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The Effects of Exposure to Antibiotic Waste Water on Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Fresh water ecosystem are frequent sinks for effluents including pharmaceutical waste water where they may be toxic to aquatic life. The aim of this study was to evaluate the acute toxicity and characterize the effect of antibiotic effluents on the morphology, haematology and activity of liver antioxidants of *Oreochromis niloticus*. *O. niloticus* fingerlings were exposed in duplicates to 20%, 40%, 60%, 80%, and 100% effluent concentrations, while juveniles with mean total length of 17 cm and mean weight of 77.89 g were exposed to sub-lethal concentrations (1/20th and 1/10th of the 96hrs LC_{50}) for 21 days. The 24hrs, 48hrs, 72hrs and 96hrs LC_{50} was 59.337 (50.69−68.79) %, 51.50 (27.19 – 74.51) %, 48.70 (41.65 – 55.49) % and 46.853 (40.04 – 53.37) % respectively. The liver weight and hepato somatic index (HSI) reduced significantly in fishes exposed to both sub-lethal concentrations compared to control ($p<0.05$). Red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb), super oxide dismutase (SOD) activity and glutathione (GSH) level decreased while malondialdehyde (MDA) level increased significantly ($p<0.05$) in liver of fishes exposed to 4.68% (1/10th of 96hrs LC_{50}) and 2.34% (1/20th of 96hrs LC_{50}). The Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of the exposed fishes were not different from the control ($p>0.05$).The study showed that antibiotic effluents was moderately toxic to *O. niloticus* fingerlinger. Sub-lethal concnetrations reduced liver weights, induced anaemia, reduced antioxidants activities and induced oxidative stress in liver of exposed fishes.

Keywords*: Tilapia, oxidative stress, antibiotics, acute toxicity, anaemia.*

Introduction

Industries are vital to the economy of a nation. Pharmaceutical industries are research and development based businesses driven by the search for novel products to improve human health. Despite the health and economic benefits of the industry, the presence of pharmaceutical materials in the environment leaves a lot to be desired. Surface and ground water systems are the final destination of active pharmaceutical materials from industrial effluents, municipal waste water, animal excretion of veterinary drugs, and landfill leachate (Heberer, 2002; Petrović *et al.,* 2003; Musson and Townsend 2009; Anyakora *et al.,* 2012; Cardoso *et al.,* 2014; Rzymski *et al.,* 2017) where they poses serious danger to aquatic ecosystems (Boxall *et al.,* 2012) and contaminate ground water (Sui *et al.,* 2015) which is a major source of drinking water in countries like Nigeria.

The fate and concentration of pharmaceuticals in aquatic ecosystem depend on a number of factors relating to the chemical nature of the compound and environmental factors such as sunlight, temperature, pH, dissolved oxygen, and microbial activities (Rzymski *et al.,* 2017). Pharmaceuticals may undergo several processes which may lead to elimination in fresh water (Yamamoto *et al.,* 2009) and can also bio-accumulate in the aquatic ecosystem (Brodin *et al.,* 2014). Diclofenac, naproxen, ibuprofen, 17α-ethinyloestradiol, and antidepressants have been reported to accumulate in fish samples (Lahti *et al.* 2011; Du *et al.,* 2014). Bioaccumulation of pharmaceuticals in fish may be influenced by limited metabolism and elimination of pharmaceuticals in fish and continuous exposure to pharmaceutical active ingredients in aquatic ecosystem (Du *et al.,* 2014).

Pharmaceutical compounds found in surface waters include antibiotics, antidiabetic, anticonvulsant, analgesic, anti-inflammatory, antiepileptic, antidepressants and psychiatric drugs (Ramirez *et al.,* 2009; Fram and Belitz 2011; Blair *et al.,* 2013). Indeed effluents generated during drug manufacturing may also contain other hazardous substances such as organic materials, metals such as zinc, silver, cadmium, and thallium, acids, alkalis, nonmetallic elements such as arsenic or selenium (Anyakora *et al.,* 2011; Ramola and Singh 2013), which may be hazardous to human health and aquatic organisms including fish (Rzymski *et al.,* 2017). The toxicity of pharmaceutical effluents is due to the present of active pharmaceutical ingredients (API). APIs are biologically active as such can interact with specific pathways in biological systems (Boxall *et al.,* 2012). Certain

antidepressants and psychiatric drugs can reduce territorial aggression, feeding rate and activity while others can increase sociality and boldness in some fish species (Brodin *et al.* 2014).

