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Haematological Profile and Serum Biochemistry of Juvenile *Clarias gariepinus* as Biomarkers of Textile Wastewater Toxicity

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ABSTRACT Pollution is a global problem with hazardous consequences especially on the biotic components of the aquatic ecosystem. Blood is a useful tool in diagnosing the health condition of fish. Hence, the aim of this project was to evaluate the effect of textile wastewater on fish blood. Fish were exposed to varying concentrations of textile wastewater for 96 hours. There were four treatments including the control and others containing 0.0005 ppm, 0.002 ppm and 0.035 ppm of textile wastewater per every 35 litres of freshwater. Each treatment had one replicate with 5 fish stocked in each replicate. The textile effluent significantly reduced ($p<0.05$) red blood cells (RBC), packed cell volume (PCV), haemoglobin concentration (HGB), and white blood cell (WBC) in the other treatments compared to the control. However, the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were not statistically different ($p>0.05$) from the control. There was a significant reduction in the value of serum metabolites (total protein, globulin, glucose) of juvenile *C. gariepinus* after exposure to textile effluent ($p<0.05$). Urea, albumin, and cholesterol increased significantly ($p<0.05$). However, creatinine did not follow any specific trend across the treatments. The activities of Aspartate transaminase (AST) and Alkaline transaminase (ALT) were significantly higher in the exposed fish compared to the control ($p<0.05$). Alkaline phosphatase (ALP) recorded a significantly higher value in the control compared to the other treatments ($p<0.05$). There were no significant differences in the concentrations of both Superoxide dismutase (SOD) and Glutathione S-transferase (GST) of fish exposed to textile effluent ($p>0.05$). The result revealed that the textile wastewater adversely affected the blood components of the exposed fish. It can be concluded that the presence of textile wastewater in aquatic environments could induce stress and consequently deteriorate the health of aquatic organisms.

Keywords: Aquatic pollution; fish health; textile effluent

INTRODUCTION

Fish is a good source of protein and micro-nutrients necessary for balanced nutrition and good health. Globally, it has been reported that of the total protein consumed by humans, fish accounted for 16.6% of animal protein intake and 6.5% of all protein consumed compared to other protein sources (FAO, 2012; 2022). Hence, the need to use our aquatic (particularly freshwater) resources sustainably cannot be over-emphasized going by its role in the ecosystem and the benefits derivable from it. Aquatic ecosystems are water-based environments in which the living components interact with the non-living components (Barange *et al.*, 2010). The influx of unwanted substances into water bodies causes alterations in the physical, chemical, and biological characteristics of the aquatic system which lead to pollution, consequently causing ecological imbalance.

According to Owa (2013), water is said to be polluted if some substances or conditions are present to such a degree that the water cannot be used for a specific purpose. Industrial effluents contribute a lot to water pollution, which can be hazardous to aquatic plants and animals (Romano *et al.*, 2001). These pollutants bio-magnify and bioaccumulate in the system of aquatic organisms with a broad range of impacts and stresses on the affected organisms (Censi *et al.*, 2006). This leads to a steady decline in the aquatic flora and fauna, particularly fishes. Ogundiran *et al.* (2007) reported that fishes are more susceptible to stressful conditions than terrestrial animals because of their intimate interaction and dependence on their surrounding environment. Aquatic organisms, like fish, accumulate pollutants directly from contaminated water (bio-accumulation) and indirectly through the food chain (bio-magnification) (Riba *et al.*, 2004; Ashraf, 2005). Once the toxicant enters the body of the fish, it may damage important tissues and organs leading to physiological and pathological disorders (Ogundiran *et al.*, 2007).

The hematological parameters are an important tool for assaying physiological and pathological changes in animals (Gabriel *et al.*, 2011). These parameters are also used to provide information about the feeding conditions and water quality in which fish lives (Fazio *et al.*, 2013). Hence, they are valuable as indicators of disease or stress due to pollutants and environmental fluctuations in fish (Bhatkar & Dhande, 2000). Routine hematological assessment of fish includes the determination of total erythrocyte count, hematocrit, hemoglobin concentration, erythrocyte indexes, total white blood cell count, and thrombocyte count (Campbell, 2004). Alterations in these blood parameters in fish due to exposure to pollutants have been numerously reported (Diwan, 2005; Lakshmanan *et al.*, 2013; Hazelton *et al.*, 2013; Kumar & Gautan, 2014; Giridhar *et al.*, 2015; Mallum *et al.*, 2016).

