



ACUTE TOXICITY ANALYSIS OF EFFLUENT FROM TANNERY INDUSTRY IN KANO METROPOLIS

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ABSTRACT

The acute toxicity of tannery effluents collected from Challawa industrial estate Kano, Nigeria was assessed using *Artemia salina* and *Clarias gariepinus*. The test was done after the evaluation of range finding test before a definitive test with 3 replication of each treatment. The varying concentrations were prepared by diluting crude effluent with borehole water on V/V% in a completely randomized design. The experiment showed *C. gariepinus* fingerling to exhibit abnormal behavior such as initial erratic movement, skin discolouration and loss of reflex. Recorded mortality and behavioral abnormalities in both species observed were dose-time-dependent. The 96 hours LC₅₀ estimated for *C. gariepinus* was 9.95% while that of *A. salina* after 24 hours and 48 hours were 4.63%, 0.73% respectively. Thus, the information from the study demonstrates the toxic effect of tannery effluent which requires proper management before its discharge into environment.

Keywords: Acute toxicity, *Artemia salina*, *Clarias gariepinus*, Effluent, Tannery

INTRODUCTION

For the last few years, particularly in Africa, there is a significant increase in human population. The resultant increase in the activities of human such as urbanization and economic activities causes detrimental effect to our environment (Bernand and Ogunleye, 2015). For developing countries like Nigeria, environmental problems are growing at faster rate due to lack of enforcement of laws (Wakawa *et al.*, 2008).

For across the globe, it is estimated that each year, tannery industries discharge 300–400 million tons of pollutants into water bodies (Chowdhury *et al.*, 2015). Historically, Nigeria has about 41 commercial tanneries designed more to process skins than hides, with most of them predominantly based in Kano (USAID, 2012). These industries witnessed boom in production, making it second to only oil in terms of foreign exchange earnings according to CBN reports by FMenv (2012). There operational processes involve transforming of raw hide or skin into leather used in the manufacturing of a wide range of products (Ado *et al.*, 2014). During the process, tanning agents are used which generate highly turbid, coloured and foul smelling effluent (Yusif *et al.*, 2016). The major problem of tannery effluent is heavy metals, toxic chemicals, chloride, lime with high dissolved and suspended salt and other pollutants it contains (Jahan *et al.*, 2014).

The number of investigation carried out with vertebrate specie on exposure to tannery effluent is low (Souza *et al.*, 2016). Although

there is considerable amount of literature published on characterization of tanneries/textile effluents in Kano as reported by Akan *et al.* (2009), Ezike *et al.* (2012), Bernand and Ogunleye (2015), and Umar *et al.* (2017) among many other researchers, there is little or no documented report on acute and chronic toxicity test of the effluent. The method of acute toxicity testing of the effluent normally involves the use of organisms such as luminous bacteria, algal, crustaceans and fish (Xiao *et al.*, 2016). It is recommended to be carried out using at least two different animal species.

The fish species (*C. gariepinus*) is of importance because of its cultivation as food and widely found free living in freshwater of Nigeria. Furthermore, brine shrimp has been proposed for toxicity testing due to its simplicity, inexpensiveness and reliable short term routine. This research focuses on assessing acute toxicity of effluent from a tannery industry in Kano metropolis using test organisms; *C. gariepinus* and a simple crustacean (*A. salina*). These will contribute to our knowledge through establishing information on toxic effects of the discharged effluent to aquatic life. Information obtained can also help in formulating control measures to discharge chemicals and monitoring the environment.

MATERIALS AND METHODS

Study Sites and Effluent Source

Located in the Northern part of Nigeria, Kano State lies between latitude 12° 40' and 10° 30' and longitude 7° 40' and 9° 40'.

Special Conference Edition, November, 2019

The effluent was collected some few meters away from point of discharge of a tannery industry in Chalawa industrial estate (an area that houses most of the well known leather finishing company). The Grab sample of effluent produced was collected using clean jerry cans washed with water and then rinsed with the effluent. A portion was analysed for physicochemical parameters (Temperature, pH, DO and salinity) using combined water quality meter model 8603.

Experimental Organisms Set-up

In accordance with Olowa and Nuneza, (2013), *Artemia salina* eggs were obtained and hatched in artificial seawater with minor modification as the first experimental organism.

Juveniles *Clarias gariepinus* of size 7.5-8.5cm and weight 2.9-3.6g were employed as second experimental organism. They were purchased from Rumbun Kifi Dorayi, Kano state. The fishes were acclimatized for fourteen days 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.