In a developing countries like Nigeria, industrial effluents are seldom treated prior to their discharge into receiving water bodies. The constituents of the effluents may become a hazard to aquatic organisms including fish by altering key physiological process which are critical for the survival of these organisms in their habitat. The aim of the study was to evaluate the effect of antibiotic effluent on the haemology and liver antioxidants in Nile tilapia *Oreochromis niloticus*.

Materials and Methods

Experimental Design

Following standard static acute toxicity test procedure (APHA, 1985), *Oreochromis niloticus* fingerlings were exposed to pharmaceutical effluents for 96 hours to evaluate the acute lethal toxicity, while adult tilapia was exposed for 21 days to sub-lethal concentrations (1/10th and 1/20th of the 96h LC_{50}) of the effluent using semi-static test procedure to assess the effect of the effluent on the haematology and antioxidants in the liver.

Collection of Pharmaceutical Effluent and Determination of Physicochemical Parameters

At the time of sample collection, the pharmaceutical industry located in South-east, Nigeria was manufacturing antibiotics particularly amplicox and ampicillin. The effluent was collected at the point of discharge into a receiving water body and stored in a 25 liter gallon. The temperature, pH, electrical conductivity, dissolve oxygen (DO), and total dissolved solids (TDS) were measured in-situ using appropriate portable meters.

Collection and Acclimatization of Fish Samples

Oreochromis niloticus of uniform length and weight was procured from the fish farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and transported to the laboratory where they were acclimatized in plastic aquarium for one week to borehole water. The fishes were fed once daily with a commercial diet at a rate of 2% of the body weight (Sprague, 1969). Faeces and unused food in the aquarium were removed daily by siphoning before feeding. After acclimation period, fishes were transferred randomly into the plastic aquaria to test the effluent.

Acute toxicity test

The test solutions with the required concentrations were prepared by diluting the raw effluents with borehole water. Ten fingerlings were exposed to five concentrations, 100, 80, 60, 40 and 20% in duplicates in a static test for 96 hours. Mortalities were recorded after 24, 48, 72 and 96 hours and assessed by prodding the animals for body movement. Dead organisms were removed quickly. The physicochemical characteristics of the test solutions were measured before and at the end of the experiment.

Sub-lethal Toxicity Test

O. niloticus were exposed to sub-lethal concentrations of the effluent on for 21 days. Five adult size tilapias were exposed per concentration in replicates and at the end of the exposure period, samples from control and test concentrations and were collected to analyze the following haemalogical indices - packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), and white blood cell (WBC). Oxidative stress markers including superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) and glutathione (GLT) concentration were analyzed in liver homogenate.

Fish biometrics

Fish biometric including body and organ weight, length (standard and total) were measured with a weighing balance and meter rule respectively. Somatic indices including hepatosomatic index (HSI), gill somatic index (GSI), and heart somatic index (HSI) were calculated as the ratio of organ weight to body weight × 100.

Haematological Analysis

Blood samples were obtained from the fish by using haematocrit capillary tubes and transferred to EDTA bottle. Haematogical parameters – RBC, PCV, Hb and WBC were analyzed using Mindray BC-2300 Hematology Analyzer. Red cell indices - MCHC, MCH and MCV were calculated using RBC, PCV, and Hb values (Dacie and Lewis 2001) according to standard formulae

$$MCHC\ (g/dl) = \frac{\text{Hb}\ (g/dl) \times 100}{\text{PCV}\ (\%)}$$

$$MCH\ (pg) = \frac{\text{Hb}\ (g/dl) \times 10}{\text{RBC}}$$

$$MCV\ (fl) = \frac{\text{PCV}\ (\%) \times 10}{\text{RBC}}$$

Antioxidant Activities Oxidative Stress Analysis

The liver of the fish were rinsed in buffered saline. Liver samples were homogenized with 5.0 mL 4.0m phosphate buffer pH 8.0

and centrifuged at 3000rpm for 10 minutes. The supernatant was collected and used for the analysis. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 at 30 °C (Magwere *et al.,* 1997). Catalase (CAT) was assayed spectrophotometrically based on the measurement of a decrease in absorbance of the test sample by the induced decomposition of H_2O_2 in the presence of an analyte enzyme (Aebi *et al.,* 1983), while reduced glutathione (GSH) was determined by the method of Jollow *et al.,* (1974). Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.,* (1993).