According to Galadima *et al.* (2011), anthropogenic-induced pollution is the major culprit in the poor quality observed in the common sources of water that are available to local communities in Nigeria. An identified aquatic pollutant is textile effluent. Textile printing and dyeing processes produce huge amounts of effluents with constituents such as starch, waxes, Carboxyl Methyl Cellulose (CMC), polyvinyl alcohol, wetting agents, sodium hypochlorite, chlorine gas, sodium hydroxide, hydrogen peroxide, acids, surfactants, sodium silicate, sodium phosphate and short cotton fiber (Ghaly *et al.*, 2014). Consequently, the bio-accumulation of these toxicants consequently leads to the food chain adverse effects, impart severe damage (Vinodhini & Narayanan, 2009), cause acute disorders (Azmat & Bibi, 2013), and finally cause death of aquatic organisms (Yusuff & Sonibare, 2004; Ogundiran *et al.*, 2010)

In view of the enormous discharge of textile effluents into Itoku tributary of Ogun River, it becomes imperative to investigate the extent of pollution in the river using blood as a biomarker.

MATERIALS AND METHODS

Procurement of experimental fish

Juvenile *C. gariepinus* were obtained from a reputable farm. They were fed to satiation 40% crude protein diet twice daily for two weeks before stocking into experimental tanks.

Experimental set-up

Forty juvenile *C. gariepinus* were divided into 4 groups of one replicate each. The fish were examined carefully for any pathological symptoms. The fishes were grouped into the following designated treatment as shown below:

Treatment 0: Control fishes maintained in 35 litres of fresh water,
Treatment 1: 35 litres of fresh water+ 0.0005 ppm of textile wastewater,
Treatment 2: 35 litres of fresh water+0.002 ppm of textile wastewater,
Treatment 3: 35 litres of fresh water+0.0035 ppm of textile wastewater.

The feeding regime adopted during the acclimatization period was maintained. Water was changed twice daily to remove uneaten food and feaces. The renewal water for each treatment always contained its required concentration. The experiment lasted for a period of 96 hours. At the end of the experiment, sedation of the fish was done using clove oil.

Collection of blood samples

Blood samples were collected via caudal vein puncture using a sterile 2 ml syringe and needle as described by Kori-Siakpere *et al.* (2005). A portion of the collected blood was dispensed into EDTA-containing vials for haematological analysis while the other portion was dispersed into vials devoid of EDTA.

Haematological analysis

Red blood cells (RBC) and white blood cells (WBC) counts were estimated using a Neübauerhaemato-cytometer using Hendricks (1952) diluting fluid for RBC counts; Shaw (1930) solution for WBC counts; Haemoglobin (Hb) concentrations were evaluated using the Sahli-Helligehaemoglobin method and pmhmn backed cell volume (PCV) was estimated using a micro-haematocrit method following the method of Hesser (1960). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values were estimated following the method of Stockham *et al.* (2002).

Serum biochemistry analysis

The blood collected was first centrifuged for five minutes to obtain the serum. Serum glucose concentrations were estimated following the glucose oxidase method as described by Morgan & Iwana (1997). Serum total triglyceride concentrations were estimated using an enzymatic method as reported by Tietz (1990) using a commercially available kit (Randox Laboratory Limited, United Kingdom). Total protein concentration, glucose, albumin, creatinine, urea, Aspartate aminotransferase activity, Alkaline Phosphatase activity, and Aspartate aminotransferase activity were determined spectrophotometrically using a Randox kit. Serum glucose was determined spectrophotometrically by using a Randox kit following the method of Barham *et al.* (1972). Superoxide dismutase was measured by the procedure of Zou *et al.* (2014).

Statistical analysis

All the data generated during the experimental work were subjected to a one-way analysis of variance (ANOVA). Duncan's multiple range test was used to test for significance at 5%.

