Vol. 20(7), pp. 287-292, July, 2021
DOI: 10.5897/AJB2018.16673
Article Number: C60685067251
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Changes in some behavioral, hematological and biochemical indices of air-breathing *Clarias gariepinus* [BUCHELL, 1822] exposed to pharmaceutical effluent

Ogundiran M. A.[1], Ayandiran T. A.[1]*, Olasunmibo A.O.[1], Olanipeku A. S.[1] and Okaseun O. T.[2]

[1]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Osun State, Nigeria.
[2]Bioresource Development Centre, National Biotechnology Development Agency, Ondo state, Nigeria.

Received 10 October 2018; Accepted 26 December, 2018

This work aimed at evaluating the behavioral, biochemical and hematological effects of pharmaceutical effluent in laboratory population of *Clarias gariepinus* using a static renewal bioassay system. Fish specimens were collected and exposed to five (0.04, 0.06, 0.08, 0.10 and 0.12 mgL^{-1}) sublethal concentrations of the effluent including a control experiment. Different dose dependent behavioral responses such as erratic swimming, gasp for breath, restlessness, and constant upward movement were observed in exposed fish. There was a steady decrease in the value of red blood cell (RBC), hemoglobin (Hb) and packed cell volume (PCV) as concentrations increase compared to the control stock. White blood cell count was found to be significantly ($p<0.05$) higher as the concentration of the test medium increases. Irregular level of lymphocytes and granulocytes across all concentrations was observed and levels of lymphocytes and granulocytes were significantly ($p<0.05$) increased in all effluent-treated fish samples during the exposure period. Different levels of total protein, glucose, cholesterol, etc., were obtained in the control and effluent-treated fish samples ($p<0.05$). The levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (serum enzymes) and lactate dehydrogenase were significantly ($p<0.05$) higher in the exposed *C. gariepinus*. The hematological and biochemical alterations in the effluent treated *C. gariepinus*, which were strongly indicative of cellular damages. This might be attributed to toxic effects of the phamarceutical effluent. Consequently, direct discharged of untreated or partially treated pharmaceutical effluent should be discouraged as this calls for public health concern.

Key words: Hematology, biochemical, pharmaceutical, *Clarias gariepinus*, effluent.

INTRODUCTION

Fishes live intimately with their environment and are exposed to varied degree of physical and chemical change in the aquatic phase and such changes can alter their general physiology and metabolism, which may be evaluated using their haematological and biochemical indices. The use of haematological indices in assessment of fish physiological has been used as an index of fish health status. These haematological indices are used as a tool to detect physiological changes, as a result of exposure to different environmental stressors such as

*Corresponding author. E-mail: taayandiran@lautech.edu.ng.

handling, pollutants, metals, hypoxia, anesthetics, season and acclimation (Ogundiran et al., 2007; Ayandiran et al., 2010).

Pharmaceutical compounds are used for several beneficial purposes in modern society but simultaneously pharma industries are releasing very toxic contaminants directly or after chemical modifications into the environment (Halling-Sorenson et al., 2005). Moreover, pharmaceutical waste compounds may enter the environment by different routes such as discharge of treated wastewater, seepage from landfills sites, sewer lines, runoff from animal wastes, etc. (Glassmeyer et al., 2005). Various physical and biological processes occurring in aquatic ecosystem may cause reduction of many pharmaceutical compounds, trace concentrations of human and veterinary pharmaceutical compounds as well as their metabolites have been detected in different water bodies like surface water, groundwater and drinking water sources (Bruce et al., 2010; Benotti et al., 2009).

Different industries including pharmaceuticals, chemicals, paints, etc., are speedily growing in Nigeria, which dispose off their effluents into the streams either directly or after partial treatment. Pharmaceutical compounds had been established to reach the environment and can be considered as environmental pollutants. Several pharmaceutical production facilities were found to be sources of much higher environmental pollutants concentration than those resulting from the applications of drugs (Larsson et al., 2007). Generally, pharmaceutical industries generate a huge quantity of wastes during manufacturing and maintenance operations and trace amount of this wastewater for longer duration in the environment may cause considerable adverse effects to human health and aquatic life (Benotti et al., 2009).

