COMPARATIVE STUDY OF HEMATOLOGICAL AND HISTOPATHOLOGICAL ALTERATIONS IN *Oreochromis niloticus* AND *Clarias gariepinus* EXPOSED TO INDUSTRIAL WASTE EFFLUENTS.

Abstract

In recent decades the level of foreign compounds in aquatic ecosystems such as heavy metals, pesticides and other persistent organic pollutants has increased alarmingly as a result of domestic, industrial and agricultural effluent. This research therefore examined the histopathological and haematological effects of industrial effluent on *Orechromis niloticus* and *Clarias gariepinus*. The histopathology and haematology parameters of the fishes exposed to graded doses of the effluent were assessed using conventional techniques. The results of the haematological assessment of the fishes shows a steady and significant ($P < 0.05$) reduction in the PCV of the tilapia from 23 % (control) to 10 % and 25 % (control) to 18 % in catfish exposed to 6.25 ml/L of the effluent, the RBC count reduced with increase in the concentration of the effluent from 2.60 x 10^6/L (control) to 1.15 x 10^6/L (tilapia) and from 2.80 to 1.72 x 10^6/L (catfish) in the sample exposed to 6.25 ml/L of the effluent. Also, the values of Hb concentration reduced from 7.73 g/dL to 3.10 g/dL (tilapia) and from 8.33 to 5.17 g/dL (catfish). However, the WBC count increased from 4800.33 x 10^3/L to 9500.30 x 10^3/L (tilapia) and from 5300.30 x 10^3/L to 7800.50 x 10^3/L (catfish) in those exposed to 6.25 ml/L of the effluent. In the histological assessment of the gills of the fishes, the tilapia fish exposed to 6.25ml/L of the effluent recorded an extensive damage, distortion and thickening of the tilapia fish gill lamellae. Also, there was loss of secondary lamellae and loss of gill raker filaments of the catfish. The liver cells of tilapia had many monocytes (kuppfer cells) while there were mild increase in the number of kuppfer cells of the catfish. The kidney artery of tilapia was filled with leucocytes while there were cellular proliferation (hyperplasia) and less of melanin deposits in the kiney cells of catfish. These are indications that the industrial effluent contain toxicants that are dangerous to the fish health.

Keywords: Industrial effluents, tilapia, catfish, toxicant, haematology, histopathology

INTRODUCTION

Increasing contamination of the aquatic environment worldwide has been associated with improper discharge of solid wastes, industrial, medical and agricultural effluents (Larsson, 2014; Alimba *et al.*, 2017), due to the deteriorating effects on surface, underground water and sediment qualities of most aquatic environment, and hence the deleterious health effects on biotic communities (Chigor *et al.*, 2012; Othman *et al.*, 2012). It has been reported that in recent decades the level of foreign compounds in aquatic ecosystems such as heavy metals, pesticides and other persistent organic pollutants has increased alarmingly as a result of domestic, industrial and agricultural effluents (Pereira *et al.*, 2013). Heavy metals occur naturally in the environment and are found in varying levels in the ground and surface water. Anthropogenic activities do, however, cause an increased discharge of these metals into natural aquatic ecosystems.

Blood characteristics are effective and sensitive indices for monitoring physiological changes in fishes. Analysis of blood indices has proven to be a valuable approach for examination of the health status of farmed fish, providing reliable information on metabolic disorders (Bahmani *et al.*, 2001) and becoming a basic part of fish health monitoring programmes (Jawad *et al.*, 2004; Animashahun *et al* ., 2006)

Blood parameters are considered good physiological biomarkers of the whole body and, therefore, they are important in diagnosing the structural and functional status of fish exposed to environmental pollutants (Seriani, 2011). Haematological parameters have become promising biomarkers for measuring the impacts of water pollution in fish, because blood variables respond to low doses of pollutants (Osman, A. 2018).

Histological study appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the liver. Exposure to heavy metals may cause histological changes in the liver, (Van der Oost *et al.*, 2003). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, including gills, kidney and liver, that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer *et al.*, 2001). The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost *et al.*, 2003) it is also one of the organs most affected by contaminants in the water.