RESULTS AND DISCUSSION

Haematological profile of juvenile C. gariepinus exposed to textile waste water

Values of haematological parameters provide insight on the health and physiological status of fish (Fazio et al., 2013). Packed cell volume (PCV) is a measure of the percentage of red blood cells in the blood. The red blood cells (erythrocytes), are oxygen-carrying devices (Chen et al., 2009) as they contain haemoglobin. The main function of haemoglobin is to transport oxygen from the gas exchange organs of the fish to the peripheral tissues (Cao & Wang, 2010). Hence, any quantitative decrease in their levels might lead to de-arrangement of oxidative metabolism (Goel & Kalpana, 1985). In this study, the result of PCV of juvenile C. gariepinus exposed to textile wastewater revealed that the pollutant caused a significant reduction in PCV levels compared to the control ($p<0.05$) (Table 1). This is similar to the report of Tak *et al.* (2014) who observed a reduction in PCV of Cyprinus carpio exposed to varying doses of dichlorvos for 24 and 96 hours. A similar trend was observed for haemoglobin, red blood cells, and white blood cells. This reduction could be an indication of anemia (Abbas *et al*., 2005) due to the destruction of blood cells because of exposure to textile wastewater. The highest value for Hb and RBC were recorded in the control treatment and decreased significantly ($p<0.05$) in treatments exposed to the varying concentrations of textile waste water. Reduced values of haemoglobin and RBC have been reported by Eriegha *et al.* (2017), when fish was exposed to sub-lethal concentrations of crude oil. These parameters are known to trap oxygen during

Table 1. Haematological profile of juvenile *C. gariepinus* exposed to textile waste water.

Haematological profile	T0	T1	T2	T3
PCV (%)	28.50±0.50^{a}	27.50±0.50^{ab}	25.50±0.50^{b}	26.55±0.55^{ab}
Hb (g/dl)	9.90±0.10^{a}	9.20±0.10^{b}	8.40±0.10^{c}	9.05±0.15^{b}
RBC (x10^12/l)	4.88±0.08^{a}	4.45±0.05^{b}	4.15±0.05^{b}	4.14±0.16^{b}
WBC (x10^9/l)	13.60±0.10^{a}	12.50±0.20^{b}	11.50±0.30^{c}	10.10±0.10^{d}
MCV (fl)	60.20±0.20^{a}	60.50±0.50^{a}	59.75±0.25^{a}	60.05±0.45^{a}
MCH (pg)	20.20±0.20^{a}	20.45±0.25^{a}	19.90±0.10^{a}	20.45±0.25^{a}
MCHC (g/dl)	33.40±0.40^{a}	33.35±0.35^{a}	33.10±0.10^{a}	33.60±0.60^{a}

*Means with different superscripts in the same row are significantly different ($p<0.05$).

respiration, hence, the reduction in their values could lead to asphyxiation due to inability of fish to get enough oxygen. White blood cells are the fighter cells as they prevent invasion of foreign bodies in the fish system. In this study, a reduction in the values of treatments containing textile wastewater decreased significantly ($p<0.05$). Similar results were observed in Oreochromis niloticus exposed to sub-lethal sodium selenite concentrations (Ranzani-Paiva *et al*., 2014).

Serum metabolites profile of juvenile C. gariepinus exposed to textile waste water

Blood serum metabolites show alterations in the blood as a result of metabolic activities. Metabolites like glucose, albumin, total protein, and globulin have been used to understand responses of fish to pollution (Sukirtha & Usharani, 2013; Giridhar *et al*., 2015; Mallum *et al*., 2016). In this study, the total protein of juvenile *C. gariepinus* exposed to textile wastewater showed that the pollutant caused a significant reduction ($p<0.05$) compared to the control (Table 2). This observation might have emanated from kidney damage or lack of nutrients in the blood due to the heavy metal composition of the textile waste water. This quantitative decrease in total protein levels might lead to a breakdown of the proteins in their body (Ahmad, 2008). Globulin is an indicator for a variety of conditions in fish including liver damage or disease and nutritional problems. Increased levels of globulins in fish tissues are often associated with infectious diseases, immune-mediated disease, and some types of cancer. In this study, except for T1, there was a significant reduction ($p<0.05$) in globulin levels compared to the control treatment. This might indicate that the textile waste water did not have any negative effect on the globulin level due to the short period of exposure. Glucose is a key source of energy for most vertebrate organisms. In the juvenile *C. gariepinus* exposed to textile wastewater, a significant reduction ($p<0.05$) was observed in all the treatments compared to the control. This indicates that there was a decline in the blood sugar of the experimental fish which can degenerate to hypo-glycaemia. This same trend was observed by Giridher *et al.* (2015) and Kumar & Gautan (2014). However, a reverse trend was recorded for cholesterol and urea where the control had lower values than the treatments exposed to the textile waste water. An increase in urea level could signify gill dysfunction as urea is excreted mainly through the gills (Aldrin, 1982). The increase in cholesterol levels did not support the findings of Samuel *et al.* (2017) in a study where *C. gariepinus* was exposed to heavy metals. Creatinine, however, did not follow any specific trend across the treatments (Table 2).