Information about the phamarceutical effluent toxicity at ecosystem level is limited and there is a need to investigate the toxicity impact of this effluent on fish. Therefore, this present study aimed at investigating the impact of sublethal concentrations of pharmaceutical effluent on catfish (*Clarias gariepinus*) with special reference to the haematological and biochemical changes.

MATERIALS AND METHODS

Experimental fish and chemicals

A total of 350 freshwater Juvenile African catfish [*C. gariepinus*] were procured from a local fish farm in Ogbomoso, Nigeria and were transported to the fisheries laboratory of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. The fish specimens were given prophylactic treatment immediately by bathing them repeatedly in 0.05% $KMNO_4$ to prevent possibility of any dermal infection. The fish stock was acclimated to the laboratory condition in six [600-L] plastic tank capacities for four weeks using non-chlorinated bore-hole water fetched from the running taps of the university. They were fed with 35% protein diet at 3% body weight daily in the early hour of the day. In order to avoid oxygen depletion, the holding tanks were continuously aerated using air pumps. The experiment was carried out indoor to maintain suitable photoperiod. The fish were treated in accordance with the rule conforming to worldwide conventional principles of laboratory animal care. The behavioral responses of the fish held in both experimental and control conditions were monitored on hourly basis. The physico-chemical analysis of the test medium was analyzed daily using standard methods of APHA (2012). Pharmaceutical effluents used were collected early in the morning at the discharge point between 7 and 8 AM from Sofak Pharmaceutical Company, Ogbomoso. The used test media from the pharmaceutical effluent was prepared by diluting the stock solution with a constant factor covering a large range.

Definitive experiment

Three hundred fish from the acclimatized batch were used for the definitive experiment. The fish were not fed 24 h prior to commencement of the exposure to the test media throughout the duration of the experiment, as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). The fish were randomly selected into six groups containing 50 fish each, regardless of sex. Each group was further randomized into two replicates, with 25 fish per replicate in 100-L capacity glass aquaria. Fish in the first group were exposed to borehole water only [control] and fish in the second group were exposed to 0.04 mg/L of the test media, respectively. The third, fourth, fifth and sixth groups were treated with 0.06, 0.08, 0.10 and 0.12 mg/L of the test media, respectively. The experiment was conducted within six weeks in a static renewal bioassay system in which the water and the effluent were changed daily to maintain constant effluent concentration. The test concentrations of the pharmaceutical effluent were prepared by dilution from the stock. The dose schedule selected was based on presumptive investigation involving a range finding test from previous reports in literatures.

Collection of blood sample and hematological analysis

At the expiration of the experiment, blood needed for the haematological investigation was collected from anesthetized fish samples in each treatment and control stocks with MS 222 [Ethyl 3-aminobenzoate methane sulfonate salt] to minimize stress. Blood was obtained by cardiac puncture using a hypodermic heparinized syringe. The collected blood samples were transferred into small vials, which were also previously rinsed with heparin. Red blood cell (RBC) counts were estimated using a Neubauer hemocytometer, as described by Rusia et al. (1992) and Allen (1993). 0.02 mL of blood was pipetted from the blood sample and added to 4 mL of the RBC diluting fluid [Toisson's solution], in a clean test tube to make a 1:20 dilution of the blood sample (Clara et al., 2004). The diluted blood sample was loaded onto a Neubauer counting chamber, and all RBCs in the five groups of 16 small squares in the central area of the Neubauer chamber was counted using a light microscope at 40X objective. The number of cells counted for each sample was multiplied by 10000 to obtain the RBC count per microliter of blood. Hematocrit [packed cell volume (PCV)] was determined using the microhematocrit method of Nelson and Morris (1989), in which the capillary tubes were filled with blood and centrifuged for 5 min at 14000× *g* using a microhematocrit centrifuge (Hawkesley & Sons, Ltd, Lancing, UK) at room temperature. Soon after centrifuging, the hematocrit was read using the microhematocrit reader.