This research therefore examined the histopathological and haematological effects of industrial effluent on *Orechromis niloticus* and *Clarias gariepinus* and it will serve as baseline information for the application of the toxicant in the aquatic environment

2. MATERIALS AND METHODS

Collection of Effluent

The Paint industrial effluents used in this study was obtained at point source of the drainage pipe of a Factory in Lagos. The samples were collected in sterile 1 L containers in the mid-stream flow of the effluent by inclining the container at an angle to allow water flow into it; and taken to the laboratory.

Set up and Pollution of Aquaria

A total of 60 juvenile fish of both catfish and tilapia fish of both sexes were used. The average weight of the fish was 5.3±0.1 g The experiments were conducted in aerated glass aquaria of (120 x 40 x 30 cm) enable easy observation of test organisms. The fishes were then exposed to the industrial effluent at sub-lethal concentrations of 0.00 (control), 1.25mls/l, 2.50mls/l, 3.75mls/l, 5.0mls/l and 6.25mls/l for 96hours and allowing one hour for acclimation to laboratory conditions. The completely randomized design was adopted for the study and each experiment was set up in triplicates (Svobodova *et al.*, 1991)

Haematological Parameters Analysis

Th blood samples were collected and analysed using standard methods described by Ebunlomo et al. (2012). Whole blood was used for the estimation of red blood cell (RBC) counts and haemoglobin (Hb) and haematocrit (Hct) concentrations by using an automated technical analyser (Celltac α MEK- 6400J/K). White blood cell (WBC) counts and WBC differentials were calculated according to the methods of Alimba *et al.* (2017). Neutrophils, lymphocytes, eosinophils and monocytes were counted using an Olympus oil immersion light microscope at 1000× magnification (Ghosh, 1984).

Histopathogical Assessment

The fixed sections of the gills, liver and kidney from the treated and control fish were dehydrated by passing through ascending order of ethyl alcohol–water concentrations, cleared in xylene and embedded in paraffin wax using rotary microtome. Four μm thick sections of the tissues were prepared on slides, stained with Hematoxylin–Eosin (H&E) and mounted in neutral DPX medium for microscopic examination at 400 X magnification by trained pathologist.

Data Analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SSPS window 7 version 25.0 software. The significance was determined at the level of $p \leq 0.05$.

RESULTS

Haematological Parameters of the fishes exposed to industrial effluent

The results of the haematological assessment of the fishes exposed to graded dose of the industrial effluent is presented in Table 1 and Table 2. Table 1 shows a steady and significant ($P < 0.05$) reduction in the packed cell volume of the experimental animals from 23 % in the control to 10 % in the sample exposed to 6.25 ml/L of the effluent, the red blood cell count reduced with increase in the concentration of the effluent from 2.60 x 10^6/L (control) to 1.15 x 10^6/L in the sample exposed to 6.25 ml/L of the effluent. Also, the values of haemoglobin concentration reduced from 7.73 g/dL in the control to 3.10 g/dL in the samples treated with the highest concentration of the effluent. However, the white blood cell count increased from 4800.33 x 10^3/L in the control to 9500.30 x 10^3/L in those exposed to 6.25 ml/L of the effluent.

The haematological parameter assessment of catfish exposed to the industrial effluent follow a similar trend to that of tilapia fish. As the concentration of the effluent increased, there were decreases in the values of red blood cells (2.80 to 1.72 x 10^6/L), packed cell volume (25 to 18 %) and haemoglobin concentration (8.33 to 5.17 g/dL). Also, there was an increase in the white blood cell count from 5300.30 x 103/L (control) to 7800.50 x 10^3/L in the sample exposed to the highest concentration of the effluent.

