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Sub-Chronic Toxicity Evaluation of Tannery Waste Water to *Clarias gariepinus* Juveniles

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ABSTRACT: This paper was conducted to investigate the sub-chronic toxicity of tannery effluents sourced from Challawa industrial estate Kano, Nigeria using *Clarias gariepinus*. The study covered physicochemical parameters, haematological, and biochemical stress enzymes assessments. The water quality results revealed mean value of Temperature (29.92±1.93ºC), pH (8.14±0.85), DO (1.60±0.38mg/l), BOD (0.77±0.16mg/l), Salinity (7.75±0.60mg/l), Nitrate (1.19 ± 0.05mg/l) and Phosphate (16.48±0.85mg/l). After termination of 21 days sub-chronic test, haematological and biochemical changes analysed were generally considered significantly different ($P<0.05$) within the treatments. The haematological indices revealed a decrease and sudden increase in concentration of estimated values of PCV, Hb, RBC, MCHC and MCH, while WBC and MCV fluctuated. These may be attributed to stress, the duration of exposure and levels of pollutants in the effluent. Furthermore, oxidative stress enzymes followed an order of SOD > CAT > GSH. This could be due to level of pollutants in the effluent. The results of the data indicated tannery effluent to have a toxic effect on the experimented organism. The information calls for a more enrich toxicity testing that should involve wide range of organisms. It should entail reproductive aspects of the species, detail relationship and enhanced methodological procedures.

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Being among the most polluting industrial sectors, tannery effluent in Nigeria is directly or indirectly discharged into rivers resulting in toxic effects on aquatic life as well as polluting the environment (Chowdhury *et al.,* 2015: Likita *et al.,* 2016). Many typical processes are used in treating and assessing level of toxicity in tannery wastewater such as biological process, oxidation process and chemical process. Among these conventional methods, physical and chemical methods are considered the most widely use but expensive and energy consuming (Jahan *et al.,* 2014). However, the contaminated wastewater, soil and vegetables physicochemical parameters vary with place and time. The analysis has certain restriction despite giving basic information of contamination, therefore, toxicity test is peculiarly important in research and producing proper indicators of industrial effluent to toxicity control (Xiao *et al.,* 2016). Principally, tannery effluent has hazardous pollutants having dark brown colour, odour, high content of toxic and varying level of persistent organic substances (Jahan *et al.,* 2014; Tadessa and Guya, 2017). Developed countries have environmental agencies with defined policy to monitors and handle wastewater discharges detecting problem before outbreak of epidemics. Contrarily, the reverse is the case in developing countries like Nigeria where environmental problems are growing at faster rate due to lack of enforcement of laws (Zungum *et al.*, 2020; Wakawa *et al.*, 2008). Observation proclaims a wide variety of reproductive and developmental problems in wildlife and humans caused by exposure to environmental contaminants that can interfere with endocrine signalling pathways (Liney *et al.*, 2005). Therefore, pollutants in discharge effluent can be quickly absorbed or ingested via food chain (Zungum *et al.*, 2019; Abalaka 2015; Parveen *et.al.*, 2017) affecting the health status of aquatic organisms. Fishes are widely used to evaluate the health and physiological risk of aquatic ecosystem serving as biomarkers of environmental pollution (Ogundiran *et al.,* 2010). Here, exposing them to the effluents has displayed histopathological degradations, haematological changes and environmental oxidative stress in response to toxicants.Since investigations carried out on tannery effluent in Kano were largely on physicochemical parameters, with little information on toxicity to biota. As reported by Akan *et al.* (2009), Ezike *et al.* (2012), Bernand and Ogunleye (2015), and Umar *et al.* (2017) among many

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other researchers on characterization of tannery effluent in Kano State, it was revealed that most of the parameters in the effluents have exceeded the maximum allowable limits by WHO, FEPA, USEPA, and NESREA for discharge into the environment. Hence the present study was designed to assess the sub-chronic toxicity of tannery effluent through haematological parameters and antioxidative stress enzymes using a common fish (*C. gariepinus*). Thus, serves as ideal tools for toxicity studies of the released tannery effluent.

MATERIALS AND METHOD

*Study Sites and Effluent Collection:*The effluent was collected from a tannery industry in Challawa industrial estates Kumbotso local government area of Kano state.Grab samples of effluent produced by the industry was collected using 50L jerry cans washed with water and then rinsed with the effluent.

