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The effect of domestic effluent on growth and haematological parameters of *Clarias gariepinus* (Burchell, 1822) (Pisces; Clariidae)

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Resumen

Efecto de los efluentes domésticos en el crecimiento y los parámetros hematológicos de Clarias gariepinus (Burchell, 1822) (Piscis; Clariidae)

Esta investigación determina el efecto toxicológico de efluentes domésticos en los parámetros sanguíneos de *Clarias gariepinus*. Se realizaron cuatro tratamientos: Control, 30, 50 y 70 mlL^{-1}. Cada tratamiento fue por triplicado. Se produjeron cambios de comportamiento en los peces expuestos a 50 y 70 mlL^{-1} del efluente, y no se observó ninguno en los peces Control y los tratados con 30 mlL^{-1}. Hubo diferencias significativas en la ganancia media de peso y longitud entre los peces expuestos al tratamiento y el control. Hubo una reducción significativa ($p<0.05$) de los glóbulos rojos. Los glóbulos blancos, el hematocrito, el volumen corpuscular medio y la hemoglobina de los peces expuestos fueron significativamente más altos que en el control ($p<0,05$). Los resultados de este estudio sugieren que los efluentes domésticos pueden afectar negativamente el crecimiento y la hematología de los peces.

Palabras clave: *Clarias gariepinus*; Parámetros hematológicos; Efluentes; Crecimiento.

Abstract

This research determines the toxicological effect of domestic effluents on blood parameters of *Clarias gariepinus*. Four treatment were set up: Control, 30, 50 and 70mlL^{-1} . Each treatment was done in triplicate. There were behavioral changes in fishes exposed to 50 and 70 mlL^{-1} of the effluent while none was observed in Control fishes and fishes undergone 30 mlL^{-1}. There were significant differences in the Mean Weight and Mean Length Gain between the fishes exposed to treatment and Control fishes. There was significant reduction in the red blood cells ($p<0.05$). White blood cells, hematocrit, mean corpuscular volume and haemoglobin in the fishes exposed were significantly ($p<0.05$) higher than Control. Results from this study suggests that domestic effluents can negatively affect the growth and haematology of fishes.

Key words: *Clarias gariepinus*; Haematological parameters; Effluents; Growth.

Introduction

Clarias gariepinus Burchell, 1822) is one of the most highly valued freshwater fishes of Africa, and among the most highly prized food fishes of West Africa (Emokaro, 2010). *C. gariepinus* fish had been used in fundamental research work for improvement of science of fisheries (Nguyen *et al.* 2000). *C. gariepinus*, like any other aquatic organism, lives in direct contact with the aquatic environment where some changes are rapidly reflected as measurable patho-physiological alterations in exposed fishes (Wilson and Taylor 1993, Musa & Omoriegie 1999, Seith & Saxena 2003). *C. gariepinus* is known to tolerate harsh aquatic conditions (Hogendoorn 1992, Bruton 1979) in terms of low dissolved oxygen concentrations by utilizing both dissolved and atmospheric oxygen (Okechi 2004), especially in fishes above 12-14 days old with functionally developed accessory respiratory organs (Peteri *et al.* 1992).

Water pollution is characterised by the addition of anthropogenic contaminants to the extent that it either cannot serve humans for drinking purposes or support the biotic communities (Agrawal *et al.* 2010). A change in the quality of water by the presence of toxins/contaminants, makes it potentially harmful to life forms, instead of sustaining them (Agrawal *et al.* 2010). The entry of toxicants into aquatic media may affect the water quality parameters which in turn leads to changes in the haematological variables of fish and other aquatic lives due to close association with the external environment (Carvalho & Fernandes 2006, Kavitha *et al.* 2010). The increasing spates of water pollution have continued to be a major problem in Nigeria and other developing countries (Adelegan 2008).