Table 1: Haematological parameters of Tilapia fish exposed to industrial effluent

TREATMENT	HB (g/dL)	PCV (%)	WBC ($x10^3$/L)	RBC ($x10^6$/L)	Neu (%)	Leu (%)	Mono (%)	Eos (%)
CONTROL	7.73±0.10^c	23	4800.33±10.05^a	2.60±0.07^d	58	42	-	-
TR1	4.33±0.05^b	15	8200.50±8.00^{bc}	1.50±0.58^{bc}	70	26	3	1
TR2	4.75±0.03^b	14	8400.67±15.06^b	1.60±0.10^c	66	31	3	-
TR3	4.63±0.00^b	14	8500.00±11.30^b	1.65±0.20^c	68	32	-	-
TR4	3.43±0.02^a	13	8900.00±10.40^b	1.40±0.15^b	72	26	2	-
TR5	3.10±0.02^a	10	9500.30±12.50^d	1.15±0.08^a	60	40	-	-

Key: HB: Haemoglobin, PCV: Packed cell volume, WBC: White blood cell, RBC: Red blood cell, TR1= 1.25mls/l, TR2= 2.50mls/l, TR3= 3.75mls/l, TR4= 5.0mls/l and TR5= 6.25mls/l.

Table 2: Haematological parameters of catfish exposed to industrial effluent

TREATMENT	HB (g/dL)	PCV (%)	WBC ($x10^3$/L)	RBC ($x10^6$/L)	Neu (%)	Leu (%)	Mono (%)	Eos (%)
CONTROL	8.33±0.03^c	25	5300.30±20.30^a	2.80±0.00^c	54	44	2	-
TR1	6.33±0.00^b	19	6100.00±15.00^b	2.15±0.05^b	60	38	2	-
TR2	6.00±0.04^b	18	6100.00±22.50^b	2.05±0.10^b	59	41	-	-
TR3	5.33±0.03^a	16	7600.33±31.05^c	1.80±0.02^a	69	30	-	-
TR4	5.75±0.03^{ab}	17	7800.50±18.40^c	1.90±0.10^a	66	33	1	-
TR5	5.17±0.02^a	18	7800.50±25.00^c	1.72±0.10^a	63	35	2	-

Key: HB: Haemoglobin, PCV: Packed cell volume, WBC: White blood cell, RBC: Red blood cell, TR1= 1.25mls/l, TR2= 2.50mls/l, TR3= 3.75mls/l, TR4= 5.0mls/l and TR5= 6.25mls/l.

Histopathological Parameters of the major organs of fishes exposed to industrial effluent

The histological assessment of the gills of the fishes is presented in plate 1A-D, plate 1A and 1C shows the normal gill rakers of tilapia fish and catfish respectively. There were long gill rakers without detachment. However, in the tilapia fish exposed to 6.25ml/L of the effluent, there was an extensive damage, distortion and thickening of the tilapia fish gill lamellae. Also, there was loss of secondary lamellae and loss of gill raker filaments of the catfish.

The histological changes in the liver of the fishes are shown in plate 2A-D, plate 2A and 2C revealed normal hepatic cells of the tilapia and catfish respectively where no apparent damages were observed. Albeit, the liver cells of tilapia exposed to 6.25 ml/L concentration of the effluent had many monocytes (kuppfer cells) while there were mild increase in the number of kuppfer cells of the catfish exposed to similar treatment.

