Assessment of Municipal Sewage Effluent on the Growth Performance and Hematological Indices of the African Catfish (*Clarias gariepinus*, Burchell 1822) Juveniles

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Abstract

The impact of municipal sewage effluent on the growth characteristics and hematological indices of an African catfish (*Clarias gariepinus*) was studied for 90 days with a view to determining potential toxicity of the risk of indiscriminate discharge of municipal sewage on the aquatic biota in a design of four exposure concentrations (0, 1.03, 2.06, 4.12 and 8.24%). The result of the study on the growth performance indices showed a predominant decrease in survival, mean weight gain (MWG), daily weight gain (DWG), and body weight gain (BWG) and a converse marginal decrease in other parameters with an increase in sewage concentration. However, hematological indices analyses showed a decrease (p>0.05) in red blood cell (RBC) count with the increased concentration of sewage effluent. Significantly higher (p<0.05) levels of white blood cell (WBC), Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelet (PLT) were observed in exposed fish compared to the control group. The study concluded that an increase in sewage has an adverse effect on the growth and haematology of the fish.

Keywords: Sewage; Municipal; Discharge; Exposure; Toxicity; hematology

Introduction

Sewage, which is daily generated by man while meeting his various living requirements, has been defined as liquid waste products generated from domestic, commercial, industrial, and institutional sources (Katyal and Satake, 2001). It consists of dissolved or suspended pollutants that include wastes generated by humans such as faeces and urine and sullage. This domestic sewage is known to be a complex mixture of constituents of organic and inorganic matter and microorganisms together with water (Henry and Heinke, 1989; Carolyn, 2010; Ademoroti and Uwidia, 2011).

Apart from the serious environmental health problems that domestic sewage pollution could pose on those in contact, if discharged untreated, or not well treated (especially in many developing parts of the world), it can also affect the physico-chemical properties of receiving water bodies and aquatic life. There have been reports of several sewage-related pollutants posing toxicity risks to aquatic organisms (Diaz *et al.*, 2002; Ogunfowokan *et al.*, 2005; Garg 2006; Gomez-couso and Mendez-Hermida, 2006; Taiwo *et al.*, 2012). The authors reported reduction in fish abundance, mortality in

fish and shell fish, impairment in reproductive development in amphibians and fish, and release of toxins that are deadly due to the presence of sewage related pollutants in aquatic environment. Therefore, bio-monitoring of aquatic pollution is quite fundamental (Aguayo *et al.*, 2004).

In toxicity studies, several researchers have used various fish species as a biosensor to several xenobiotics compounds (Abrahamson *et al.*, 2007; Kerambrun *et al.*, 2011; Abdel-Moneim *et al.*, 2012; Al-Ghais, 2013; Maier *et al.*, 2014; Vincze *et al.*, 2014; 2015). Noteworthy is the African Catfish (*Clarias gariepinus*) which is one of the dominant fish found in rivers, dams, lakes, swamps, muddy waters, flood plains, and often culture (Cambray, 2003). The fish species have been widely used *in situ* as bioindicators of xenobiotic compounds in the aquatic environment (Sironka and Drastichova, 2004; Ramesh *et al.*, 2009; Nwani *et al.*, 2010; Sevcikova *et al.*, 2011; Fakolujo *et al.*, 2018; Akinbadewa *et al.*, 2020). This is due to their culturable attributes which include the fish's hardiness, bioaccumulation potential, and their wide tolerance to adverse environmental conditions.

Various biomarkers such as immune responses, histological alterations, changes in enzyme levels, gene expression alteration, etc., have been used in assessing the effect of various pollutants on organisms (Moore *et al.*, 2004; de la Torre *et al.*, 2007). However, analysis of blood samples, among other several biochemical analyses, has revealed several alterations or changes in the physiology of any organism (Bhatia *et al.*, 2004). This is simply because blood serves as a medium through which inter-and- intracellular substances are transported from various tissues and organs in the body. Several authors have used this means to expose the effect of environmental pollutants such as pesticides and heavy metals on fish (Bhatia *et al.*, 2004; Johal and Grewal, 2004).

Continual discharge of municipal sewage effluent into aquatic environments in developing countries especially Nigeria does not only damaging the quality of the environment by rendering water bodies unsuitable, it also threatens the health of aquatic biota. There is, therefore, a need to evaluate the effect of long-term discharge of sewage effluent on aquatic fauna especially on growth and heamatological indices of *Clarias gariepinus* a food fish in Nigeria, hence this study.

Materials and Methods

Sampling Area

Raw sewage samples were collected from the oxidation pond, Obafemi Awolowo University Ile – Ife, Osun State Nigeria, (Latitude 07° 30. 450' N and Longitude 004° 30. 710' E) (Figure 1).

Collection of test organism

One hundred and twenty (120) *Clarias gariepinus* juveniles $(7.65 \pm 0.02g)$ were sourced from a Bioresources Farm, Ile-Ife, Osun State, Nigeria. The fish were transported in an aerated chamber to the Fish Culture Laboratory, Department of Zoology, Obafemi Awolowo University where they were acclimatized for 14 days in twelve 40 L aquaria (90 x 45 x 45 cm) made of glass, filled to 30L mark with unchlorinated water each containing ten juveniles. To minimize waste production in the aquaria tanks, fish feeding was stopped a day to commence the experiment (Obiezue *et al.*, 2014).

Municipal sewage sample collection

The sewage discharge, used for the toxicity test, was obtained from the sewage treatment oxidation pond of the Obafemi Awolowo University Ile-Ife, Nigeria where municipal sewage pools. Sewage samples were collected in batches between late rain (September) and early dry season (December)

from the discharge point where it runs into the nearby river. The sewage effluent was then kept in the refrigerator to prevent the continuation of microorganisms' activities before the experiment commences. All the sewage samples weekly collected for each month were poured together in a large container during the experiment to attain homogeneity and avoid variability in concentration.