Data Analysis

Lethal concentration was determined by probit analysis (Finney 1952). The mean values of the weight of the organs, somatic indices, haematological indices and antioxidants of the exposed fishes was compared with control groups using one-way ANOVA while turkeys test post hoc test was used to obtain the specific significant differences among the experimental group. All analysis was computed with SPSS version 19 except the dose response graph which was plotted with Statistica version 10. Acute Toxic Unit (Tua) was calculated using the formular; $Tua=1/ LC_{50}$

Results

The physicochemical characteristics of the pharmaceutical effluent and the test solutions are presented in Table 1 and Table 2 respectively.

Table 1 shows the physiochemical properties of the waste water sample compared with the NESREA standard. The waste water had a coffee brown colour and was objectionable while the NESREA 2009 regulation stated industrial effluents be colourless and odourless prior to their discharge into a receiving water body. The pH of the waster water was within the standard however there is no permissible limits for temperature, total dissolved solids, dissolved oxygen and electrical conductivity specified in NESREA 2009 regulation.

Table 1: Physicochemical Properties of Antibiotics Effluent

	pH	Temperature (ºC)	Total Dissolved Solids (mg/l)	Dissolved Oxygen (mg/l)	Electrical Conductivity (µcm/s)	Appearance
Effluent Sample	6.1±0.36	30.24±0.91	75.00±5.00	9.30±0.76	155.00±5.00	Coffee brown colour with characteristic antibiotics odour
NESREA 2009	6-9	NS	NS	NS	NS	Colourless, odourless and not objectionable

NS-Not specified

Table 2: Physicochemical Properties of Test Solutions During the Acute and Sub-lethal Toxicity Test

Toxicity Test	Concentration	pH	Temperature (ºC)	Total Dissolved Solids (mg/l)	Dissolved Oxygen (mg/l)	Electrical Conductivity (µcm/s)
Acute toxicity test solution	Control	7.03±0.68	27.10±0.21	125.0±101.60	4.20±1.05	250.00±201.79
	20%	7.20±0.87	27.08±0.15	108.33±81.0	2.50±2.01	216.33±161.82
	40%	7.17±0.93	27.09± 0.18	135.67±98.52	2.20±1.91	272.00±197.58
	60%	7.30±1.04	27.08±0.15	216.67±155.60	1.83±1.70	443.00±319.65
	80%	7.07±0.91	27.08± 0.15	235.67±168.63	1.53±1.53	465.67±331.37
	100%	7.20±1.04	27.10±0.20	276.67±136.10	1.37±1.42	502.00±388.73
Sub-lethal toxicity test Solution	Control	7.03±0.38	26.67±0.58	93.50±46.41	1.80±1.02	186.50±91.11
	1/20th of 96hr LC_{50}	6.85±0.07	26.33±1.15	73.00±12.73	1.20±0.28	147.50±26.16
	1/10th of 96hr LC_{50}	7.10±0.12	26.33±1.15	80.20±24.56	0.60±0.12	160.60±48.49
	Control	7.03±0.68	27.10±0.21	125.0±101.60	4.20±1.05	250.00±201.79
	20%	7.20±0.87	27.08±0.15	108.33±81.0	2.50±2.01	216.33±161.82
	40%	7.17±0.93	27.09± 0.18	135.67±98.52	2.20±1.91	272.00±197.58
	60%	7.30±1.04	27.08±0.15	216.67±155.60	1.83±1.70	443.00±319.65
	80%	7.07±0.91	27.08± 0.15	235.67±168.63	1.53±1.53	465.67±331.37
	100%	7.20±1.04	27.10±0.20	276.67±136.10	1.37±1.42	502.00±388.73
Sub-lethal toxicity test Solution	Control	7.03±0.38	26.67±0.58	93.50±46.41	1.80±1.02	186.50±91.11
	1/20th of 96hr LC_{50}	6.85±0.07	26.33±1.15	73.00±12.73	1.20±0.28	147.50±26.16
	1/10th of 96hr LC_{50}	7.10±0.12	26.33±1.15	80.20±24.56	0.60±0.12	160.60±48.49

The physicochemical properties of the test solutions is presented in table 2 and showed that the water quality parameters were not significantly different from the control ($p>0.05$)

Mortality Rate of *O. niloticus* Fingerlings Exposed to Antibiotic Effluent

Table 3 shows the mortality pattern of exposed fishes with little mortality occurring at effluent concentration of 20%, and 40%, partial mortality at 60%, and total mortality at 100% leachate concentration after 96hrs of exposure. The lethality of 20% and 40% effluent concentration was statically the same, 60% leachate is significantly lethal compared to 20% and 40% concentration, while 80% and 100% effluent had similar potency.