The result was expressed as the percentage of whole blood. Hemoglobin determination was done using the cyanmethemoglobin method (Blaxhall and Daisley, 1973). About 0.02 ml of blood was

Table 1. Physico-chemical characteristics of the pharmaceutical effluent used.

Parameter	Effluent value	Standard value
Dissolved Oxygen (mg/L)	0.83±0.03	6.0
pH	5.70±0.01	6.5-9.5
Total Solids (mg/L)	318.67±8.82	<1000
BOD (mg/L)	127.00±1.53	50
COD (mg/L)	241.33±9,91	ND
Alkalinity	3.83±0.08	ND
Total Hardness	72.87±0.81	<200
Conductivity (µS)	979.67±0.88	NP
SO_4^- (mg/L)	18.17±0.12	500
Cl^- (mg/L)	34.67±0.11	ND
PO_4^- (mg/L)	16.50±0.17	5.0
Lead (mg/L)	0.05±0.02	<0.01
Copper (mg/L)	0.06±0.01	<2.0
Zinc (mg/L)	0.04±1.01	<1.0
Iron (mg/L)	0.08±0.02	<1.0

ND, Not determined.

mixed with 4 mL of Drabkin's solution and this was allowed to stand for 10 min to attain full color development. Absorbance was read at 540 nm with a Unican spectrophotometer against the blank. For determination of leucocytes, 0.02 mL of blood was pipetted into a small test tube containing 0.38 mL of white blood cell (WBC) diluting fluid (Turk's solution) to make a 1:20 dilution of the blood sample. The diluted sample was loaded onto the Neubauer counting chamber, and all cells on the four corner squares were counted using a light microscope at 10X objective. The total number of WBCs was calculated in $mm^3 \times 10^4$. Aspartate aminotransferase (AST) and alanine aminotranferease (ALT) were analyzed according to the method of Reitman and Frankel (1957), alkaline phosphatase (ALP) according to Bessey et al. (1946) and lactate dehydrogenase (LDH), according to the method of Wahua (1999).

Statistical analysis

The data obtained from the results of all the analysis were subjected to statistical analysis using SPSS 16.0 software. Data were subjected to one-way analysis of variance, Duncan multiple range test was used to determine the significant difference at the 5% probability level, and the results were expressed as the means standard error.

RESULTS AND DISCUSSION

Physico-chemical analysis of the test medium

The physico-chemical characteristics of the effluent used is shown in Table 1 and virtually all the analyzed parameters were found to exceed (WHO, 2011) standards for effluent discharge into any category of water bodies. The results of the hematological and biochemical responses of the test organism to varying concentrations of pharmaceutical effluent are show in Tables 2 and 3, respectively, while the activities of some selected serum enzymes are shown in Table 4. Most of the physico-chemical attributes of the effluent used showed deviations from the WHO (2011) of the permissible level of physical, chemical and heavy metals in any categories of water. Thus, continuous discharge of the effluent into water bodies over time might lead into the bioaccumulation of metals in fish tissues and organisms in nearby water bodies.

Behavioral responses

Exposure of the test organism *C. gariepinus* to pharmaceutical effluent caused visible behavioural changes in the fish. After 60 min, their swimming activity slowed down, the test fishes felt suffocation, they tried to stay at upper water surface to gasp for air, irregular and jerky movement, and loss of body equilibrium were also pronounced. Also, they settle down at the bottom of the aquaria and those that are not able to withstand the situation died. Fishes of control group were free from such behavioural changes. This conforms to the submission of Adewoye (2010).

Hematological and biochemical response

Fishes are in direct contact with their surrounding environment and any change in the environment will be reflected as changes in their physiological processes and survival. Fishes possess shorter development time compared to mammalian species. The present study revealed an interesting pattern of response of the haematological variables in effluent exposed fish. In

Table 2. Hematological indices of *Clarias gariepinus* exposed to pharmaceutical effluent.