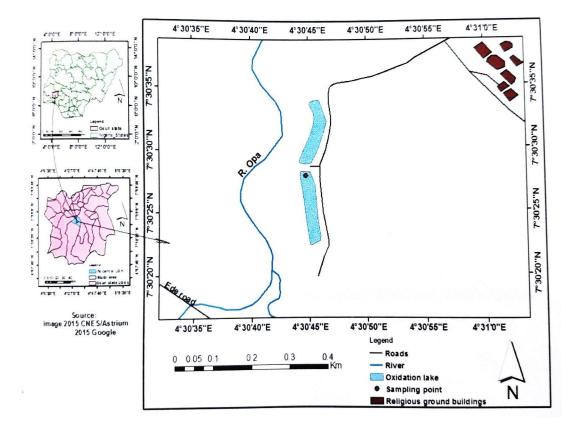


Fig. 1: Map showing the study area. (Inset: Map of Nigeria showing Osun State, Map of Osun State showing Ife Central Local Government Area and Map of oxidation pond and sampling point)

Physico-chemical analysis of the sewage sample and homogeneous media

The mixture of sewage effluent was analysed for physicochemical parameters before the commencement of the laboratory experiment. In the experimental setup, some of the physico-chemical parameters were measured *in situ* daily which include Temperature, pH, Conductivity, Salinity, Total dissolved solid using a Gowe[®] Multi-Function Water Quality Test Monitor as well as Dissolved Oxygen using Milwaukee D.O. meter. Other physico-chemical parameters such as biological oxygen demand, nitrate, total suspended solids, alkalinity, total solids, total acidity, phosphate, and chemical

oxygen demand were determined once before every water turnover according to the method of APHA *et al.* (1992; 1995) and Golterman *et al.* (1978).

Acute toxicity test

The method of Rand (2008) was employed in range finding test for the definitive concentrations used in evaluating acute toxicity of the sewage effluent on the *Clarias gariepinus* juvenile. The pooled sewage effluent samples were properly mixed before been used at the different concentration of exposure in five labelled tank A to E. The different concentrations used A = 0 (ordinary water, serving as experimental control), B = 7.5L, C = 15L, D = 22.5L and E = 30L which were 0%, 25%, 50%, 75% and 100% of sewage sample respectively were prepared by the appropriate dilution with tap water. Ten fishes were exposed to each concentration for 96 hours during which the behavioural responses, percentage mortality at 96 hours, mortality after 96 hours, and LC₅₀ (concentration at which half or 50% of the test organism died on exposure) of the fishes were evaluated. The 96-h LC₅₀ of the fish was determined from the graph of percentage mortality against concentration (Figure 2).

Chronic toxicity test

The fish specimens were exposed to varying fractions concentration of the 96-hr LC_{50} value obtained from the acute toxicity test which were $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ and 0 of the 96-h LC_{50} value obtained which were 8.24L, 4.12L, 2.06L, 1.03L and 0L respectively and 50%, 25%, 12.5%, 6.25% and 0% respectively in concentration. The test solution was changed every 48 hours for 90 days.

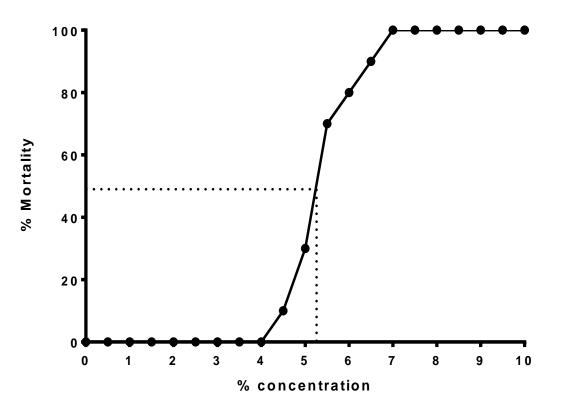


Fig 2: 96h LC₅₀ value for *Clarias gariepinus* fingerlings exposed to different concentration of sewage effluent during the period of study

Evaluation of fish growth parameters

The experimental fish was weighed on a sensitive weighing balance (in grams). Fish in each experimental tank were fed 5% of their body weight twice daily, six days a week. Sampling done fortnightly for growth indices determinations were also used for ration allotment to allow for fish weight gain. According to Pitcher and Hart (1982) and Burel *et al.* (2000) as shown in Table 1, the fish growth performance, the quantity of the feed consumed, and the feed utilization indices were determined using the data generated.

Collection of blood and hematological analysis

2 ml of experimental fish blood sample obtained from the caudal vein using 5ml sterile plastic syringe fitted with 0.8 x 40 mm hypodermic needle into heparinized bottle coated with anticoagulant. After blood collection, the test fish was then separately placed in an aerated tank for necessary recovery. The fish hematological method as designed by Blaxhall (1973) was used in this study. Erythrocytes counted from the small squares of the improved Neubauer hemocytometer were used to estimate the Red blood cell (RBC) count (RBC $\times 10^6 \,\mu$ l). The hematocrit (PCV, %) level of the fish blood sample was determined by collecting the blood sample through capillary action into heparinized capillary tubes in duplicate and centrifuging at 13000 rpm for 4 minutes in a centrifuging machine. Hemoglobin concentration (Hb, g/dl) evaluation was done using the photometrical cyanohemoglobin method following the standard formula by Svobodova (2001). The routine clinical method of Wintrobe (1978) was followed in evaluating the white blood cell (WBC) count of the test fish.

From the data generated for RBC, PCV and Hb, the Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Mean Corpuscular Volume (MCV) were calculated according to the established formula of Torts *et al.* (1988).

Statistical analysis

Data generated were presented as means and standard errors of the mean (\pm SEM). The statistical analysis was performed using Graph Pad Prism Graphical – Statistical Package Version 5. The differences in the mean values of the determined parameters across the treatments were determined by one-way ANOVA and comparisons of the means were done using the Bonferroni test. Mean values are considered to be significantly different at p < 0.05 level.