Table 3: Mortality Rate of *O. niloticus* Exposed to Different Concentrations of Antibiotic effluents

Duration	Concentration	Total Exposed	Mortality
24hrs	0% (Control)	20	0
	20%[a]	20	1
	40%[a]	20	2
	60%[b]	20	10
	80%[c]	20	15
	100%[c]	20	20
48hrs	0% (Control)	20	0
	20%[a]	20	1
	40%[a]	20	3
	60%[b]	20	13
	80%[c]	20	18
	100%[c]	20	20
72hrs	0% (Control)	20	0
	20%[a]	20	1
	40%[a]	20	4
	60%[b]	20	14
	80%[c]	20	19
	100%[c]	20	20
96hrs	0% (Control)	20	0
	20%[a]	20	1
	40%[a]	20	5
	60%[b]	20	14
	80%[c]	20	19
	100%[c]	20	20

Concentrations with different alphabet are significantly different, ($p<0.05$)

Acute Toxicity of Antibiotic Effluent

The results of the 24, 48, 72 and 96 hours LC_{10}, LC_{50} and LC_{95} is presented in table 4 below, while Figure 1 is a plot of % mortality vs concentration showing the probit regression equations f or 24, 48, 72 and 96 hours exposure time.

Table 4: Acute lethal Concentrations of Antibiotic Effluents

Lethal Concentration/ Toxicity Unit	Exposure Time			
	24Hrs	48Hrs	72Hrs	96Hrs
LC_{10} (95% C.I) %	32.289 (21.894 – 39.773)	30.873 (3.792 – 44.145)	29.495 (21.096 – 35.641)	28.712 (20.584 – 34.649)
LC_{50} (95% C.I) %	59.337 (50.693 – 68.789)	51.497 (27.188 – 74.511)	48.699 (41.653 – 55.485)	46.853 (40.036 – 53.369)
LC_{95} (95% C.I) %	129.573 (102.929 – 201.033)	99.307 (70.283– 707.207)	92.692 (77.769 – 125.584)	87.842 (73.949 – 118.146)
TUa (95% C.I) %	1.69 (1.46-1.98)	1.95 (1.34-3.68)	2.06 (1.81-2.4)	2.14 (1.88-2.5)

Results indicates lethal concentration (95% confidence interval)

The 24hrs, 48hrs, 72hrs and 96hrs LC_{50} was estimated to be 59.34 %, 51.50 %, 48.70 % and 46.85 % of the antibiotic effluent respectively. The LC_{50} decreased as the exposure time increased.