Oxidative stress enzyme of juvenile C. gariepinus exposed to textile wastewater

The values for oxidative stress enzymes of juvenile C. gariepinus exposed to textile wastewater are shown in Table 3. The activities of Aspartate transeaminase (AST) and Alkaline transeaminase (ALT) were significantly higher in the exposed fish compared to the control with a value increase from 72.50±0.50 (T0) to 95.50±0.50 (T3) and 35.50±0.50 (T0) to 45.25±0.25 (T3) respectively although AST value for T2 was significantly lower than the control ($p<0.05$). Alkaline phosphatase (ALP) recorded a significantly higher value in the control compared to the other treatments ($p<0.05$). There were no significant differences in the concentrations of both Superoxide dismutase (SOD) and Glutathione S-transferase (GST)

Table 2. Serum metabolites profile of juvenile *C. gariepinus* exposed to textile wastewater.

Serum metabolites profile	T0	T1	T2	T3
T.P (g/dl)	4.45±0.45^{a}	3.20±0.20^{b}	4.30±0.30^{ab}	4.20±0.20^{ab}
ALB (g/dl)	2.45±0.25	2.60±0.20	2.50±0.10	2.25±0.15
GLOB (g/dl)	2.10±0.10^{a}	0.70±0.10^{b}	2.05±0.05^{a}	2.05±0.05^{a}
GLUC (mg/dl)	55.20±0.20^{a}	23.05±0.05^{d}	34.20±0.20^{c}	41.15±0.15^{b}
UREA (mg/dl)	0.00±0.00^{c}	3.50±0.20^{b}	4.35±0.25^{a}	3.25±0.15^{b}
CHOL (mg/dl)	64.40±0.40^{d}	103.10±0.10^{c}	121.40±0.40^{b}	124.10±0.10^{a}
CREAT (mg/dl)	2.65±0.15^{b}	4.25±0.15^{a}	2.20±0.20^{bc}	1.65±0.15^{c}

*Means with different superscripts in the same row are significantly different ($p<0.05$).

Table 3. Oxidative stress enzyme of juvenile *C. gariepinus* exposed to textile waste water.

Oxidative stress enzyme	T0	T1	T2	T3
AST (U/L)	72.50±0.50^b	70.50±0.50^c	26.50±0.50^d	95.50±0.50^a
ALT (U/L)	35.50±0.50^d	50.50±0.50^a	38.50±0.50^c	45.25±0.25^b
ALP (U/L)	34.45±0.45^a	24.15±0.15^c	32.40±0.40^b	31.20±0.20^b
SOD (U/L)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
GST (U/L)	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.01

*Means with different superscripts in the same row are significantly different ($p<0.05$).

of fish exposed to textile effluent ($p>0.05$). Oxidative enzymes are biomarkers of stress responses. ALT and AST are enzymes indicating damage in liver cells. AST is also released in the blood when there is muscle injury. The result from this study is in agreement with the report of Das *et al*. (2004) but contradicts the report of Sukirtha & Usharani (2013) who observed an increase in levels of ALP of Danio rerio exposed to dichlorvos in an acute test.

CONCLUSION AND RECOMMENDATION

Conlusion

Textile production is an avenue for boosting the economy, however, the discharge of wastewater from this production is capable of endangering aquatic resources as evidenced in this present study. This study has been able to use blood as a biomarker showcasing the hazardous effect of textile water on fish, a biological resource relevant in ensuring food security.

Recommendation

To minimize this hazard, textile activities can be regulated through the enforcement of proper water treatment of wastewater from textile activities before discharge into the environment.

AUTHORS' CONTRIBUTION

AFD is doing research, ideas, data generation; TAO is written manuscript and data analyze; HOA is doing manuscript preparation and written manuscript.

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