Results

The result of the physico-chemical parameters of the sewage effluent and the homogenous media is shown in Table 2. The analysed physicochemical parameters of the raw sewage effluent revealed that the conductivity, total solids and total dissolved solids level of the sample were higher compared to the other parameters such as BOD₅, salinity, nitrate, phosphate and dissolved oxygen analysed in the sewage sample.

Generally, among the physico-chemical parameters analysed in the homogenous media, temperature and pH were the only parameters with no significant difference (p>0.05) across the treatment, however highest temperature and pH values (27.09 \pm 0.04 °C and 7.13 \pm 0.04 respectively) were recorded in exposure treatment with 2.06 and 8.2% sewage concentration respectively (Table 2).

Apart from dissolved oxygen and biological oxygen demand levels of the culture media which significantly (p<0.05) decreased with increasing sewage concentration, all other determined

parameters (with the exception of pH and temperature) increased (p < 0.05) as the sewage concentration increases (Table 2).

S/N	Parameter	Formula	Reference		
	Growth parameter Indices				
1.	Specific Growth Rate (SGR)	(Log Wf - Log Wi)X 100	Brown (1957)		
2.	Daily Weight Gain (DWG)	$\frac{D}{D}$	Pitcher and Hart (1982)		
3.	Mean Weight Gain (MWG)	W_{f} - W_{i}	Pitcher and Hart (1982)		
4.	Percentage Survival	Nf x 100	Deyab et al. (2009)		
5.	Body Weight Gain (BWG)	MWGx 100 Wi	Davies and Ezenwa (2010)		
_	Feed Utilization Indices	NAMA	W/1 (1000)		
5. 7.	Protein Efficiency Ratio (PER) Food Conversion Ratio (FCR)	MWG <u>PI</u> TFI <u>MWG</u>	Wilson (1989) Burel <i>et al.</i> (2000)		
8.	Protein Intake (PI)	Diet Protein Content x DFI	Sveier <i>et al</i> . (2000)		
9.	Total Feed Intake (TFI)	TWF	Abdel-Hakim et al. (2008)		
10	Daily Feed Intake (DFI)	Nc TFI D	Abdel-Hakim et al. (2008)		

3 8 7 4 9 3 9

Where; W_f = Final Fish Weight; W_i = Initial Fish Weight; D = Duration of Fish Rearing; N_f = final number of fish after the experiment, Ni = initial number of fish at the start of the experiment, TWF = Total Weight of feed fed; Nc = number of currently exposed fish, MWG =Mean Weight Gain, TFI = Total feed intake

The total suspended solid (73.67 ± 14.47) and nitrate (0.31 ± 0.09) levels of the homogenous media with 1.03% as compared with the control (62.67 \pm 12.81 and 0.27 \pm 0.04 respectively) were the only parameters that were not significantly different (p>0.05) among the exposed treatments.

Generally, conductivity levels were higher irrespective of the treatment compared to other determined physicochemical parameters. This was closely followed by total solids (Table 2).

Fish growth performance and feed utilization

Fish in the control tank had the least mortality rate (Table 3). The exposed groups had lower survival rate with increase in concentration through the 90 days exposure period (Table 3). Generally, as shown in Table 3, when compared to the fish exposed to varied sewage concentration, the fish in the control tank exhibited considerably (p>0.05) higher growth and feed utilization indices.

Except the feed conversion ratio which increases (p<0.05) with increasing sewage concentration, all other fish growth and feed utilization indices decreased (p<0.05) as sewage concentration increases. Among the exposed treatments, the treatments with highest sewage concentration had significantly (p<0.05) poor growth performance and feed utilization indices (Table 3).

Parameters	Raw Municipal	Sewage concentration					FEPA (1991)	NESREA	USEPA
	Sewage	0 %	1.03 %	2.06 %	4.1%	8.2%	- Limit	(2009) Limit	(2009) Limit
Temperature (°C)	27.00±0.21	26.95	26.80	27.09	26.77	26.93	< 40	-	-
		$\pm 0.02^{a}$	$\pm 0.02^{a}$	$\pm 0.04^{a}$	$\pm 0.01^{a}$	$\pm 0.01^{a}$			
рН	7.24±0.07	6.88 ± 0.05^{a}	6.99 ± 0.05^{a}	7.05 ± 0.03^{a}	7.11 ±0.04 ^a	7.13 ±0.04 ^a	6.50-8.50	6.00-9.00	6.50-8.50
Conductivity (µS/cm)	626.83±67.75	164.72 ± 4.07^{a}	259.33 ±12.33 ^b	345.50 ±20.17°	426.28 ± 27.14^{d}	512.67 ±35.38 ^e	-	1000	-
Dissolved Oxygen (mg/l)	2.75±1.60	$9.50 \pm 0.65^{\rm d}$	2.99 ±0.16 ^c	$2.18\pm\!0.37^{b}$	1.74 ± 0.50^{ab}	1.38 ± 0.08^{a}	5.00	2 - 5	-
Chemical Oxygen Demand (mg/l)	35.40±2.11	3.27 ± 0.26^{a}	8.32 ± 0.54^{b}	12.11 ±0.39 ^c	15.90 ± 0.69^{d}	25.36 ±0.47 ^e	-	1000	-
Biological Oxygen Demand (mg/l)	0.07±0.01	6.10 ± 0.15^{d}	4.13 ±0.31 ^c	4.56 ±0.11 ^c	2.21 ±0.09 ^b	1.07 ± 0.01^{a}	50.00	50	250
Salinity (psu)	0.56±0.02	$\begin{array}{c} 0.08 \\ \pm 0.02^d \end{array}$	0.13 ±0.09 ^c	0.21 ± 0.04^{c}	0.33 ±0.02 ^b	$\begin{array}{c} 0.38 \\ \pm 0.02^{a} \end{array}$	-	-	-
Alkalinity (mgCaCO ₃ /l)	209.67±22.20	21.18 ± 2.84^{a}	58.63 ± 4.25^{b}	97.75 ±7.20 ^c	135.39 ±9.65 ^d	168.30 ±11.42 ^e	45	45	-
Acidity (mgCaCO ₃ /l)	89.00±14.26	12.80 ± 1.87^{a}	31.38 ± 2.90^{b}	$47.86 \pm 4.56^{\circ}$	62.05 ± 6.81^{d}	74.86 ±7.21 ^d	-	-	-
Total Dissolved Solids (mg/l)	407.00±35.10	87.33 ±3.94 ^a	129.5 ±4.12 ^b	$159.33 \pm 6.53^{\circ}$	191.00 ±6.22 ^d	224.33 ±8.69 ^e	2000.00	500	500
Total Suspended Solids (mg/l)	200.17±29.42	62.67 ± 12.81^{a}	73.67 ±14.47 ^a	125.00 ±24.95 ^b	168.50 ± 28.16^{bc}	172.33 ±7.83 ^c	30.00	50	-
Total Solids (mg/l)		157.39	227.06	314.28	389.78	468.00	_		
i otal bolido (ing i)	607.17±36.32	$\pm 4.94^{a}$	$\pm 9.44^{b}$	$\pm 17.24^{\circ}$	$\pm 21.02^{d}$	$\pm 28.72^{e}$		-	-
Nitrate (mg/l)	0.87±0.12	0.27	0.31	0.59 ± 0.09^{b}	0.66	0.76	20.00	10	10
		$\pm 0.04^{a}$	$\pm 0.09^{a}$		$\pm 0.05^{\mathrm{b}}$	±0.02 ^c		-	-
Phosphate (mg/l)	4.99±0.11	0.04 ± 0.01^{a}	0.08 ± 0.01^{b}	0.95 ± 0.08^{c}	2.48 ± 0.07^{d}	3.69 ±0.10 ^e	-	5	-