The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent, and run-off from agricultural fields (Adeyemo 2005). Stress response is characterized by physiological changes and the effect of pollutants on fish is assessed by acute and chronic toxicity tests (Heath, 1991). Effluent originates as human waste from certain activities including using toilet facilities, bathing, preparing foods and washing laundry (Olojo *et al.* 2005). This includes grey water from washing machines and sinks. Sewage usually travels from a building plumbing either into sewer, which will carry it elsewhere, or into an on-site sewage facility (FAO 2007). A global estimate by United Nations is that 90% of all wastewater generated is released into the environment untreated (Corcoran *et al.* 2010). It is common for this effluent to be discharged into nearby rivers and streams. These effluents pose a serious threat to the environment and quality of life in the receiving waters. Effluent at different concentrations was found to impair the swimming pattern, skin colouration, feeding rate and general behaviour of fish which suggests that fish can tolerate low concentrations of pollutants with reduced mortality (Dahunsi *et al.* 2011). These pollutants, which have a negative effect on fish, are released by agriculture, industrial wastewater discharge, raw sewage extraction, chemical waste, and oil spills due to fishing vessels (Velusamy *et al.* 2014).

The use of haematological technique in fish culture for toxicological research, environmental monitoring and fish health conditions have grown rapidly in recent times (Gabriel *et al.* 2007a, Akinrotimi 2008, Akinrotimi *et al.* 2011). Haematological indices are of different sensitivity to various environment factors and chemical (Akinrotimi *et al.* 2013). Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in values of one or more haematological parameters of aquatic organisms (Akinrotimi *et al.* 2007, Gabriel *et al.* 2007b).

Therefore this study investigated the effect of domestic effluent on growth and haematological parameters like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and so on of *Clarias gariepinus*.

Materials and methods

Experimental fishes and collection

One hundred and fifty (N=150) healthy four weeks old, juvenile of *C. gariepinus* of the mean weight 10.1±1.2 g and length of 8.4±0.63 m was purchased from Grace Land Fish Farming Gombe State, Nigeria and transported in a container to the laboratory of Federal University of Kashere, Gombe State. The fishes were stocked in a tank containing borehole water for acclimatization for a period of 7 days. The fishes were fed twice daily (7.00 am and 6.00 pm) with Coppens feed of 3 mm of size and the ratio of feeding was changed every week by 5% of their body weight. Twelve

juveniles were randomly distributed into four different containers. Their growth was checked every week and each set-up was in triplicate. Experimental tanks were covered with 4 mm mesh size net to prevent them from jumping out. Concentrations of pollutants media and fresh water in the experimental tanks were changed every three days to avoid deterioration. Daily monitoring of the fish specimens was carried out to assess the state and behavioral attitude of fish within the pollutant media; and mortality was monitored and recorded daily throughout experimental period. Growth measurement and monitoring were carried out weekly throughout the eight weeks of experimental period. Measurements on total length (cm), was carried out using measuring rule; and weight (g) gained by specimen were measured using digital measuring scale.

Effluent collection

The effluent used in this study was obtained at the point source from the male hostel D in Federal University of Kashere in clean 40,000 cm^3 keg and transported to the laboratory. The effluent contains bathing water, water from washing cloths and plates. Analysis was carried out for parameters with short holding times including temperature, pH, turbidity, total dissolved solid (TDS) and dissolved oxygen (DO) using hand held meters.

Experimental set up

The experimental set up consists of twelve plastic tanks of 60 L capacity. Each tank consists of domestic effluent treatments of 0% (control), 30%, 50% and 70%; all in triplicates. The control consisted of borehole water. The plastic bowls were cover with net to prevent the juveniles from jumping out of the container and escaping.

Growth performance

Minimal mortality was observed during the experimental periods. The weight and length of *C. gariepinus* was measured before exposure and during the exposure. Mean length gain (MLG) and mean weight gain (MWG) were calculated as follows:

Mean weight gain =FMW-IMW
FMW- Final mean weight
IMW- Initial mean weight
Mean length gain= FML- IML
FML- Final mean length
IML- Initial mean length

Blood collection

Blood samples were collected via caudal vein puncture as described by Kori-Siakpere *et al.* (2005) from the experimental fishes. Fish was held by the person to collect the blood in a slanting and/or vertical position with the ventral part facing the person. Blood samples were collected with sterile 2 ml syringe and needle. The needle was introduced on the ventral mid line between the anal opening and the beginning of the anal fin to assess the caudal vein beneath the vertebral column.