Parameter	0.00 (mg/L)	0.04 (mg/L)	0.06 (mg/L)	0.08 (mg/L)	0.10 (mg/L)	0.12 (mg/L)
Packed cell volume (%)	26.00±2.00	24.5±0.5	24.00±1.00	22.00±2.00	23.00±1.00	21.00±1.00
Red blood cell (µl)	3.10±0.10	2.95±0.25	2.85±0.05	3.15±0.05	1.10±0.10	1.00±0.10
White blood cell (µl)	1270±0.10	2200±0.35	3000±0.05	9200±0.01	10000±0.10	8700±0.10
Hemoglobin (g/dl)	7.29±0.65	5.21±2.22	5.01±0.01	4.75±1.10	4.33±1.11	4.01±1.19
Granulocyte (%)	37.00±2.01	27.50±2.50	30.5±2.50	32.00±2.00	28.50±0.50	27.50±1.50
Leucocyte (%)	70.00±2.11	72.50±2.50	69.50±2.50	58.00±2.00	71.51±0.55	72.50±1.35

Table 3. Biochemical indices of *Clarias gariepinus* exposed to pharmaceutical effluent.

Parameter (mg/L)	Total protein	Glucose	Cholesterol	Albumin	Chloride	Potassium	Calcium
Control	35.00±3.00	86.10±4.00	100.0±2.21	16.00±0.01	94.50±2.50	3.60±0.02	2.35±0.05
0.04	32.51±1.50	79.00±1.00	108.0±2.01	19.50±1.50	91.00±0.01	3.40±0.20	2.10±0.20
0.06	31.00±3.00	73.00±1.00	115.0±0.09	19.00±1.00	88.50±1.50	3.35±0.05	2.00±0.15
0.08	33.00±2.00	74.00±0.01	112.0±0.04	23.50±0.05	89.50±0.01	3.15±0.05	2.21±0.02
0.10	27.00±1.00	61.11±2.00	126.1±5.50	21.55±1.45	77.91±2.11	3.11±0.01	1.21±0.01
0.12	25.15±2.31	55.01±2.22	119.0±13.29	27.01±1.56	75.55±1.00	3.00±0.07	1.11±0.02

Table 4. Response of serum enzyme of *Clarias gariepinus* to varying concentrations of pharmaceutical effluent.

Parameter (µkat^{1})	0.00 (mg/L)	0.04 (mg/L)	0.06 (mg/L)	0.08 (mg/L)	0.10 (mg/L)	0.12 (mg/L)
Alanine aminotransferase	0.45±0.10	0.39±0.51	0.33±0.01	0.42±0.11	0.46±0.05	0.49±1.09
Aspartate aminotransferase	3.99±0.11	2.12±0.15	2.21±0.55	2.01±1.15	2.00±0.00	1.74±1.13
Alkaline phosphate	1270±0.10	2200±0.35	3000±0.05	9200±0.01	10000±0.10	8700±0.10
Lactate dehydrogenase	15.21±1.23	11.39±2.05	12.46±0.19	10.25±1.21	8.20±2.00	9.22±4.65

addition, different concentrations resulted in an anemic condition in fish, as shown from decreased RBC, PCV and hemoglobin (Hb) values as effluent concentration increases. From the present results, reduction in RBC count, PCV and Hb concentrations of tested fish compared to control may be due partly to the presence of heavy metals and other pollutants in the utilized effluent and the toxicity of this effluent may cause RBC lysis. Previous studies had shown a decrease in RBC counts, relative PCV and Hb values when fish were exposed to cassava effluent (Adekunle et al., 2007), diazinon (Svoboda et al., 2001), textile dyes (Al-sabti, 2000) and soap and detergent effluent (Ogundiran et al., 2007). These findings are in agreement with the present results. On the contrary, an increase in RBC, Hb and Ht values were recorded in African catfish on exposure to Gold crew (Alagoaa et al., 2009), to copper (Mazon et al., 2002) and in Nile Tilapia and catfish exposed to lead (Al-Akela et al., 2000). Deformed RBCs were detected on exposure to cadmium (Witeska et al., 2006) and environmental pollution (Pacheco and Santos 2002). Moreover, marked reduction in the value of hematological indices as documented in this work may also be attributed to a reduction in the level of cellular iron resulting in reduced oxygen carrying capacities of the blood which eventually stimulates erythrocytic degradation.