Table 2: Physico-chemical properties of the Municipal Sewage and the Homogenous Media

FEPA-Federal Environmental Protection Agency NESREA-National Environmental Standards and Regulations Enforcement Agency USEPA-United States Environmental Protection Agency for maximum permissible limits for effluent from wastewater

*Mean values within the same row with the same superscript are not significantly different (p>0.05)

Effects of municipal sewage on the haematological indices of Clarias gariepinus juveniles

In all the heamatological parameters determined after 90 days of exposure, only red blood cell count (RBC) levels of the exposed fish were insignificant (p<0.05) across the treatment (Table 4).

However, the white blood cell count (WBC) and mean corpuscular volume (MCV) increase (p<0.05) with increase in sewage concentration in the exposed fish (Table 4). The hemoglobin (Hb) level of the fish in highest sewage concentration (8.20%) was found to be lower (p<0.05) than the Hb level of the fish in other exposed groups and control which were not significantly different (p>0.05) from each other (Table 4). In addition, the fish in the control had highest heamatocrit level (p>0.05) compared to the exposed fish group up to 2.06% (Table 4).

The MCH and MCHC of the fish varied significantly (Table 4). Fish exposed to different sewage concentration had higher platelet count (p<0.05) compared to the control, however, the fish exposed to 2.06% sewage concentration had highest PLT count (Table 4).

Discussion

The pH of Sewage treatment during the exposure period ranged from 6.9 to 7.2 which was within safe limits of 6.0 to 8.5 as suggested by FEPA (2009), NESREA (2009), and USEPA (2009). The level of conductivity across the treatment tanks was also within the acceptable limits for aquatic organism (WHO, 1996; FEPA, 2009; NESREA, 2009; USEPA, 2009). While the recorded dissolved oxygen (DO) concentrations in all treatment tanks during the period of exposure were lower than permissible limits for aquatic life (FEPA, 2009; NESREA, 2009, USEPA, 2009).

The distress responses exhibited in the behaviour of the C. gariepinus during the toxicity test could be due to the effects of the chemical and biological constituents of the effluent which caused a quick depletion in oxygen concentration as C. gariepinus juveniles are known for high DO consumption. Higher levels of Biological Oxygen Demand (BOD) recorded in this study increase the rate of oxygen depletion due to the enormous quantity of organic matter that are biodegradable. This corresponds with the opinion of El-Sayed (2011) who reported that, DO rapidly depletes in sewage polluted water because of high BOD. However, this requires the critical necessity to assess the quantity of pollutants present in a given body of water. (El-Sayed, 2011). The result of low DO and high BOD in this study could explain the irregular swimming, frequent surfacing, and gasping for breath observed in the test organism's response to the environment which increased with exposure duration and concentration. The weakness and eventual comatose observed in the test fish over a long period of exposure with higher mortality in the high concentrations (75% and 100%) compared to (25% and 50%) could be linked to lower levels of oxygen and reduced amounts of chemical and biological constituents present. Consequently, abnormal behaviour and mortality of organism increase as concentration of pollutant and exposure duration increase. This corresponds with the submissions of Shobha et al. (2007) and Dahunsi et al. (2011) who opined that fish exposure to different concentrations of effluent can affect the feeding rate, color of the skin, pattern of swimming, and general behaviour of such fish.

High Total Solids (TS) and Alkalinity, was observed in the study throughout the duration of exposure. This is an indication that indiscriminate discharge of such effluent is potential source of toxicity to aquatic environment. The submissions of Adewoye *et al.* (2005) corroborate the observed effluent characteristics features as a result of organic loads in the wastewater. High Chemical Oxygen Demand (COD), may probably be due to high level of human faecal deposits and domestic wastes from the sewers, (Abdel-Satar, 2005; Abdo, 2010; Saad *et al.*, 2011) and an increase in water temperature