Haematological Analysis

The blood in the EDTA anticoagulant tubes was placed on a centrifuge. A centrifuge is a heavy machine that spins test tubes at a high speed of about 17,000 RPM. This centrifuge force pulls particles to the bottom from the plasma as well as any substances. Blood was centrifuge for 10 min.

The centrifuge blood was subjected to automatic machine of KX 21N Haematology Analyser. The sample access number was enter to the machine and the entry button was press, then, the sample was moved into the probe and the machine was allowed to aspirate the sample. The sample was removed from the probe and it was allowed to analysed the result and print it out. The subsequent samples were repeated with the same procedure. Concurrently, the total red blood cells (RBC), white blood cells (WBC), haematocrit or packed cell volume (PCV), haemoglobin (Hb), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets from each blood sample were determined.

Mean corpuscular volume (MCV): Average volume of a single RBC count which is calculated from the data obtained for RBC and PCV using standard formula from Torts & Torres (1988):

MCV = PCV×10/RBC (106) fL (femtrolitres)

Mean corpuscular haemoglobin (MCH): Quantity or amount of haemoglobin present in one RBC. It is the amount of Hb expressed in relation to the volume of one RBC and is calculated from the data obtained for Hb and RBCc using standard formula from Torts & Torres (1988):

MCH = Hb×10/RBC (106) pg (picograms)

Mean Corpuscular Haemoglobin Concentration (MCHC): Concentration of Hb in one RBC.

It is the amount of Hb expressed in relation to the volume of one RBC and also calculated using the formular propounded by Torts & Torres (1988):

MCHC = Hb×100/PCV g/dl

Evaluation of water quality parameters

The physico-chemical parameters were measured in the experimental tanks on weekly basis. Temperature was evaluated by using mercury in glass thermometer. The water pH was measured using pH meter, total dissolved solid (TDS) was determined using electrical conductivity meter. Alkalinity, dissolved oxygen (DO), was determined using the methods described by APHA (1998), while biological oxygen demand (BOD) was determined using Winklers' method.

Statistical Analysis

All the data generated during the experimental work were subjected to one way analysis of variance (ANOVA) at 5% of significance.

Results

The result of physio-chemical parameters is shown in the table 1. Temperature ranged from 27-32 °C during the study period and the mean was 30.2 °C. The highest temperature observed was in 30m1 concentration of domestic effluent which is 30.2 °C while the lowest temperature observed was in 70 ml concentration of domestic effluent which is 27.2 °C. Dissolved oxygen content was maintained at 2.2-3.7 mgL^{-1}. pH readings were between 5.59 -6.92 °C. The highest pH value was in 30 $m1L^{-1}$ concentration of domestic effluent which is 6.92 °C while lowest pH value was observed in 70ml concentration of domestic effluent which is 5.59 °C. The highest BOD observed in 70 ml concentration of domestic effluent which is 38.0 mg L^{-1} while the lowest was in 30 ml concentration of the effluent which is 24.2 mgL^{-1}.

Behavioral responses of fishes were observed daily. The control and the group exposed to 30 mlL^{-1} showed normal swimming behavior and natural coloration of skin during the experimental period. Abnormal behavioral changes, such as swimming near the water surface, loss of equilibrium, erratic swimming, circling movement, and staying motionless on the bottom of container, were however observed in fish exposed to 50 ml L^{-1} and 70 mlL^{-1}.

The mean length of the experimental fishes exposed to domestic effluent is shown in the table 2, that there is no significant difference of their length in the first week at all concentrations while in the sixth week there is significant differences in 50 and 70 mlL^{-1} compared to control but not significant in 30 mlL^{-1} concentration. However, in the seventh and eighth weeks all the experimental fishes showed a level of significant difference ($p<0.05$) from the control.