Using standard analytical methods (APHA, 2005), a portion of effluent was used for in-situ measurement of physicochemical parameters; Temperature, pH, DO, BOD_5 (measured after 5 days and calculated as DO_1- DO_5) and salinity using Hanna Waterproof pH/TDS/EC/ Temperature Meters (HI 991300) and combined water quality meter model 8603.

Test Organisms:Clarias gariepinusn Juveniles of size 7.5-8.5cm and weight 2.9-3.6g were employed as purchased from Rumbun Kifi Dorayi, Kano state. The fishes were acclimatized by renewal bioassay using borehole water for fourteen days in Biological Science Departmental Aquarium prior to toxicity test and fed with commercial feed of 1mm size. Feeding was stopped for 24 hours prior to and during exposure period that lasted for 96 hours. The experiment was set up in a completely randomized design manner (Tijani and Ajani, 2017).

*Sub-Chronic Toxicity Assay:*After a preliminary testing, ten acclimatized fishes were subjected to varying concentrations of 12.5%, 6.25%, 3.16%, and 1.56% of tannery effluent in replicates using static renewal bioassay (ASTM, 1990). The fishes are fed after the effluent at the set concentrations were replenished every 24 hours for 21 days.

*Haematological Studies:*The method employed for collection of blood was in accordance to Dahunsi and Oranusi (2013) where blood was sampled from three *C. gariepinus* survivors of each treatment at the end of experiment. By severe caudal puncture, blood was collected using 1ml insulin syringe to estimate numbers of erythrocytes (RBC) and leukocytes (WBC), haemoglobin (Hb) and Haematocrit (Hct) using appropriate described method from Tijjani and Ajani (2017). From the results of R b B8C count, WBC count, Hb and PCV, the mean corpuscular volume (MVC), mean corpuscular haemoglobin (MVH) and the mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulas (Dacie and Lewis, 1984):

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

$$\text{MCH (pg/cell)} = \frac{\text{Hb(G/dl)} \times 10}{\text{RBC count in million mm}-6}$$

$$\text{MCV (fl/cell)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC count in million mm}-6}$$

*Biochemical Analysis:*Antioxidative stress enzymes were determined from the supernatant fraction of the liver homogenates of sacrificed *C. gariepinus* with normal saline. Catalase Activity (CAT) was measured using Abebi's method (1974), Reduced glutathione (GSH) was done according to Ellman (1959) as described by Rajagopalan *et al.* (2004) and Superoxide dismutase (SOD) was determined by the method describe by Fridovich (1989).

*Statistical Analysis:*Data obtained from haematological and biochemical analyses were analysed using SPSS 16.0 software. Duncan Multiple Range tests (DMRT) as post-hoc test was done after confirmation using one-way ANOVA at 0.05 level of significance.

RESULTS AND DISCUSSION

The physicochemical results of untreated tannery effluent compared to international and national standard limit are shown in Table 1. Temperature and pH mean value were 29.92±1.93ºC and 8.14±0.85 respectively, revealing that they were within the standard permissible limit. The nitrate, DO, and BOD_5 mean values recorded were below standards while phosphates do not conform to NESREA (2009) and WHO (2012) standards.

Results of haematological parameters were presented in Table 2 with mean values significantly different ($p>0.05$) among and across treatment levels. The mean values of blood parameters as Hb, PCV, RBC, MCV generally decreased with increase in concentration while WBC fluctuate and a slight fluctuation in MCV was observed. The values of RBC had an increase at 3.16% concentration but continued to decline with increase in concentration.

Table 1: Mean Physicochemical Characteristics of the Crude Tannery Effluent as Compared toStandards

Parameters	Effluent Discharge	WHO Standard (2012)	NESREA Standard (2009)
Temperature (ºC)	29.94±1.93	30-36	-
pH	8.13±0.85	6.5-8.5	6.5-8.5
DO (mg/l)	1.60±0.39	10	6
BOD_5 (mg/l)	0.77±0.16	20-40	-
Salinity (mg/l)	7.75±0.60	-	-
NO_3^-(mg/l)	1.19 ± 0.05	-	10
PO_4^{-3} (mg/l)	16.48±0.85	-	3.5
References	This work	Onyidoh (2017)	Adeogun and Chukwuka (2012)

Key: DO = Dissolved Oxygen, BOD_5 = Biochemical Oxygen Demand, NO_3^- = Nitrate, PO_4^{-3} = Phosphate

When compared with the control, MCHC had a decline at the least concentration (1.56%) with another sharp elevation at the highest concentration (12.5%). Even though MCV decreased as concentration increases, there was increase in mean value at 6.25%. The pattern of blood parameters implied a concentration dependent relationship with the control recording the highest values at almost all blood parameters except for WBC, MCHC and MCH.