Table	e 3: Growt	h perform	ance and F	eed utiliza	tion of <i>C</i> . g	ariepinus (exposed to	varying co	oncentrati	on of sewa	ge effluent.
Concentrations	Initial Weight	Final Weight	MWG (g)	DWG (g)	SGR (%)	DFI (g)	TFI (g)	FCR	PI	PER	Mortality %
0%	7.65 ±0.02 ^a	76.79 ± 3.82^{d}	69.14 ±3.68 ^e	0.77 ±0.05 ^c	1.11 ± 0.17^{d}	0.08 ±0.01 ^c	6.87 ±1.15 ^c	0.09 ±0.01 ^a	3.20 ± 0.04^{d}	21.61 ±2.56 ^b	8
1.03%	7.65 ± 0.02^{a}	$60.76 \pm 5.69^{\circ}$	53.11 ±4.85 ^d	$0.59 \\ \pm 0.07^{b}$	0.99 ±0.04 ^c	0.07 ±0.03 ^{bc}	6.46 ±1.36 ^{bc}	$\begin{array}{c} 0.12 \\ \pm 0.01^{b} \end{array}$	2.80 ±0.16 ^c	18.97 ± 4.07^{ab}	25
2.06%	7.65 ±0.02 ^a	56.84 ± 3.16^{b}	49.19 ±1.38°	0.55 ±0.13 ^b	0.97 ±0.06 ^{bc}	$\begin{array}{c} 0.07 \\ \pm 0.02^{\mathrm{bc}} \end{array}$	6.06 ±1.13 ^{bc}	$\begin{array}{c} 0.12 \\ \pm 0.02^{\text{b}} \end{array}$	2.80 ±0.10 ^c	17.57 ±3.22 ^a	33
4.1%	7.65 ±0.02 ^a	52.84 ±4.03 ^b	45.27 ±2.05 ^b	$0.50 \\ \pm 0.11^{b}$	0.93 ± 0.03^{b}	$\begin{array}{c} 0.06 \\ \pm 0.01^{\text{b}} \end{array}$	$5.87 \\ \pm 0.58^{\rm b}$	0.13 ±0.05 ^{bc}	$\begin{array}{c} 2.40 \\ \pm 0.09^{b} \end{array}$	18.86 ± 5.01^{ab}	42
8.2%	7.65 ±0.02 ^a	35.38 ±6.33 ^a	27.73 ±5.17 ^a	0.31 ±0.03 ^a	0.74 ±0.15 ^a	0.04 ±0.01 ^a	4.35 ±0.46 ^a	0.16 ±0.02 ^c	1.60 ±0.17 ^a	17.33 ±3.08 ^a	58

MWG – Mean Weight Gain; DWG – Daily Weight Gain; SGR – Specific Growth Rate; DFI – Daily Feed Intake; TFI – Total Feed Intake; FCR – Feed Conversion Ratio; PI – Protein Intake; PER – Protein Efficiency Ratio *Mean values with the same superscript for same parameter are not significantly different (P<0.05)

Concentration (L)			Parameters					
	WBC (µl)	RBC (µl)	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLT (µl)
Control	$14.54{\pm}0.90^{a}$	$3.03{\pm}0.39^{b}$	10.04 ± 0.21^{b}	35.22±0.23 ^c	105.82 ± 3.82^{a}	$39.84{\pm}1.89^{b}$	$32.84{\pm}0.83^{a}$	25.46 ± 0.28^{a}
1.03	$31.42{\pm}1.78^{b}$	$3.20{\pm}0.27^{b}$	11.52 ± 0.74^{b}	33.69 ± 1.40^{bc}	111.39±4.70 ^b	34.25 ± 2.42^{a}	37.41±0.34 ^c	64.38±1.54 ^c
2.06	34.31±0.23 ^c	2.96 ± 1.91^{b}	11.20±0.60 ^b	$34.04{\pm}1.58^{c}$	$115.34{\pm}1.07^{b}$	41.14 ± 0.98^{b}	36.01 ± 0.64^{c}	$69.87{\pm}0.24^d$
4.12	$39.14{\pm}1.58^{d}$	$2.88{\pm}0.89^{b}$	$10.54{\pm}0.40^{b}$	32.01 ± 0.89^{b}	120.27±2.61 ^c	41.12 ± 0.74^{b}	36.29 ± 0.42^{c}	$64.24 \pm 0.98^{\circ}$
8.24	41.32±0.82 ^e	2.51±0.94 ^b	6.64 ± 0.62^{a}	29.02±1.25 ^a	122.94±1.74 ^c	46.98±1.68 ^c	34.43±1.26 ^b	60.65 ± 1.40^{b}

Table 4: Heamatological indices of *Clarias gariepinus* exposed to varying Municipal sewage concentrations for 90 days

Table 4: Heamatological indices of *Clarias gariepinus* **exposed to varying Municipal sewage concentrations for 90 days.**RBC = Red Blood Cell; HCT = Hematocrit; WBC = White Blood Cell; Hb = Haemoglobin; PLT=Platelets; MCH = Mean Corpuscular Haemoglobin; MCV-Mean Cell Volume MCHC = Mean Corpuscular Haemoglobin Concentration.

*Mean values with the same superscript within the same column are not significantly different (P>0.05).

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during culture, which probably accelerated the rate of oxidation of organic matter (Abdo, 2004 and 2010).

Pollutants or presence of toxicants in culture media has been reported to affect the quantity of food consumed by fish (Ugwu et al., 2006 and Elevele et al., 2017). The introduction of the sewage effluent in the media tank could also be the reason for the decrease in the feed consumption (TFI) level in the exposed group which consequently led to a decrease in DWG, MWG and BWG of exposed fishes with increase in exposure period and concentration. However, fish under the control treatment apparently fed better than the exposed fish which was in support of Ugwu et al. (2006) and Elevele et al. (2017) findings. The increase count of WBC with increase in sewage effluent concentration recorded in the exposed fishes may be due to the effect of the test fish fighting against the pollutants which resulted in the higher antibodies (WBC) production to enhance or promote the organism's good health status. This conforms to the report of Dahunsi and Oranusi (2013) that WBC count increase during acute and sub-lethal treatment probably because of lymphomyeloid tissue that is stimulated as a defense mechanism for toxicity tolerance in fish. The observed least level of hematocrit (HCT), Haemoglobin (Hb) and red blood cell (RBC) in exposed fish with highest sewage concentration compare to other treatments in this study could be as a result of the pollutant's influence on body fluid, which matches the trend seen in other fish species exposed to pollution as reported by Sancho et al. (2000), Seth and Saxena (2003), Adhikari et al. (2004), Gholami-Seyeskolaei et al. (2013) and Bacchetta et al. (2014). An increase in MCV, MCH and MCHC as seen in the fishes exposed to high sewage concentration compare to the control can be attributed to and explained as an anemic response to erythropoiesis and haemosynthesis suppression, as well as an increase in the rate at which erythrocyte is being destroyed in haematopoietic organs (Svoboda et al., 2001; Gabriel and Ugbomeh, 2016). Platelets elevation in the exposed fish with increase in sewage effluent concentration suggests an interference of the pollutant with thrombocytopoiesis in the bone marrow as suggested by Gabriel and Ugbomeh (2016).