The table 3 shows the result of the mean weight of the experimental fishes exposed to domestic effluent. The result showed that from the first week of exposure to the fifth weeks there is no significant differences observed in concentration of the treated organisms, while in the 6th weeks there is significant difference in 50ml, and 70ml concentrations as compared with the control but there is no significant difference in 30ml. However, in the eight weeks all the experimental fishes showed a level of significant difference ($p<0.05$) from the control.

The table 4 shows the result of the haematological parameters of the *C. gariepinus* after exposure to different concentrations of the domestic effluent for about 8 weeks. These are the WBC, RBC, Hb, PCV, MCH, MCHC and MCV. In comparison with the control, all the parameters showed an increase in values obtained as the concentration increased for the same parameter except for WBC and MCH which showed

Parameters	Control	30mlL^{-1}	50mlL^{-1}	70mlL^{-1}	WHO Specification
Temperature (ºC)	32.8±3.2^{c}	30.2±2.8^{b}	29.9±2.6^{b}	27.2±1.7^{a}	36
pH	6.98±2.2^{b}	6.92±2.2^{b}	5.9±2.1^{b}	5.59±1.8^{a}	6.5
TDS (mgL^{-1})	577±1.6^{a}	608±1.8^{a}	1270±2.4^{b}	1554±2.6^{c}	500
Turbidity (NTU)	7.8±0.35^{a}	8.9±0.63^{a}	15.3±1.1^{b}	18.0±1.6^{c}	5.0
DO (mgL^{-1})	3.87±2.4^{b}	3.50±2.2^{b}	2.70±1.8^{a}	2.32±1.7^{a}	4.00
BOD (mgL^{-1})	24.2±1.0^{a}	28.3±1.5^{b}	32.1±2.4^{c}	38.03±3.0^{d}	25.0

TDS: Total dissolve solid; DO: Dissolve oxygen, BOD: Biological oxygen demand
Values bearing the same letters along the same column are not significantly different at p≤0.05

Tabla 1. Parámetros fisicoquímicos de las concentraciones de tratamientos de los efluentes domésticos.

Table 1. Physico-chemical parameters of the treatment concentrations of the domestic effluents.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	9.17±0.5^{a}	11.60±0.4^{a}	13.33±0.3^{a}	15.60±1.1^{a}	18.53±1.9^{b}	23.20±1.0^{b}	24.27±1.0^{b}	25.47±0.5^{a}
30mlL^{-1}	7.17±0.6^{a}	10.87±0.2^{a}	12.3±1.2^{a}	14.53±0.3^{a}	17.43±0.4^{ab}	20.03±1.5^{ab}	17.77±1.2^{b}	21.17±0.9^{a}
50mlL^{-1}	8.4±0.63^{a}	10.90±0.3^{a}	12.00±0.3^{a}	13.76±0.9^{a}	16.93±0.7^{a}	17.63±1.4^{a}	17.13±0.5^{a}	18.17±1.6^{a}
70mlL^{-1}	7.4±0.35^{a}	10.63±0.5^{a}	12.13±1.1^{a}	13.73±0.9^{a}	16.13±1.1^{a}	16.10±1.5^{a}	15.73±1.1^{a}	19.20±0.5^{a}

Values bearing the same letters along the same column are not significantly different at p≤0.05

Tabla 2. Longitud media de los peces de experimentación.

