakbi, M. Trabelsi, N. Attia, A. Milled and M. Hammami, 2010. Hepatoxicity induced by sub-acute exposure, of rats to 2, 4-Dichlorophenoxyacetic acid based herbicide 'Desmournedlourd'. J. Hazard. Mater., 180: 225-233.
21. Ayoola, S.O., 2008a. Histopathogical effects of Glyphosate on Juvenile of African Catfish (*Clariasgariepinus*). American- Eurasian J. Agric. & Environ, Sci., 3(4): 362-367.
22. Nwani, C.D., S.N. Naresh, K. Ravindra, K. Basdeo, K. Pavan and S.L. Wazir, 2010a. Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish *Channapunctatus* (Bloch). Aquat Res., 2: 105-111.
23. Nwani, C.D., W.S. Lakra, S. Naresh, R. Nagpure, R. Kumar, K. Satish and S. Kumar, 2010b. Toxicity of the herbicide Atrazine: Effects on lipid Petroxidation and activities of Antioxidant Enzymes in the freshwater Fish Channapuctatus (Bloch) Int. J. Environ. Res. Public Health, 7: 3298-3312.

24. Olafedehan, C.O., A.M. Obun, M.K. Yusuf, O.O. Adewumi, A.O. Oladefedehan, A.O. Awofolaji and A.A. Adeniji, 2010. Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits Proceedings of 35th Annual Conference of Nigerian Society for Animal Production, pp: 212.
25. Dogan, D. and C. Can, 2011. Hematological, Biochemical and Behavioral Responses of *Oncorhynchusmykiss* to Dimethoate. Fish Physiology and Biochemistry, 37: 951-B.A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids, Integrative Comparative Biology, 42: 517-525.
26. Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids, Integrative Comparative Biology, 42: 517-525.
27. Atamanalp, P., M.S. Keles and H.I. Aras, 2001. The effects of Cypermethrin (a Synthetic Pyrethroid on some Biochemical Parameters (Ca, P, Na, Tp) on the Trout (*Oncorhynchusmyleiss*). Canadian Journal of fish and Aquatic Sci., 45: 219-222.
28. Mallya, 2007. The effects of Dissolved oxygen on fish growth in aquaculture.The United Nations University, pp: 30.
29. EPA, 2001. Parameters of Water Quality: Interpretation and standards.Environmental Protection Agency, Ireland, pp: 133.
30. Fagbenro, O.A., T.S. Olaniran and A.O. Esan, 1991. Some aspects of the biology of the catfish, *Heterobranchus bidorsalis* Geoffrey Saint Hilaire, 1809 (Clariidae) in river Ogbese, Nigeria. J. Afr. Zool., 105: 363-372.
31. Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids, Integrative Comparative Biology, 42: 517-525.
32. Banaee, M., A.R. Mirvaghefei, B. Majazi Amiri, G.R. Rafei and B. Nematdost, 2011. Hematological and Histopathological Study of Experimental Diazinon Poisoning in common carp fish (*Cyprinuscarpio*). Journal of Fisheries (Iranian Journal of Natural Resources), 64(1): 1-14.
33. Okoh, F.A.A., 2015. Behavioral Responses of African Freshwater Fish *Clariasgareiepinus*to Different Concentrations of a Comercial Herbicide, Orizoplus. Journal of Biology, Agriculture and Healthcare, 5(8): 59-63.
34. Ramesh, M., R. Rinivasan and M. Saravanan, 2009. Effect of atrazine (Herbicide) on blood parameters of common carp *Cyprinuscarpio* (Actinopterygii: Cypriniformes). African Journal of Environmental Science and Technology, 3(12): 453-458.
35. Aderemi, F.A., 2004. Effects of replacement of wheat bran with cassava root sieviate supplemented or unsupplemented with enzyme on the haematology and serum biochemistry of pullet chicks. Tropical Journal of Animal Science, 7: 147-153.
36. Ovuru, S.S. and I.K.E. Ekweozor, 2004. Haematological changes associated with crude oil ingestion in experimental rabbits. African Journal of Biotechnology, 3(6): 346-348.
37. Mmereole, F.U.C., 2008. The Effects of Replacing Groundnut Cake with Rubber Seed Meal on the Haematological and Serological Indices of Broilers. International Journal of Poultry Science, 7(6): 622-624.
38. Olafedehan, C.O., A.M. Obun, M.K. Yusuf, O.O. Adewumi, A.O. Oladefedehan, A.O. Awofolaji and A.A. Adeniji, 2010. Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits Proceedings of 35th Annual Conference of Nigerian Society for Animal Production, pp: 212.
39. Afolabi, K.D., A.O. Akinsoyinu, R. Olajide and S.B. Akinleye, 2010. Haematological parameters of the Nigerian local grower chickens fed varying dietary levels of palm kernel cake Proceedings of 35th Annual Conference of Nigerian Society for Animal Production, University of Ibadan, Nigeria, 14th - 17th March, pp: 247.
40. Blahova, J., H. Modra, M. Sevcikova, P. Marselek, L. Zelnikova and M. Svobodova, 2014. Evaluation of Biochemical, haematological and histopathological responses and recovery ability of common carp (*Cyprinuscarpio*) after acute exposure to atrazine herbicide. Biomed Research International, 14: 1-8.
41. Alimba, C.G., A.A. Bakare and O.O. Aina, 2012. Liver and kidney dysfunction in wistar rats exposed to municipal landfill leachate. Resources and Environment, 2: 150-163.
42. Bakare, A.A., O.A. Alabi, A.M. Gbadebo, O.I. Ogunsuyi and C.G. Alimba, 2013. *In vivo* Cytogenotoxocity and Oxidative Stress Induced by Electronic Waste Leachate and Contaminate Groundwater. Challenges, 4: 169-187.

43. Banaee, M., A.R. Mirvaghefei, B. Majazi Amiri, G.R. Rafei, and B. Nematdost, 2011. Hematological and Histopathological Study of Experimental Diazinon Poisoning in common carp fish (*Cyprinuscarpio*). Journal of Fisheries (Iranian Journal of Natural Resources), 64(1): 1-14.
44. Van Der Oost, R., J. Beber and N.P.E. Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Phamacology, 13: 57-149.
45. Olufayo, M.O. and O.H. Alade, 2012. Acute toxicity and histological changes in gills, liver andkidney of catfish, *Heterobranchus bidorsalis* exposed to cypermethrin concentration. African Journal of Agricultural Research, 7(31): 4453-4459.
46. Fischer-Scherl, T., A. Veeser, R.W. Hoffmann, C. Kuhnhauser, R. Negele and T. Ewringmann, 1991. Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchusmykiss*), Archives of Environmental Contamination and Toxicology, 20(4): 454-46.
47. Yang, L., J. Zha, W. Li, Z. Li and Z. Wang, 2010. Atrazine affects kidney and adrenal hormones (AHs) related genes expressions of rare minnow (Gobiocyprisrarus). Aquatic Toxicology, 97: 204-211.