Conclusion

This study revealed that even at the lowest level of sewage concentration exposure, significant impacts which were detriment to fish growth, feed utilization and anomaly in blood parameters was observed which indicate the high levels of pollutants in the sewage. This study therefore concluded that improper discharge of sewage effluent into aquatic environment does not only inhibit fish growth nor cause anomaly in blood parameters with resultant negative eco-physiology of the fish *C. gariepinus*, it also alters the physicochemical properties of water and with continuous discharge overtime the aquatic environment will become unsafe for the aquatic organism.

References

- Abdel-Moneim A.M, Al-Kahtani , M.A., and Elmenshawy O.M. (2012). Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia. *Chemosphere* 88(8):1028–1035. doi:10.1016/j.chemosphere.2012.04.001
- Abdel-Satar, A. M. (2005). Water quality assessment of River Nile from Idfo to Cairo. *Egyptian Journal of Aquatic Research*, 31(2):200 223.
- Abdel-Hakim N.F., Lashin M.E., Al-Azab A.A., Nazmi H.M. (2008). Effects of Replacing Soybean Meal with other Plant Protein Sources on Protein and Energy Utilization and Carcass Composition of Nile Tilapia (*Oreochromis niloticus*). 8th International Symposium on Tilapia in Aquaculture p. 979-95
- Abdo, M. H. (2004). Seasonal variations of some heavy metals in macrophytes and water of Damietta branch, River Nile, Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 8:195-211.
- Abdo, M. H. (2010). Environmental and water quality evaluation of Damietta branch, River Nile, Egypt. *African Journal of Biological Sciences*, 6(2):143-158.
- Abrahamson A., Andersson C., Jönsson M.E., Fogelberg O, Örberg J, Brunström B, and Brandt I. (2007). Gill EROD in monitoring of CYP1A inducers in fish—a study in rainbow trout (*Oncorhynchus mykiss*) caged in Stockholm and Uppsala waters. *Aquatic Toxicology*, 85(1):1–8. doi:10.1016/j.aquatox.2007.07.013
- Ademoroti, C.M.A, and Uwidia I. E. (2011). Characterisation of Domestic Sewage from an estate in Warri, Nigeria. *International Journal of Chemistry*, 3(3): 81.
- Adhikari, S, Sarkar, S., Chatterjee, A., Mahapatra, C. T., and Ayyappan S. (2004). Effects of Cypermethrin and Carbofuran on certain hematological parameters and Prediction of other recovery in a freshwater teleost, *La-beo rohita* (Hamilton). *Ecotoxicology and Environment Safety*, 58(2):220-226.
- Adewoye, S.O., Fawole, O.O., Owolabi, O.D., and Omotosho, J.S., (2005). Toxicity of cassava wastewater effluent to African catfish: *Clarias gariepinus. Ethiopian Journal of Science*, 28 (7):189-194.
- Aguayo S., Muñoz M.J., De La Torre A., Roset J., De La Peña E., and Carballo M. (2004). Identification of organic compounds and ecotoxicological assessment of sewage treatment plants (STP) effluents. *Science of the Total Environment*, 328(1–3), 69–81. doi:10.1016/j.scitotenv.2004.02.013.
- Akinbadewa A. O., Adewole, H.A., Olaleye, V.F., and Ogundepo, G.E. (2020). Sublethal Effect of Glyphosate [N-(Phosphonomethyl) Glycine] on Growth Performance and Biochemical Activities in Some Organs of *Clarias gariepinus* (Burchell, 1822) Fingerlings. *Journal of Agricultural Sciences*, 31(2):122-130
- Al-Ghais S.M. (2013). Acetylcholinesterase, glutathione and hepatosomatic index as potential biomarkers of sewage pollution and depuration in fish. *Marine Pollution Bulletin*, 74(1):183–186. doi:10.1016/j.marpolbul.2013.07.005
- APHA (American Public Health Association), AWWA (American Water Works Association), WPCF (Water Pollution Control Facilities) (1992). Standard Methods for the Examination of Water and Wastewater, 18th Ed. American Public Health Association Washington, D.C.:1289