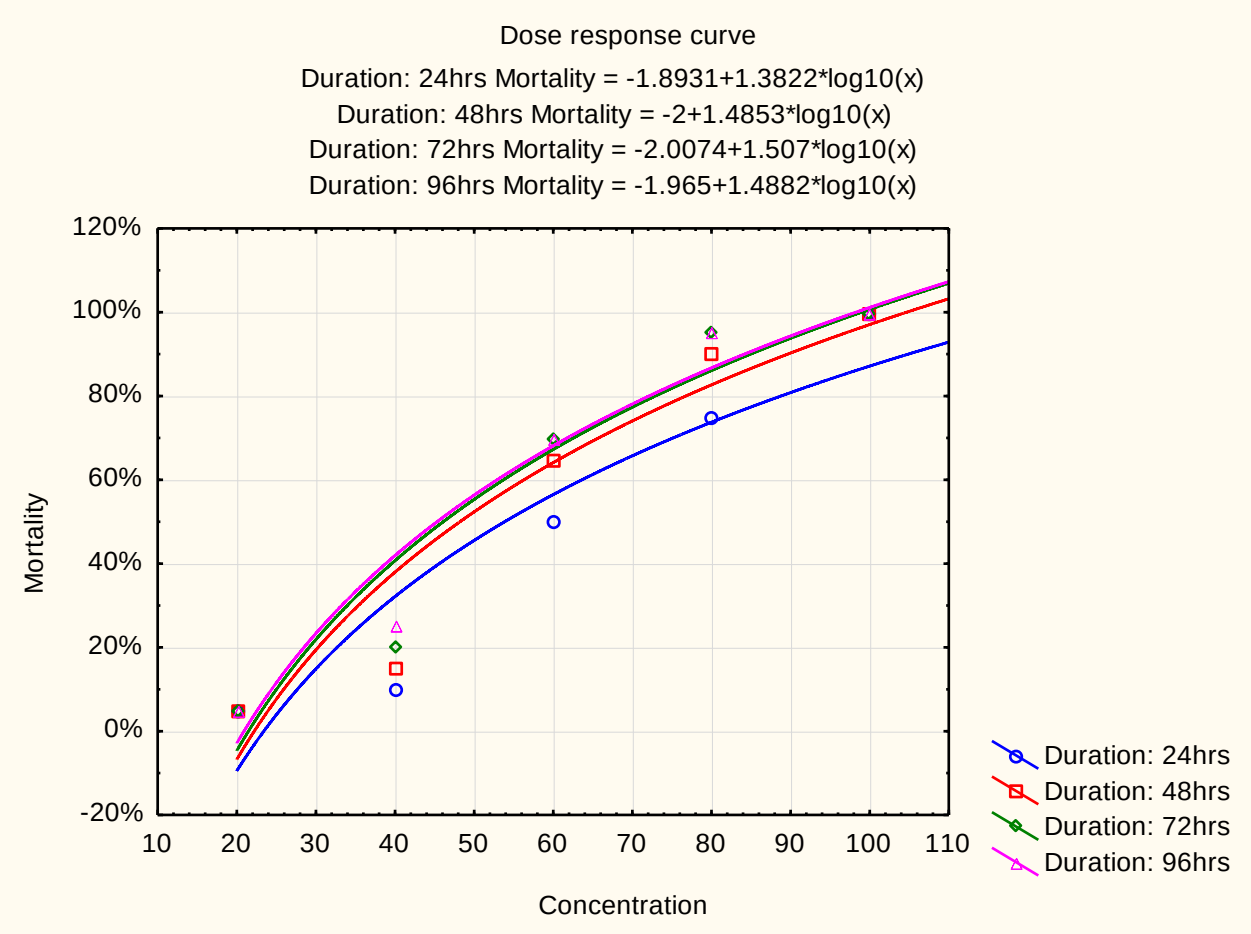

Figure1: Dose response graph of *Oreochromis nitolitus* exposed to antibiotic waste water

Figure 1 depits the comparison of the concentration –mortality curve after 24, 48, 72 and 96 hours of exposure and the regression estimates. Mortality increased as both concentration and exposure time increased. The closeness of the 72hrs and 96hrs curve suggest mortlity peaked after 72 hrs of exposure.

Morphological Indices of Exposed *Oreochromis niloticus*

The result of the effect of the antibiotic waste water on fish biometrics and somatic index is presented in table 5.

Table 5: Comparison of Mean Morphological Indices Between Different Treatment Groups and Control after Antibiotic Exposure

Morphological Indices	Experimental Group		
	Control (0%)	1/10th of 96 hr LC50 (4.68%)	1/20th of 96 hr LC50 (2.34%)
Total length (cm)	18.50±1.32	16.13±1.03	17.25±0.61
Standard length (cm)	15.33±1.61	13.83±1.46	13.83±0.46
Body weight (g)	85.70±22.63	72.58±5.74	79.53±8.33
Weight of gill (g)	6.60±2.83	4.93±1.04	5.18±0.19
Weight of liver (g)	3.53 ± 0.01^{a}	0.75 ± 0.13^{b}	1.10 ± 0.22^{b}
Weight of heart (g)	0.095 ± 0.007^{ab}	0.09 ± 0.005^{b}	0.082 ± 0.007^{a}
Gill somatic index	7.5±1.3	6.8±1.3	6.5±0.5
Hepato somatic index	4 ± 1^{a}	1 ± 0.2^{b}	1 ± 0.2^{b}
Heart somatic index	0.11±0.04	0.14±0.008	0.1±0.01

Results indicate mean± standard deviation, n=5, results with different alphabets in each row are significantly different ($p<0.05$)

Total length, standard length, body weight, gill and heart weight as well as the gill somatic index and hepato somatic index of the exposed fishes were not significantly different from the control fishes ($p>0.05$). However, there was significant decrease in the weight of the liver by 77.48% and 66.97% and the hepato somatic index by 66.67% and 66.7% in fishes exposed to 4.68% and 2.34% of the effluent respectively compared to the control group ($p<0.05$). Also height weight of fishes exposed to 4.68% effluent was significantly different from fishes exposed to 2.34%.