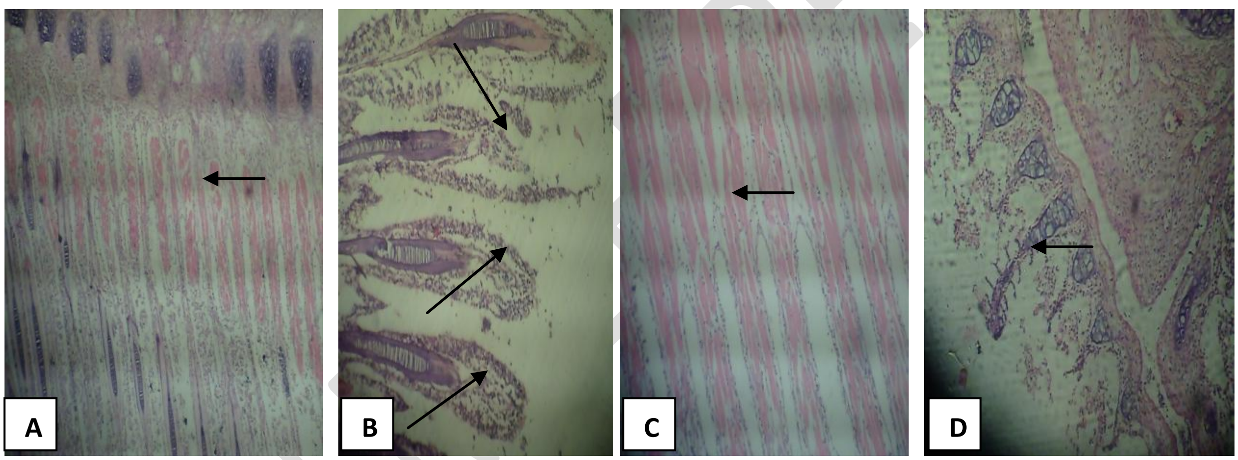

Plate 1: Showing the histopathology effect of industrial effluent on gills of fish

Key: A (control): Normal gill of tilapia with long gill raker and no detachment,
B (6.25 ml/L): Extensive damage, distortion and thickening of the tilapia fish gill lamellae
C (control): Normal gill cartilages of catfish
D (6.25 ml/L): Loss of secondary lamellae and loss of gill raker filament of catfish

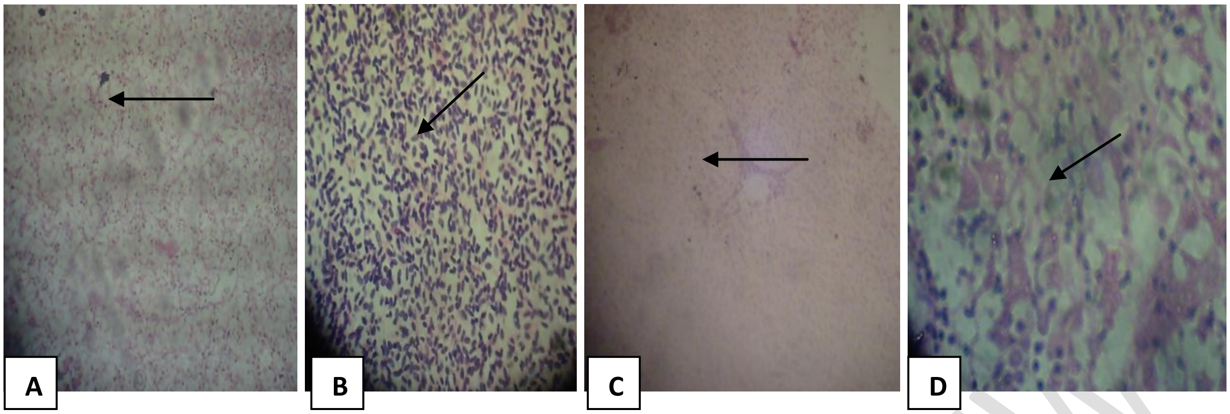

Plate 2: Showing the histopathology effect of industrial effluent on gills of fish

Key: A (control): Normal liver cells of tilapia fish
B (6.25 ml/L): Hepatocytes filled with many monocytes (kuppfer cells) of tilapia fish
C (control): Normal hepatic cell of catfish
D (6.25 ml/L): Mild increased in the number of kuppfer cells of catfish

The histological changes in the kidney of the fishes are given in plate 3A-D, plate 3A and 3C revealed normal kidney with few melanocytes and distinct cells of tilapia fish and catfish respectively. But, the kidney artery of tilapia exposed to 6.25 ml/L concentration of the effluent was filled with leucocytes while there were cellular proliferation (hyperplasia) and less of melanin deposits in the kiney cells of catfish.