- APHA (1995). Standard Methods for the Examination of Water and Wastewater, 19th Edition, American Public Health Association, New York
- Bacchetta, C., Rossi, A. Ale, A. Campana, M., Parma M. J., and Cazenave J. (2014). Combined toxicological effects of pesticides: A fish multi- biomarker approach. *Ecological Indicators*, 36:532-538.
- Bhatia N. P, Sanhu, G. S, and Johal, M. S. (2004). Haematological alterations in *Heteropneuses fossilis* upon exposure to endosulfan. *Pollution Research*. 23(4):633-636.
- Blaxhall P. C. and Daisley K. W. (1973). Routine haematological methods for use in fish blood. *Journal of Fish Biology*, 5(6):771-781.
- Brown, M. E. (1957). Experimental studies on growth. In: *The Physiology of Fishes*. Academic Press, New York. Vol. 1, 361-400 pp.
- Burel C., Boujard T., Kaushik S.J., Boeuf G., Van der Geyten S., Mol K.A., Kuhn E.R., Quinsac A., Krouti M., and Ribaillier D. (2000). Potential of plant-protein sources as fish meal substitutes in diets for turbot (*Psetta maxima*): growth, nutrient utilisation and thyroid status. *Aquaculture* 188:363–382. <u>https://doi.org/10.1016/S0044-8486(00)00342-2</u>
- Burel C, Boujard, T. Tulli, F., and Kaushik S. J. (2000). Digestibility of Extruded Peas, Extruded Lupin and Rapeseed Meal in Rainbow Trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture, 188 (3-4):285 298.
- Cambray, J.A. (2003). The need for research and monitoring on the impacts of translocated sharptoothed catfish, *Clarias gariepinus*, in South Africa. *African Journal Of Aquatic Science*, 28(2):191-195.
- Carolyn, J. M. B. (2010). Fish communities near municipal wastewater discharges in the Grand river watershed. MSc thesis, University of Waterloo, Ontario, Canada. P. 13
- Dahunsi, S.O, Oranusi, S.U and Ishola, R.O. (2011). Biochemical profile of *Clarias gariepinus* exposed to sub-lethal concentrations of chemical additives effluent. *International Journal* of Research in Environmental Science and technology, 1 (4):52-58.
- Dahunsi, S.O. and Oranusi, U. S. (2013). Haematological response of *Clarias gariepinus* to rubber processing effluent. *Annual Review and Research in Biology*, 3(4):624-635.
- Davies, O. and Ezenwa N. (2010). Groundnut cake as alternative protein source in the diet of *Clarias gariepinus* fry. International Journal Science and Nature, 1:73-76
- de la Torre F.R., Salibián A., and Ferrari, L. (2007). Assessment of the pollution impact on biomarkers of effect of a freshwater fish. *Chemosphere*, 68(8):1582–1590. doi:10.1016/j.chemosphere.2007.02.033.
- Deyab E., Samy M. and Hayam T. (2009). Nutrition evaluation of sesame seed meal, Sesamum indicum (L.) as alternative protein source in diets of juvenile mono-sex Nile Tilapia (Oreochromis niloticus). Egyptian Journal of Aquatic Biology and Fisheries. 13. 93-106. 10.21608/ejabf.2009.2026.
- Diaz, P., Gappa, J. J. L. and Piriz M. L. (2002). Symptoms of eutrophication in intertidal macroalgal assemblages of Neuvo Gulf, Patagonia, Argentina. *Marine Botanical*, 45(3): 267-273.
- Eleyele, O. A., Aladesanmi, O. T., Akinwumi, K. O., and Okonji E. R. (2017). Toxicity of multivitamins production effluent. A study of oxidative stress in *Clarias gariepinus* (Burchell 1822). *International Research Journal of Public and Environmental Health*, 4 (6):96-111.
- EL-Sayed, S. (2011). Physicochemical studies on the impact of pollution up on the River Nile branches, Egypt. M.Sc. Thesis Faculty of Science, Benha University, Egypt. Pp 125

- Fakolujo, O.I., Adewole, H.A., Obuotor, E.M., and Olaleye, V.F. (2018). Growth performance and selected biochemical indices assessment in the serum and liver of *Clarias gariepinus* juveniles exposed to varying concentrations of water-soluble fraction of crude oil. *Borneo Journal of Science and Technology*. 39(1):16-27.
- FEPA (1999) Federal Environmental Protection Agency. National Environmental Protection (Effluent Limitations) Regulations 1991 as cited by Odiete 1991. In: Environmental Physiology of Animals and Pollution, Published by Diversified Resources Ltd., Lagos, Nigeria. Pp. 157 – 219.
- Gabriel, O. and Ugbomeh, A. (2016). Effects of cypermethrin on haematological parameters of African Freshwater Catfish, *Clarias gariepinus. Journal of Aquatic Science*, 31(2B):433-442.
- Garg, S. K. (2006). Sewage Disposal and Air Pollution Engineering. In: Environmental Engineering. 18th Edn., *Khanna Publishers*, New Delhi, 2: 228-278.
- Gholami-Seyeskolaei, S.J., Mirvaghefi, A. Farmhand H., and Kosari, A. A. (2013). Effect of a glyphosate based herbicide in *Cyprinus carpio*: Assessment of acetylcholinesterase activity, haematological responses and serum biochemical parameters. *International Journal of Ecotoxicology and Environmental Safety*, 98:135-141.
- Golterman, H.L., Clymo, R.S. and Ohnstad, M.A.M. (1978). Methods for Physical and Chemical Analysis of Freshwater. Oxford, Edinburgh, London, Melbourne. Blackwell Science Publication, 214p.
- Gomez-Couso, H., and Mendez-Hermida, B. (2006). Cryptosporidium contamination in harvesting areas of bivalve molluscs. *Journal of Food Protection*, 69(1):185-190.
- Henry, J. G., and Heinke, G.W. (1989). Environmental Science and Engineering, Prentice Hall, Eaglewood Cliffs, N.J. 478 p.
- Johal, M. S. and Grewal, H. (20040. Toxicological study on the blood of *Channa punctatus* (Bloch) upon exposure to carbaryl. *Pollution Research*, 23(1):601-606.
- Katyal, T. and Satake, M. (2001). *Environmental Pollution*. New Dehli, Anmol Publications, Pvt. Ltd.
- Kerambrun E., Sanchez W., Henry F., and Amara R. (2011). Are biochemical biomarker responses related to physiological performance of juvenile sea bass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) caged in a polluted harbour? *Comparative Biochemistry and Physiology Part C: Toxicology*. 154(3):187–195. doi:10.1016/j. cbpc.2011.05.006
- Maier D., Blaha L., Giesy J.P., Henneberg A., Köhler H.R., Kuch B., Osterauer R., Peschke K., Richter D., Scheurer M., and Triebskorn R (2014). Biological plausibility as a tool to associate analytical data for micropollutants and effect potentials in wastewater, surface water, and sediments with effects in fishes. *Water Research*. doi:10.1016/j. watres.2014.08.050
- Moore M.N., Depledge M.H., Readman J.W. and Paul-Leonard D.R. (2004). An integrated biomarker-based strategy for ecotoxicological evaluation of risk in environmental management. *Mutat Res Fundam Mol Mech Mutagen*, 552(1–2):247–268. doi:10.1016/j.mrfmmm.2004.06.028
- NESREA National Environmental Standards and Regulation Enforcement Agency (2009). Federal Republic of Nigeria Official Gazette, National Environmental (Sanitation and Waste Control). Federal Government of Nigeria Printer, Abuja, Nigeria, FGP 112/102009/L000 (OL54). No. 60 (96):1057 – 1102.