Table 2: Haematological Characteristics of *C. gariepinus* Exposed to Varying Concentrations of the Tannery Effluents After Chronic Assay

Concentration of effluent (%)	Estimate Count of Blood						
	PCV (%)	**Hb (g/dl)**	**WBC (10^3)µl)**	**RBC (10^6)µl**	**MCHC** (g/dl)	**MCV**(fl/cell)	**MCH** (pg/cell)
12.5	$12.33±1.16^a$	$7.80±0.46^a$	$9352.67±220.73^b$	$1.67± 0.16^a$	$63.40±2.81^c$	$73.89±3.11^a$	$46.81±1.65^c$
6.25	$22.00±1.00^b$	$8.60±0.10^{ab}$	$8006.67±640.81^a$	$2.71± 0.07^b$	$39.13±1.33^b$	$80.93±11.06^a$	$31.74±0.49^a$
3.16	$25.33±3.51^b$	$9.53±0.93^{bc}$	$10598.67±237.20^{cd}$	$3.13±0.01^c$	$37.80±2.30^{ab}$	$77.30±6.14^a$	$30.46±2.89^a$
1.56	$29.00±1.73^c$	$10.13±0.95^c$	$11016.67±10.41^d$	$3.01±0.03^c$	$34.90±1.28^a$	$96.32±4.91^b$	$33.65±2.88^a$
0.00	**$30.33±1.16^c$**	**$12.13±0.59^d$**	**$10085.67±138.05^c$**	**$3.13±0.04^c$**	**$40.01±1.48^b$**	**$97.00±2.77^b$**	**$38.80±1.48^b$**

Mean of 3 replicates ± SD
Values with the same superscript along the same column are not significantly different ($p>0.05$)

Table 3 gives result of antioxidative stress enzymes (CAT, GSH and SOD) in comparison with the control showing increase and decline in values. The mean values found to be significantly different ($p>0.05$), highest value of CAT (20.76±0.41 µ/mg protein) and GSH (54.13±0.35µ/ml) was recorded at 12.5%. The least mean value of 14.83±0.90µ/mg protein was recorded for CAT at 1.56% and 45.77±0.25µ/ml at 6.25% for GSH. In case of SOD, the highest value was recorded at 6.25% (15.00±0.30 µ/ml) and the lowest was at 12.5% (12.07±1.86 µ/ml).

Table 3: Antioxidative Stress Enzymes of *C. gariepinus* Liver Exposed to Varying Concentrations of the Tannery Effluents After Chronic Assay

Concentration of effluent (%)	CAT (µ/mg protein)	GSH (µ/ml)	SOD (µ/ml)
12.5	$20.76±0.41^c$	$54.13±0.35^d$	$12.07±1.86^a$
6.25	$16.33±0.25^b$	$45.77±0.25^a$	$15.00±0.30^b$
3.16	$15.37±0.42^a$	$46.17±0.35^a$	$14.47±1.02^b$
1.56	$14.83±0.90^a$	$47.67±0.15^b$	$14.77±0.36^b$
0.00	**$17.87±0.35^d$**	**$48.87±0.41^c$**	**$13.70±0.40^{ab}$**

Mean of 3 replicates ± SD; Values with the same superscript along the same column are not significantly different ($p>0.05$).CAT= Catalase, GSH= Reduced glutathione, SOD= Superoxide dismutase

*Physicochemical Characteristics:*Determination of physicochemical characteristics is an important tool in maintaining quality and healthy aquatic ecosystem. In the research, Temperature and pH means were reported to be 29.92±1.93ºC and 8.14±0.85 (Table 1). This is in line with Ezike (2012) that reported Temperature and pH to be within permissible limit discharged from major tanneries of Kano. The observed low average mean DO of 1.60±0.38mg/l was a common characteristic of textile and tanneries stressing aquatic life. The researcher considered depletion of Oxygen as among the factors for high mortality, oxidative stress, histopathological and haematological effects subsequently observed. It also explains why the effluent possess a strong odour as suggested by Olorunfemi *et al.* (2014). Increase salinity is an evident of high TDS (Bernand and Ogunlaye, 2015) and it discharge leads to alterations in osmotic conditions (Wosnie and Wondie, 2014) stressing life of some aquatic species. Nitrate has mean value of 1.19 ± 0.05mg/l lower than standard limits. In high concentrations, nitrate may produce asphyxiation or in infant a condition as methemoglobinemia or cancer (Ho *et al.,* 2012)**.** The mean level of phosphate was recorded as 16.48±0.85mg/l exceeding limit set by NESREA (2009). Jahan *et al.* (2014) reported similar mean value of 17.1 mg/l. The present result gives concern for eutrophication in that, according to some research high phosphate may inhabits the growth of algae.