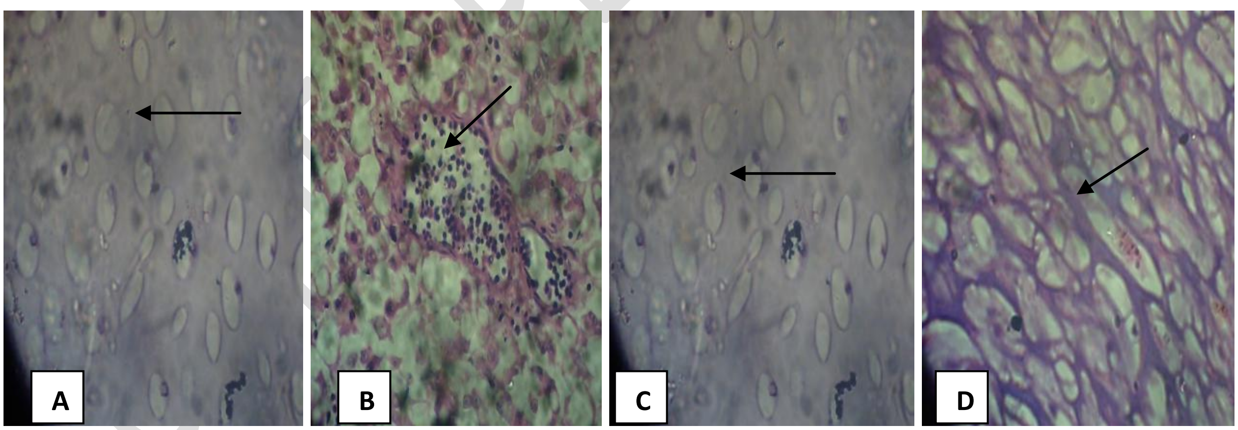

Plate 3: showing the histopathology effect of industrial effluent on kidney of fish

Key: A (control): Normal kidney with few melanocytes and distinct cells of tilapia fish
B (6.25 ml/L): Renal artery filled with leucocytes tilapia fish
C (control): Normal kidney with few melanocytes and distinct cells of catfish
D (6.25 ml/L): The kidney cellular proliferation (hyperplasia), less of melanin deposits of catfish

DISCUSSION

Haematology and histopathology are clinical tools used to detect specific changes in the health status of animals particularly to assess biological tissue damages or changes. Histopathological assays are used all over the world as investigative tools in forensics, autopsy, diagnosis of pathological changes that may have been caused by diseases, infectious agents and/or exposure to pollutants (Alimba *et al.*, 2017). A chief concern in marine food supply is fish contamination from industrial effluent which may contain toxic substances that is capable of accumulating in the body of animals in the aquatic environment. The toxicants in the effluent are usually absorbed into the blood and then bioaccumulate in issues and different internal organs, thereby causing damages that hampers the health of the fish (Larsson, 2014). These pathology changes are used as benchmarks to assess the quality and safety of the aquatic habitat.

The result of the present study which showed that there was a great decline in the values of PCV, RBC and Hb is in line with the reported effect of toxicant on blood parameters in freshwater fish *Clarias batrachus* by Joshi *et al.* (2002). Haemoglobin and parked cell volume (PCV) have been suggested as tests that can be carried out on routine basis in fish farming as a check on health status (Aderemi *et al.*, 2004). The increase observed in the WBC count of the two species of fish corroborates the findings of Joshi *et al.* (2002) who stated that survival of fish can be correlated with increase in antibody production which helps in the survival and recovery. The reduction that was observed in the PCV, Hb and RBC in the fishes exposed to the effluent is indicative of blood loss from the fishes.

Haematological characteristics have been widely used in clinical diagnosis of diseases and pathologies ofhuman and domestic animals. The applications of haematological techniques have proved valuable for fishery biologists in assessing the health of fish and monitoring stress response. Some of the values were significantly lower in the experimental fishes which may be due to the toxicants present in the effluent. It has been reported that during a stress situation, RBC count is one of the first parameters that is affected (Gernhofer *et al.*, 2001).

Increase in WBC observed in both tilapia and catfish exposed to the industrial effluent may be ascribed to boost in the formation of leucocytes in the blood cell producing tissues of the fish. These cells function in the production of antibodies which are the first line defense of the animal against infectious agents.