Table 2. Mean length of the experimental fishes.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control	10.17±1.2^{a}	22.47±1.7^{a}	28.33±1.5^{b}	39.97±3.6^{b}	50.00±7.0^{b}	67.67±8.2^{b}	82.00±2.7^{b}	95.00±4.7^{c}
30mlL^{-1}	11.77±0.7^{a}	20.47±2.7^{a}	27.27±1.7^{ab}	34.47±2.1^{ab}	38.33±3.0^{a}	54.3±3.9^{ab}	56.33±6.7^{a}	67.67±5.7^{b}
50mlL^{-1}	11.03±2.1^{a}	17.67±2.5^{a}	22.87±1.7^{a}	30.07±3.6^{a}	36.67±3.8^{a}	45.33±5.2^{a}	53.00±6.3^{a}	50.0±5.5^{ab}
70mlL^{-1}	8.30±0.3^{a}	18.47±2.3^{a}	26.20±4.0^{a}	32.20±2.4^{a}	35.67±3.2^{a}	42.67±5.5^{a}	45.67±5.7^{a}	47.67±4.8^{a}

Values bearing the same letters along the same column are not significantly different at p≤0.05

Tabla 3. Peso medio de los peces de experimentación.

Table 3. Mean weight of the experimental fishes.

Parameters	Control	30mlL^{-1}	50mlL^{-1}	70mlL^{-1}
WBC (x10 cells/ mm^{3})	3.09±0.81^{a}	4.2±0.55^{b}	6.8±0.91^{c}	11.3±0.65^{d}
RBC (x10^{6} cells/ mm^{3})	1.98±2.9^{c}	1.43±0.58^{b}	1.12±1.4^{b}	0.58±1.3^{a}
Hb (g/dL)	8.53±0.20^{a}	10.5±0.46^{c}	9.27±0.18^{b}	9.27±0.18^{b}
PCV (%)	29.2±0.29^{a}	33.4±0.24^{d}	35.5±0.23^{c}	30.8±0.12^{b}
MCH (pg)	44.47±0.260^{b}	45.83±0.033^{c}	43.43±0.120^{a}	48.83±0.441^{d}
MCHC (g/dL)	29.3±0.27^{b}	31.5±0.25^{c}	26.0±0.56^{a}	31.0±0.23^{c}
MCV (fL)	147.47±0.87^{a}	233.57±0.61^{b}	316.6±0.96^{c}	531.03±0.89^{d}

WBC: White blood cells; RBC: Red blood cells; Hb haemoglobin; PVC:Haematocrit; MCH: Mean corpuscular haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; MCV: Mean corpuscular volume

Mean values with the same letters for same parameter are not significantly different (p<0.05). Values are average from three test organisms. (g/dl)= gramme/decilitre, pg= picogram, (µl)= microlitre, mg/l= milligramme/litre.

Tabla 4. Media y desviación estándar de los parámetros de *Clarias gariepinus* expuestos a diferentes concentraciones de efluentes domésticos durante 8 semanas.

Table 4. Mean and standard deviation for haematological parameters of *Clarias gariepinus* exposed to different concentrations of domestic effluent for 8 weeks.

decrease in values as concentration increases. The parameters values for the treated organisms all showed a level of significant difference (p<0.05) from the control at the highest concentrations.

Discussion

The result for most of the domestic effluent parameters analysed showed a number of deviations from WHO standards on guidelines for effluent discharges for aquaculture, the physiochemical parameters showed higher temperature (32.8 ºC) in control, and (18.0 NTU) and TDS (1554) in 70mlL^{-1}; lower DO (2.32 mg/l) and pH (5.59) in 70mlL^{-1}. The physicochemical properties of the domestic effluent showed that temperature (32.8 ºC), total dissolved solids (1554 mg/L) and dissolved oxygen (3.87 mg/L) were high. This is similar to the report of Agboola & Fawole (2014). The high DO could cause fish death.

The result obtained from the studies shows that there was significant difference between the growth rate of African catfish exposed to each concentrations of domestic effluent (p<0.05). Catfish exposed to 70m1L^{-1} concentration of domestic effluent had the highest reduction while Catfish exposed to 30mlL^{-1} concentration of domestic effluent had lowest reduction. The reduction in the growth rates of the exposed catfish supports the findings of Esenowo and Ugwumba, (2010) who reported that concentration of a domestic detergent in an aquarium tank reduce the weights of catfish (*C. gariepinus*) exposed. The most likely explanation for the growth reduction in this study is increase in metabolism due to detoxification and impaired health which lead to loss of appetite during the exposure. Growth reduction cannot be explained entirely by lack of food, it may be due to energy loss also. At higher concentrations the behavioural activities of the organism is disrupted. Domestic effluent effect is however noted to be increased with increased concentration. Ayoola (2008) reported that the