The concentration of hemoglobin decreased significantly ($p< 0.05$) in the blood of fish exposed to the pharmaceutical effluent. Heavy metals have been reported to alter the properties of haemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable (Witeska et al., 2006; Ogundiran et al., 2007; Vinodhini and Narayanan 2009) that probably results in cell swelling deformation and damage observed. It is evidently shown that cadmium influences the differential blood count (Gill et al., 1993). The results are in good concurrence with earlier works (Vinodhini and Narayanan 2009; Vutkuru 2005) that reported a significant decrease in RBC's hemoglobin and packed cell volume of fresh water fish exposed to heavy metals. The perturbation in these blood indices may be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis. The

related decreases in hematological indices implicate the toxic effect of the pharmaceutical wastewater that affects both metabolic and hematopoietic activities of *C. gariepinus*. Exposure to high concentration of the toxicant affected feeding behavior of *C. gariepinus*, with fish exposed to 14.1589 m/L effluent consuming less than 80.0% of the food supplied. And consequently, the observed dose-based reduction in haematological parameters in *C. gariepinus* in this study therefore conforms to the report of Tacon (1993) and Osuigwe et al., (2005) that nutritionally deficient diets cause decrease in haemaglobin concentration, reduced haematocrit and red blood cell count. Physiologically, haemoglobin is crucial to the survival of fish, being directly related to the oxygen binding capacity of blood. However, the reduction observed in this study may not have had a deleterious/lethal effect on *C. gariepinus,* given that the values are within the normal range recorded for African catfish (Musa and Omoregie 1999; Ayandiran et al., 2010; Yekeen and Fawole 2011). The present haematological data provides valuable information in assessing the health of *C. gariepinus* and in monitoring stress responses to the metal wastewater.

Glucose and total protein concentrations in blood of the *C. gariepinus* exposed to different sublethal concentrations of pharmaceutical effluent showed that there was a significant decrease as the concentration of the effluent increases (Table 2). The glucose concentration in animals exposed to sub-lethal concentrations of pharmaceutical effluent was significantly different ($p<0.05$) from the concentrations in control animals. Albumin concentrations in blood of the *C. gariepinus* exposed-fish showed that there was a slight significant ($p<0.05$) increase in the concentrations in the exposed animals as compared to the controls. The blood electrolyte [Sodium, Potassium and Calcium] recorded a significant ($p<0.05$) decrease in their respective values as the concentration of the effluent increases. All the anomalies recorded in the biochemical response of pharmaceutical effluent-exposed *C. gariepinus* may be stress due to the potency of the effluent-used.

The activities of serum enzymes (AST, ALT, ALP and LDH) were significantly ($p<0.05$) higher in the exposed *C. gariepinus* compared to the control. The significant ($p<0.05$) decrease in the activities of serum enzymes [AST, ALT and ALP] in the exposed fish compared to the control stock may have resulted from cellular damage in these fish, which might have arisen from the potency of the pharmaceutical effluent. This observation was similar to what Ozgur et al., (2011) reported where they observed significant increase ($p<0.05$) in serum enzyme activities of Nile tilapia *Oreochromis niloticus* exposed to a pesticide cypermetrin and two metals copper and lead. Serum activities also increased significantly in African catfish when Nkpondion et al. (2016) exposed the fishes to detergent. This is because serum enzymes are cytoplasmic in nature and are only released into blood circulation after cellular damage (Nkpondion et al., 2016).