The decline in PCV, RBC and haemoglobin values in the two fishes observed in this study is in accord with the reports of Joshi *et al.* (2002) in *Clarias batrachus* treated with various concentrations of toxic substances ranging from 1 mg/ml to 10 mg/ml. Sampathy *et al.* (1998) also reported a lowering of some haematological indices of *Oreochromis mossambrius* exposed to copper and zinc contamination. According to Joshi *et al.* (2002), the low haemoglobin level might lead to reduction in the ability of fish to carryout cellular respiration effectively which may eventually lead to death of the fish.

It was observed that all the parameters evaluated were significantly different between the two species of fish. The Hb, PCV and RBC were significantly reduced in the tilapia fish compared with that of catfish and also, the WBC were higher in the tilapia compared with that of the catfish despite exposing them to the same level of effluent concentrations. This suggests that tilapia may be more sensitive to contaminants than the catfish. They may therefore be used in monitoring water pollution.

It is generally understood that if there is lowering of red blood cell count and concentration of haemoglobin will ultimately lead to a deficiency of oxygen required by the fish for cellular respiration. Changes in the key blood indices may be connected with the type and species of fish as well as the toxicants they are exposed to (Ololade and Ogini, 2010).

The observations in this study is in line with earlier observation of Akinrotimi *et al.* (2011) who studied acute toxicity of atrazine in *Sarotherodon melanotheron* and concluded that the elevation of white blood cells in the fish is indicative of immunological reaction to the atrazine. Results from this study revealed that the red blood cells and packed cell volume of the test animals were lower at higher concentrations of the effluent, this is in consonance with the submission of Oriakpono *et al.* (2012) who studied tha response of *Sarotherodon melanotheron* to crude oil contamination and concluded that the lowering of different blood indices may have been due to faulty haematopoietic system of the fish that may have been occasioned by the exposure to the toxicant. The decline in the packed cell volume of the fish suggests there's deterioration of the fish health and the emergence of anaemia.

In the histopathological assessment, the gills, liver and kidneys of the fishes exposed to the industrial effluent showed significant distortions compared with those of controls. The extensive damage, distortion and thickening of the tilapia fish gill lamellae as well as loss of secondary lamellae and gill raker filaments of the catfish suggests that the effluent contain highly toxic substances which may have deleterious effects on this most important organ in fishes. Gill is a complex organ in both morphology and physiology with multifunction in osmoregulation, exchange site for the gas and detoxification. The capacity of the gill to carry out these functions may be greatly hampered by the presence of the effluent in the water body.

This result is in line with earlier report that lamellar fusion frequently occur in fish secondary lamellae due to toxicants. Some earlier studies reported that, some abnormalities in secondary lamellar occurred as clubbed tips and slight curvature of lamellae indicated that the fish was in stress condition.

According to Camargo and Martinez (2007), fish livers usually suffer physical changes when exposed to toxic substances probably due to its function as the seat of detoxification in the animal body. Hepatic fatty degeneration evidenced by buildup of kuppfer cels within the liver cells are frequent reactions of fishes to exposure to toxic substances (Agamy, 2012). These types of changes are generally connected with inhibition of protein synthesis, energy exhaustion and/ or a change in food usage. Also, earlier researches suggests that in Tilapia, several functions controlled by pulmonary and renal processes in higher animals are carried out by the gills. Thus, the gill and liver of the fish are vital organs appropriate for histopathology assessment of damages in polluted aquatic environment.

Conclusion

Based on the results obtained in this research, it is evident that the industrial effluent contains certain toxic substances that elicit various responses in the two fishes. The red blood cells, packed cell volume and haemoglobin levels were significantly reduced while the white blood cell count spiked in both species of fish. There were also indications that the liver, kidneys and gills of the fishes were negatively affected as shown by the histopathology results. Comparatively, Tilapia species were more sensitive to the toxicants in the industrial effluent than catfish. Therefore, tilapia may be used for monitoring aquatic environment pollution.

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