Effect of Antibiotic Waste Water on the Haematology of *Oreochromis niloticus*

The result of the effect of the antibiotic waste water on the haematological indices of *O. niloticus* is presented in table 6

Table 6: Comparison of Haematological Indices Between Different Treatment Groups and Control after Antibiotic Exposure

Haematological Indices	Experimental Group		
	Control (0%)	1/10th of 96 hr LC50 (4.68%)	1/20th of 96 hr LC50 (2.34%)
Red Blood Cell ($\times 10^{12}$/l)	4.10±0.39[a]	3.36±0.27[b]	3.17±0.26[b]
Packed Cell Volume (%)	41.17±2.33[a]	34.54±2.633[b]	31.09±1.38[b]
Haemoglobin (g/dl)	12.53±0.73[a]	10.41±0.59[b]	10.08±0.94[b]
White Blood Cell ($\times 10^{9}$/l)	9.22±1.01	9.88±1.17	10.67±1.34
Mean Corpuscular Volume (Fl)	100.79±7.41	102.93±5.81	98.66±8.29
Mean Corpuscular Haemoglobin (pg)	30.70±2.95	31.07±3.13	32.01±3.97
Mean Corpuscular Haemoglobin Concentration (g/dl)	30.43±0.68	30.21±2.17	32.40±2.02

Results indicate mean ± standard deviation, n= 5, results with different alphabets in each row are significantly different ($p<0.05$)

There was significant decrease in the Red Blood Cell (RBC) count, Packed cell volume (PCV) and Haemoglobin (Hb) of the exposed fishes compared to the control group ($p<0.05$). RBC count decreased by 18.05% and 22.68%; PCV decreased by 16.10% and 24.48%; while Hb decreased 16.92% and 19.55% in fishes exposed to 4.68% and 2.34% of the effluent respectively compared to the control. Increase in white blood cell (WBC) count by 7.16% and 15.73% and Mean Corpuscular Haemoglobin (MCH) by 2.12% and 2.11% in fishes exposed to 4.68% and 2.34% of the effluent respectively compared to the control was not significant ($p>0.05$)

Antioxidant Activity and Oxidative Stress in Exposed *Oreochromis niloticus*

Glutathione concentration decreased significantly ($p<0.05$) by 12.6% and 18% in fishes exposed to 4.68% and 2.34% of the effluent respectively compared to the control. Similarly, superoxide dismutase activity decreased significantly ($p<0.05$) by 19.8% and 21.6% exposed to 4.68% and 2.34% concentrations respectively compared to the control. However decrease in catalase activity by 12.5% and 8.3% was not statistically significant ($p>0.05$). Malondialdehyde levels increased significantly by 81% and 92.9% in fishes exposed to 4.68% and 2.34% of the effluent respectively compared to the control (Figures 2).

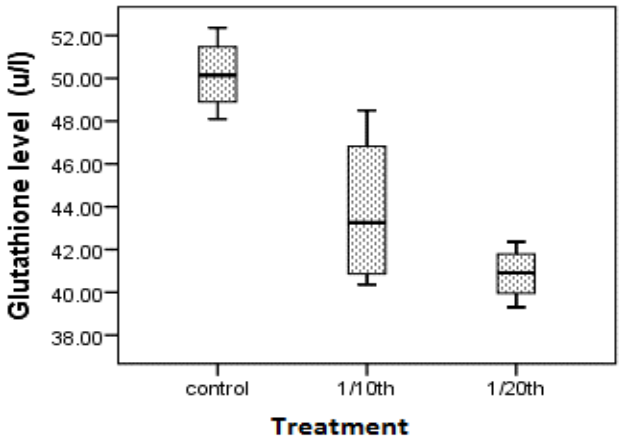

Figure 2A: Box plot of glutathione level of control compared to exposed fishes

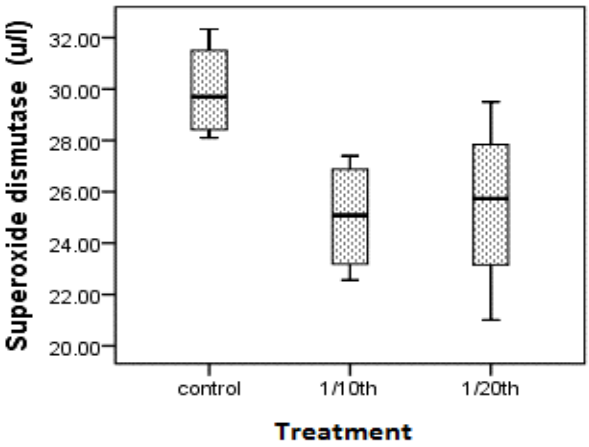

Figure 2B: Box plot of superoxide dismutase activity of control compared to exposed fishes