Conclusions

The effluent-water levels had negative direct or indirect effects on the haematological and biochemical parameters of *C. gariepinus*. The blood parameters of *C. gariepinus* not only revealed cellular disturbances but also adaptive responses. Increase in leukocytes implies a mobilization of cell defense, although the reduction of the small lymphocytes percentage suggests a secondary effect of effluent. On the other hand, the decrease in red blood cell parameters [RBC, Hb and PCV] indicates an aneamic condition which gradually progresses upon prolonged exposure eventually cause hypochromic macrocytic anemia attributed to the swelling of the red blood cells, haemodilution and impaired haemoglobin synthesis. The increase in developing haemocytoplast and myelocytes emphasizes the compensatory and defensive reaction of fish to pollution. Contamination of aquatic environment by heavy metals whether as a consequence of acute or chronic events constitutes additional source of stress for aquatic organisms. Toxicants and pollutants can result in several physiological dysfunctions in fish which could induce changes in blood parameters. Therefore, the reduction and increase in these blood parameters are indication of hyperglycaemia and hypoproteinemia caused by exposure to the phostoxin concentrations. Generally, the effects of the pharmaceutical effluent were more pronounced in serum enzymes activities than in the haematological responses of the exposed *C. gariepinus*. However, significant ($p<0.05$) increase in serum enzymes and WBC counts of the wild *C. gariepinus* were indicative of cellular damages in exposed fishes. Therefore, indiscriminate consumption of fish most especially from a polluted environment should be discouraged.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to appreciate the effort and the contributions of the Laboratory and Technical staffs of the Fisheries and Hydrobiology Unit of Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria in the course of this research work.

REFERENCES

Adekunle IM, Arowolo TA, Omoniyi IT, Olubambi OT (2007). Risk assessment of Nile tilapia (*Oreochromis niloticus*) and African mud catfish (*Clarias gariepinus*) exposed to cassava effluent. Chemistry and Ecology 23:383-392.

Adewoye SO (2010). Haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract. Advances in Applied Science Research 1(1):7479.

Alagoaa KJ, Ekweozorb IKE (2009). Sublethal effect of the dispersant Goldcrew on selected blood parameters of the African cat-fish *Clarias gariepinus*. Toxicological and Environmental Chemistry 91(2):339-343.

Al-Akela AS, Shamsia MJK (2000). A comparative study of the toxicity of lead and its impact on the carbohydrate metabolism and some haematological parameters of cichlid fish, *Oreochromis niloticus* and catfish, *Clarias gariepinus* from Saudi Arabia. Toxicological and Environmental Chemistry 74(1):19-28.

Allen P (1993). Effect of acute exposure to cadmium II chloride and lead II chloride on the haematological profile of *Oreochomis aureus* (Steindachner). Comparative Biochemistry and Physiology Part C Comparative Pharmacology 105(2):213-217.

Al-Sabti K (2000). Chlorotriazine reactive azo red 120 textile dye induces micronuclei in fish. Ecotoxicology and Environmental Safety 479(2):149-155.

American Public Health Association, Washington, DC APHA (2012). Standard methods for examination of water and wastewater, 22nd edition.

Ayandiran TA, Fawole OO, Adewoye SO, Ogundiran MA (2010). Bioconcentration of metals in the head capsule and skeleton of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluents. Agriculture and Biology Journal of North America 1(5):796-802.

Benotti MJ, Brownawell BJ (2009). Microbial degradation of pharmaceuticals in estuarine and coastal seawater. Environmental Pollution 157(3):994-1002.

Blaxhall PC, Daisley KW (1973). Routine haematological methods for use with fish blood. Journal of Fish Biology 5:771-781.

Bruce GM, Bruce RC, Pleus SA, Snyder SA (2010). Toxicological relevance of pharmaceuticals in drinking water Environmental Science & Technology 44(14):5619-5626.

Clara M, Strenn B, Kreuzinger V (2004). Carbamazepine as a possible anthropogentic marker in the aquatic environment: investigations on the behavior of carbamazepine in wastewater treatment and during groundwater infiltration. Water Research 38(4):947-954.

Gill TS, Epple A (1993). Stress related changes in the hematological profile of the American eel (*Anguilla rostrata*). Ecotoxicology and Environmental Safety 25(2):227–235.

Glassmeyer ST, Glassmeyer ET, Furlong DW, Kolpin JD, Cahill SD, Zaugg SL, Werner E (2005). Transport of chemical and microbial contaminants from known wastewater discharge: potential for use as indicators of human fecal contamination. Environmental Science & Technology 39(14):5157-5169.