level of toxicity of any toxicant depends on its bioaccumulation. No adverse behavioural changes or any mortality were recorded in the control experiment throughout the practical period. While in the exposed fishes, several abnormal behavioural responses were observed such as pronounced gasping for breath, restlessly, frequent surface to bottom movement, and sudden change of direction during movement, resting at the bottom, loss of skin colouration ,loss of equilibrium and stagnant in length. Our observation was similar to the observation of Dahunsi & Oranusi, (2013), Ogaga *et al.* (2015) and Kori-Siakpere *et al.* (2009). Loss of the equilibrium observed may be due to depletion of energy in the body of the exposed catfish. The catfish that cannot tolerate the change of the environment died. Also, the rate of mortality became greatly increased with increased in the concentration of the domestic effluent. The temperature reading fell between 27-32 °C which was within the limit for fishes. This agrees with the findings of Samson (2013) showed that toxicity increased with temperature thus the overall toxicity recorded for detergent was not influenced by temperature which was within the normal range. The oxygen stress encountered by the catfish that is responsible for the respiratory distress and death was due to their inability to withstand the oxygen depletion of the water induced by the active organic compound in the effluent. Adewoye & Fawole (2002) and Adewoye *et al.* (2005) had earlier reported that indiscriminate deposition of effluent into an aquatic system might decrease the dissolved oxygen concentration, which stand to impair respiration leading to asphyxiation (which is an indication of unconsciousness or death produced by failure of the blood to become properly oxygenated in the lungs) and may ultimately result into organ architectural degradation.

The high WBC count could be due to attempt by the fishes to fight against the antigens (pollutants) and this led to the production of more antibodies (WBC) to improve the health status of the organism. This agrees with *Ates et al.* (2008) that the increase in WBC during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defence mechanism of the fish to tolerate the toxicity. The increase in leucocyte count indicates the stimulatory effects of the toxicant on immune system and also depends on the toxicant stress. It is similar to the present finding, Saravanan *et al.* (2011) reported a significant increase in WBCs in *Cyprinus carpio* (L., 1758), exposed to pharmaceutical drugs clofibric acid and diclofenac. Significant increase in WBCs has also been reported in *Cirrhinus mrigala* (Hamilton, 1822) (Saravanan *et al.* 2012) after exposure to different concentrations of pharmaceutical drug ibuprofen. The observed reduction in haematocrit and haemoglobin concentration of the organism on exposure to the effluent could be a result of the bioaccumulation of the toxicant in the body. This decrease in the two indices was as a result of uncontrolled lysis of the RBC due to the toxicity level of the effluent; while the decrease in haematocrit compared to the haemoglobin standards was attributed to shrinkage of the erythrocytes. These are in agreement with Dahunsi & Oranusi (2013) that the decrease in haemoglobin content during stress condition may indicate a decrease in the rate of haemoglobin synthesis which lead to impaired oxygen supply to various tissues resulting in decrease in the number of RBC through hemolysis. The lysis of erythrocyte leads to a reduction in haematocrit value. The decreasing trend in mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were evident in the organisms kept in different concentrations and this correlates with the findings of (Abdel *et al.* 2011) that MCHC is an indicator of RBC swelling and the lowered MCHC during treatment might have resulted from release of young erythrocytes containing less haemoglobin into circulation.

Conclusion

It is clear from the research that exposure of *Clarias gariepinus* juvenile to even low concentrations of domestic effluent lead to significant reduction in the growth rate, which dependent on the period of exposure and concentration of the effluent. In view of the effect of this effluent, it can be inferred that, indiscriminate discharge of domestic effluents can affect growth of African catfish. This might make all the living entities in polluted environment vulnerable to diseases, and eventually leads to their death.

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