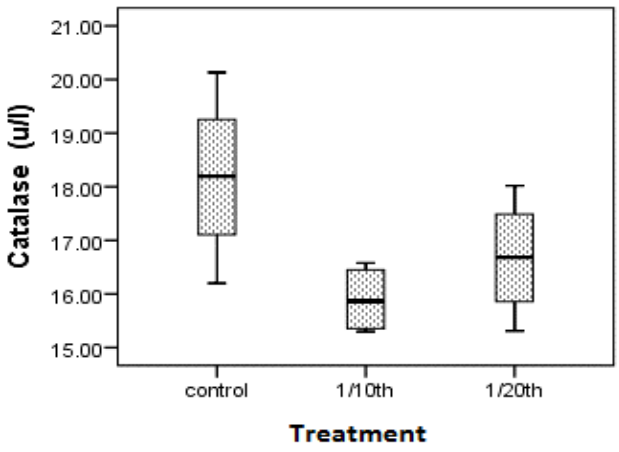

Figure 2C: Box plot of catalase activity of control compared to exposed fishes

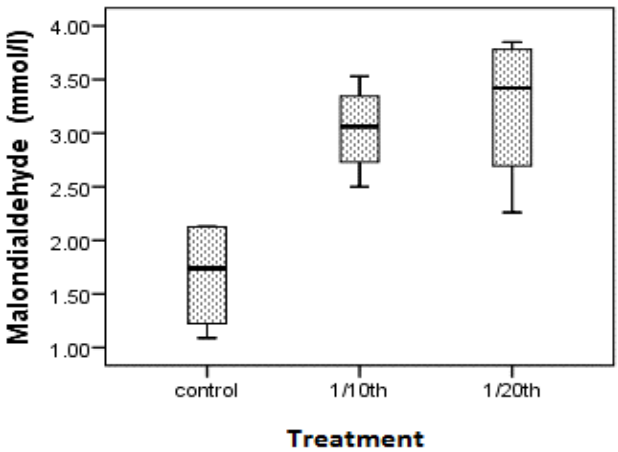

Figure 2D: Box plot of malondialdehyde level of control compared to exposed fishes

Discussion

Antibiotics are among the most common pharmaceutically active compounds found in the environment (Buřič *et al.,* 2018). In this study, the potency (toxicity) of 20% and 40% effluent concentrations may be similar, as mortality caused by both concentrations were low and statistically the same. However 60%, 80% and 100% effluent concentration caused significant mortality compared to 20% and 40%. The potency of 80% and 100% effluent concentration may be similar given that the mortality caused by both concentrations were not significantly different.

The 24, 48, 72, and 96 hours LC_{50} and the acute toxicity unit (TUa) of the current study indicate that the toxicity of the cloxacillin and ampicillin waste water to *Oreochromis niloticus* vary from low to moderately toxic as the duration of exposure increases. Industrial effluents can be classified on the basis of their LC_{50} (%) and TUa as advanced toxicity if the LC_{50} is ≤1 and TUa >100, highly toxic if LC_{50} is 1~10 and TUa is 10~100, moderately toxic if LC_{50} is 10~50 and TUa is 2~10, low toxicity if LC_{50} is 50~100 and TUa is1~2 and micro-toxic or non-toxic if LC_{50} is >100 and TUa is ≤1 (Xie and Shen 2017). Similar 24, 48, 72, and 96 hours LC_{50} values of 63.10%, 53.73%, 41.69% and 40.74% respectively which corroborates this study was reported by Xie and Shen (2017) for Zebrafish exposed to amoxicillin wastewater. In contrast, no abnormalities and mortality was recorded in Zebrafish exposed to similar concentrations of oxytetracycline wastewater (Wu and Shen, 2018).