- Nwani C.D., Nwachi, D.A., Ogokwu, O.I., Ude, E.F. and Odoh, G.E. (2010). Heavy metals in fish species from lotic freshwater ecosystem at Afikpo, Nigeria. *Journal of Environmental Biology*. 31(5):595-601.
- Obiezue, R. S., Ikele, C. B., Mgbenka, B. O., Okoye, I. C., Attamah, G. N., Uchendu, C., Ezeamachi, E., and Onyia, C. Q. (2014). Toxicity study of diethyl phthalate on *Clarias* gariepinus fingerlings. *African Journal of Biotechnology*. 13(7):884-896.
- Ogunfowokan, A. O., Okoh, E. K., Adenuga, A. A., and Asubiojo, O. I. (2005). An assessment of the impact of point source pollution from a university sewage treatment oxidation pond on a receiving stream a preliminary study. *Journal of Applied Science*, 5(1):36 43.
- Pitcher, T. J. and Hart, P. J. B. (1982). *Fisheries Ecology*. Chapman and Hall, London, UK. ISBN 0-7099-2057-1.
- Ramesh, M., Saravan, M. and Kavitha, C. (2009). Hormonal responses of the fish, *Cyprinus carpio*, to environmental lead exposure. *African Journal of Biotechnology*, 8(17):4154-4158.
- Rand, G. M. (2008). Fish toxicity studies. In: Di-Giulio RT, Hinton DE (Eds.) The toxicology of fishes. CRC Press, New York, USA:659-682.
- Saad, S. M. M., El- Deeb, A. E., Tayel, S. I. and Ahmed, N. A. M. (2011). Haematological and histopathological studies on *Clarias gariepinus* in relation to water quality along Rossetta branch, River Nile, Egypt. *Journal of Experimental Biology* (Zool.), 7(2):223 – 233.
- Sancho, E., Ceron, J. J. and Ferrando, M. D. (2000). Cholinesterase activity and haematological parameters as biomarkers in sublethal molinate ex-posure in *Aguilla anguilla*. *International Journal of Ecotoxicology and Environmental Safety*, 46(1):81-86.
- Seth, N. and Saxena, K.K. (2003). Haematological response in a freshwater fish, *Channa punctatus* due to fenvalerate. *Bulletin of Environmental and Contamination Toxicology*, 71(6):1192-1199
- Sevcikova, M., Modra, H, Slaninova, A., and Svobodova, Z. (2011). Metals as a case of oxidative stress in fish: A review. Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic Veterinarni Medicina, 56(11):537-546.
- Shobha, K., Poornima, A., Harini, P., and Veeraiah, K., (2007). A study on biochemical changes in the fresh water fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant Cadmium chloride. *Kathmandu University Journal of Science, Engineering and Technology*. 1(4):1-11.
- Siroka, Z. and Drastichova, J. (2004). Biochemical markers of aquatic environment contamination-cytochrome P450 in fish. A review. *Acta Veteriniria Brno*, 73:123-132
- Sveier H., Raae A.J., and Lied E. (2000). Growth and protein turnover in Atlantic salmon (*Salmo salar* L.); the effect of dietary protein level and protein particle size. *Aquaculture* 185: 101-120.
- Svoboda, M., Luskova, V., Drastichova, J. and Llabok V. (2001). The effect of diazinon on hematological indices of common carp (*Cyprinus carpio*). Acta Veterinaria Brunensis (Brno), 70, 457-465.
- Taiwo, A.M., Olujimi, O. O., Bamigbose, O., and Arowolo, T. A. (2012). Surface Water Quality Monitoring in Nigeria: Situational Analysis and Future Management Strategy, Water Quality Monitoring and Assessment, Dr. Voudouris (Ed.), ISBN: 978-953-51-0486-5.

- Torts L, Torres, P. and Hidaglo, J. (1988). The effects of sub-lethal concentrations on the haematological parameters of dogfish. *Scyllorhinus canicule*. *Journal of fish Biology*, 32:277-282.
- Ugwu, L. L. C., Mgbenka, B. O, Eneje, L. O, Ude E. F, and Nwenga J. I. (2006). Toxicity, Growth and Survival of *Clarias gariepinus* juveniles exposed to different concentrations of crude oil fractions-polluted water. *International Journal of Animal Research*, 3(2): 466-472.
- USEPA, (2009). Drinking Water Contaminants. Washington, DC. (Available online: http://water.epa.gov/drink/contaminants/index.cfm#Lis.)
- Vincze K, Graf K, Scheil V, Köhler H-R. and Triebskorn, R. (2014). Embryotoxic and proteotoxic effects of water and sediment from the Neckar River (Southern Germany) to zebrafish (*Danio rerio*) embryos. *Environment Science*, 26(3):1–13.
- Vincze K, Scheil V, Kuch B, Köhler, H.R., and Triebskorn R. (2015). Impact of wastewater on fish health: a case study at the Neckar River(Southern Germany) using biomarkers in caged brown trout as assessment Tools *Environmental Science and Pollution Research* 22:11822–11839. DOI 10.1007/s11356-015-4398-6
- WHO (1996). Guidelines for drinking water quality. 2nd (Ed.). Health criteria and supporting information, WHO, Geneva Switzerland.
- Wilson, R. P. (1989). Amino Acids and Proteins. pp 111–151. In: *Fish Nutrition. Second Edition*, J. E. Halver (ed). Academic Press, New York.
- Wintrobe M. M (1978). Clincal haematology, London, H. Kimpton press.;448.