*Haematological Indices:*A change in water quality is one of the major factors responsible for individual variations in fish haematology (Adeyemo, 2005). The reduction with slight fluctuation in packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) with increase in white blood cells (WBC) and mean corpuscular volume (MCV) observed from the Table 2 is in agreement with the study of Sreelekshmy and Miranda (2016) with *Arius nenga* exposed to industrial effluent. And to support the present findings is the work of Parveen *et al.* (2013) that reported similar result with tannery effluent on *Channa punctatus*. The observed increase trend in MCV and decrease of MCH and MCHC is in good concurrence with Praveena *et al.* (2013) that attributed the change observed in *L. rohita* exposed to tannery effluent to defence mechanism against the toxic effect of heavy metals leading to decrease RBC's, Hb and PCV. According to the reports of Adeyemo (2005) decrease in MCV with low HB indicated shrinkage of RBC due to destructive action of pollutants on erythrocytes. WBC was observed to fluctuate, it means the increase in WBC counts maybe attributed to attempt of the fish to fight against antigens (Adewoye, 2010) whereas increase-decrease WBC might be indication of stress (Ajani and Awogbade, 2012). Furthermore, low WBC indicated breakdown of vital metabolic activities as a result of possible blockage in the metabolic pathway (Dahunsi and Oranusi, 2013). The observed low oxygen and varying concentrations of tannery effluent in this study may have interfered and generated the changes in blood of experimented fish.

*Oxidative Stress Enzymes:*It is well known that heavy metals induce the formation of free radicals within an organism leading to oxidative stress, if prolonged may result in oxidative damage (Farombi *et al.,* 2007; Al-balawi *et al.,* 2013). In table 3, there is variation in recorded values of CAT, GSH and SOD. Studies reveal SOD and CAT to have a cooperative mechanism (Piexoto *et al.,* 2013). Adeogun *et al.* (2012) observed increase in SOD and CAT activity due to presence of environmental pollutants as defence mechanism against oxidative stress. Moreover, increased in GSH levels in the liver were reported as an evidence of adaptive and protective role against oxidative stress induced by pollutants. However, this was not the case in the present study which could be attributed to elevated oxidative stress coupled with prolong exposure duration to the effluent. The outcome of CAT activity may be as a result of weakness and malfunctioning of the liver due to oxidative stress activity. On the other hand, inhibition of SOD could lead to increased oxidative stress damaging activities of the superoxide radicals reducing the activity of the enzyme CAT (Eleyele *et al.,* 2017). The order of enzymes in the study is SOD > CAT > GSH. Aich *et al.* (2011 and 2015) showed capability of tannery effluent to alter stress enzymes in fish organs as adaptation mechanism. All things considered, observed alterations might be due to influx of pollutants, high production of peroxide radicals or associated to duration of the fish exposed to effluent. In addition, the elevated trend and depression were evident of low DO level. It also demonstrated defensive mechanism to overcome stressful conditions generated by pollutants for metabolic impairment in the aquatic organisms.

*Conclusion:*The physicochemical parameters: temperature, pH, DO, BOD, conductivity, salinity and nitrate conform to safety limits except phosphate which exceeds NESREA permissible limit. Haematological indices: the PCV, Hb, MCHC and MCH values were attributed to anaemia from low oxygen. WBC and MCV fluctuation are an indication of stress in the fish to fight against antigens.Whereas, the fluctuating trend in GSH, SOD and CAT observed in the fishes could be attributed to pollutants in the effluent such that oxidative stress enzymes can be a sensitive indicator (biomarker) of aquatic pollution. Therefore, this study further accentuates toxicity potency of tannery effluent on *Clarias gariepinus.* And beg for more enrich toxicity assessment, covering wider range of organisms entailing: reproductive aspectand enhanced methodological procedures.

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