Larsson DGJ, de Pedro C, Paxeus N (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals Journal of Hazardous Materials 148(3):751-755.

Mazon AF, Monteiro EAS, Pinheiro GHD, Fernandes MN (2002). Hematological and physiological changes induced by short-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. Brazilian Journal of Biology 62(4a): 621-631.

Musa SO, Omoregie E (1999). Haematological changes in the mudfish, *Clarias gariepinus* (Burchell) exposed tomalachite green. Journal of Aquatic Sciences 14(1):37-42.

Nelson DA, Morris MW (1989). Basic methodology. Haematology and coagulation; part IV. In: Nelson DA, Henry JB (eds.), Clinical diagnosis and management by laboratory methods, 17th edn.Philadelphia: WB Saunder pp. 578-625.

Nkpondion NN, Ugwumba OA, Esenowo IK (2016). The toxicity effect of detergent on enzymatic and protein activities of African mud Catfish (*Clarias gariepinus*). Journal of Environmental and Analytical Toxicology 6(3):1-5.

Ogundiran MA, Fawole OO Adewoye SO (2007). Effects of Soap and detergent effluents on the haematological indices and biochemical profiles of *Clarias gariepinus.* Science Focus 12(1):84-88.

Osuigwe DI, Obiekezie IA Onuoha GC (2005). Some haematological changes in hybrid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal. African Journal of Biotechnology 4(9):1017-1021.

Ozgur F, Hikmet Y, Cogun Tuzin A, Yuzerer O, Gulbin G, Ozge F, Ferit K, Yasemin K (2011). A comparative study on the effect of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Orechromis niloticus.* Fish Physiology and Biochemistry 37(3):657-666.

Pacheco M, Santos MA (2002). Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (*Anguilla anguilla* L.). Ecotoxicology and Environmental Safety 53(3):331-347.

Reish DL, Oshida OS (1987). Manual of Methods in aquatic environment research. Part 10. Short-term static bioassays . FAO Fisheries Technical Paper No 247. Rome. 62 p.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 28(1):56-63.

Rusia V, Sood SK, Kanai L Mucerjee (1992). Routine hematological tests. In: medical laboratory technology (Ed.), Vol.I. Fifth reprint. Tata McGraw Hill Publishing Company Limited, New Delhi pp. 252-258. www.abebooks.com

Svoboda M, Luskova V, Drastichova J, Zlabek V (2001). The effect of diazinon on haematological indices of common carp (*Cyprinus carpio* L.). Acta Veterinaria Brno 70:457-465.

Tacon AGJ (1993). Nutritional fish pathology:morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Tech. Paper No. 330. Rome, FAO.

Vinodhini R, Narayanan M (2009). The Impact of toxic heavy metals on the hematological parameters in common Carp (*Cyprinus carpio* L.). Iranian Journal of Environmental Health Science and Engineering 6(1):23-28.

Vutkuru SS (2005). Acute effects of Hexavalent chromium on survival, oxygen consumption, Hematological parameters and some biochemical profiles of the Indian Major Carp, Labeo rohita. International Journal of Environmental Research and Public Health. 2(3-4):456-462.

Wahua TA (1999). Applied statistics for scientific studies. Afrika Link Books, Aba, Nigeria P 365.

Ward GS, Parrish PR (1982). Manual of Methods in Aquatic Environmental research. Part 6. Toxicity Tests . FAO Fisheries Technical Paper, No 185 FIRI/T185.

World Health Organization (WHO) (2011) Guideline for Drinking Water Quality. 4th edn., NLM Classification: WA 675, World Health Organization, Geneva, Switzerland pp. 307-433, ISBN: 978 924 1548151.

Witeska M, Jezierska B, Wolnicki J (2006). Respiratory and hematological response of tench, *Tinca tinca* (L.) to a short-term cadmium exposure. Aquaculture International 14(1-2):141-152.

Yekeen TA, Fawole OO (2011). Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus*. African Journal of Biotechnology 10(64):14090-14096.