Morphological indices are useful tools in assessing the health conditions of fish. The present study revealed that the cloxacillin and ampicillin effluents had no effect on the studied morphological parameters except liver weight and the hepato somatic index. The liver is a vital organ that performs important physiological functions such as conversion of glucose to glycogen, biotransformation of xenobiotics, and destruction of old red blood cells. Hepato somatic indices serves as an indicator of the liver condition and energy reserve in the liver. In this study, the significant decrease in liver weight and hepato somatic index (HSI) of fishes exposed to cloxacillin and amplicillin waste water suggests increased metabolic activity and depletion of energy reserves as the liver of *O. niloticus* copes with the stress of antibiotics metabolism. Decrease in HSI of crucian carp (*Carassius auratus*) caged in a river receiving effluent containing pharmaceutically active compounds for 21 days was reported by Liu *et al.,* (2015) However increased HSI was reported in rainbow trout exposed to

clotrimazole (Burkina *et al.,* 2014), while no significant changes in HSI were observed in rainbow trout exposed to carbamazepine (Li *et al.,* 2010).

Haematological analysis can be used as indicators of the physiological health status of fish (El-Sayed *et al.,* 2007). 5 (Kanu *et al.,* 2016). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values of the exposed fishes were not different from the control, this indicates that normocytic normochromic anaemia may have occurred in fishes exposed to 4.68% and 2.34% of the effluent. This finding is consistent with the report of Li *et al.,* (2011) in which normocytic anaemia was observed in rainbow trout exposed to verapamil. Similarly, insignificant levels of MHC and MCHC was observed in *Cyprinus carpio* exposed chloramphenicol (Kasagala and Pathiratne, 2008) but significant decrease in MCV in *Clarias gariepinus* exposed to chloramphenicol was reported Nwani *et al.,* (2015). Although in the current study white blood cells increased by 7.16 and 15.73% in fish exposed to 4.68 and 2.34% of the effluent respectively, it was not statistically significant. Previous studies (Kasagala and Pathiratne, 2008; Nwani *et al.,* 2015) reported significant increase in white blood cells in fishes exposed to pharmaceuticals. Changes in white blood cell count may depend on duration, concentration and nature of the toxicant.

The liver has an enormous potential to generate oxidative species partly because the biotransformation of most xenobiotics including drugs involve oxidation reactions which generate superoxide anion ($O_2^{\cdot-}$) (Mello *et al.,* 2016). Superoxide dismutase (SOD) enzyme can convert the superoxide anion to hydrogen peroxide, which into can be converted into water and hydrogen by catalase (CAT). Liver cells contain the highest concentration of glutathione which plays important role in the protection of cells against oxidative stress and detoxification and elimination of xenobiotics (Kaplowitz 1981; Pavarino *et al.,* 2013). In the current study, decrease in hepatic glutathione, superoxide dismutase and catalase activities suggests exhaustion of the antioxidants and possible inhibition by sustained stress from antibiotics metabolism. The depletion of hepatic glutathione may be linked to its roles during biotransformation while SOD enzyme may have being overwhelmed by the production of superoxide anions. Enzyme activity can decrease as negative feedback either from enhanced production of free radicals or damage induced by oxidative modification leading to a deficiency in the antioxidant system (Lushchak, 2011; Steinbach *et al.,*

2014). The depletion of hepatic glutathione, SOD and even CAT in this study is corroborated by the study by Yonar *et al.*, (2011) who reported reduction in hepatic glutathione, SOD, and CAT in *Oncorhynchus mykiss* exposed to oxytetracycline. However, the activities of hepatic SOD and CAT of *O. mykiss* were not affected by atenolol (Steinbach *et al.*, 2014). Loss of equilibrium between pro-oxidants and anti-oxidants may lead to oxidative stress and cells under oxidative stress manifest several biochemical markers due to attack by free radicals on important macromolecules like lipids, proteins and DNA. Increase in malondialdehyde levels in fishes exposed to 4.68% and 2.34% of the effluent clearly indicates oxidative stress. Earlier studies have reported oxidative stress in liver of fishes exposed to antibiotics (Yonar *et al.*, 2011; Nunes *et al.*, 2014).Thus the cloxacillin and ampicillin effluents overwhelmed hepatic antioxidants and which resulted in oxidative stress.

Conclusion

In summary, pharmaceutical effluents containing ampicillin and amplicox metabolites is moderately toxic to Nile tilapia, *Oreochromis niloticus.* The effluent altered the morphology, haematology and hepatic antioxidant activities of *O. niloticus.* In particular, dilute concentrations reduced liver weight, induced anemia, reduced the activities of SOD, CAT and GSH and induced oxidative stress in the liver. *O. niloticus* biomarkers including liver weight, liver somatic indices, hepatic antioxidants and hematological parameters could be useful as early warning signals of sub-lethal effects of antibiotic to help monitor aquatic ecosystems.

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