Acute Toxicity Assay

At first, range finding test was evaluated with six concentration of the effluent at dilution 6.25%, 12.5%, 25%, 50%, 100% using clean filtered artificial seawater with adjusted salinity of 30ppt. Using static bioassay, ten *Artemia salina* larvae of less than 24 hour old were transferred to petri dishes containing 10ml of prepared samples. The test was run in three replicates for 48 hours and immobilization/mortality was recorded (Krishnakumar *et al.*, 2007). The second test was carried out following the same process with proper care with additional

concentrations; 3.16% and 1.56% to reduce error and obtain a credible result.

For the second experimental organism, range finding test was carried out using non static renewal bioassay with 6.25%, 12.5%, 25%, 50%, 100% and 0 % concentrations and 10 fishes per chamber set in duplicate. A definitive concentration at 25%, 12.5%, 9.00%, 6.25%, 3.16%, 1.56% was later employed. The experiment was set up in triplicate placing 6 fishes per chamber so as to reduce and refine for result credibility. Careful observations were made by taking into account the behavioral/morphological responses and mortality after 12, 24, 48, 72 and 96 hours (Olorunfemi *et al.*, 2014) of test organisms.

Statistical Analysis

Data was analysed using SPSS version 16.0 for probit facility. The mortalities was calculated, corrected and converted to probit (LC₅₀) (Kelle *et al.*, 2013). The LC₅₀ toxic value was transformed into toxic unit and safe concentrations using the equation;

- $TU = 100/LC_{50}$
- Safe concentration limit = $LC_{50} \times 0.1$
Where 0.1 is application factor (Workagegn, 2013)

RESULTS AND DISCUSSION

In Table 1 below, temperature and pH mean value were 29.92±1.93°C and 8.14±0.85 respectively, revealing that they were within the standard permissible limit of WHO (2012). The mean value of DO recorded was below standards and could cause stress to aquatic life. Dilution water (borehole water) physicochemical parameters measured were within the permissible limits (WHO, 2012).

Table 1: Some Major Physicochemical Parameters (mean ± SD) of the Tannery Effluent and Borehole Water as Compared to WHO Standard

Parameters	Effluent discharge	Borehole Water	WHO (2012)
Temperature (°C)	29.92±1.93	28.53±0.65	30-36
pH	8.14±0.85	6.88±0.72	6.5-8.5
DO (mg/l)	1.60±0.38	7.02±0.04	10
Salinity (mg/l)	7.75±0.60	0.03±0.02	-

Key: DO = Dissolved Oxygen

Behavioral Response:

During acute test with *C. gariepinus*, organisms exhibited distress behavioral responses. They were observed directly to have shown sudden change in the organism's response to the environment such as restlessness, gasping for breath and frequent surfacing (prominent during the first hours) which increases as the concentration increases. As time went on, the test organisms were observed to get weaker with ventral surface turned upward and

eventually die. This observed behavior corresponds with the study of Adewoye (2010) that observed visible behavioural change after 60 minutes of *C. gariepinus* exposure to *Trephosia vogelii* extracts. Similar symptoms of toxicosis was reported by Emere and Balogun (2014) with subacute behaviours including agitated swimming, loss of balance, hitting against the edges of the tank, air gulping, quiescence and death peeling off of the skin and swollen abdomen.

Special Conference Edition, November, 2019

Also of noticeable changes observed at the end of test were spine curvatures, discoloration of body, trace of haemorrhage and cannibalism at higher concentrations, with normalcy in behaviour only at concentration from 9.0% downward. Moreover, weights of the test organisms were found to have undergone reduction with shrinkage noticed at the head region. The result was in consistent with findings of many authors when respectively studying acute toxic effect of different effluent to *C. gariepinus*. Olaifa *et al.* (2004), Navraj and Yasmin (2012), Adeboyejo *et al.* (2013), Dahunsi and Oranusi (2012), Dahunsi and Oranusi (2013), Olorunfemi (2014), Agboola and Fawole (2014) and Olorunfemi *et al.* (2015).

The stressful behavior of respiratory impairment observed prior to mortality may be related to depletion of oxygen as suggested by Ariyomo *et al.* (2017). The research identified behavioral response and mortality (Table 2) to be concentration-dependant, increases and decreases as exposure time increase. However, observed behavioral response reinforce evidence that there are three phases of response to effluent, viz; active, fatigue and collapse.

The mortality details recorded from both of the acute assays were presented in Table 2 and Table 3. However, result obtained was further unveiled in Table 4 to shows estimated LC₅₀, toxic unit and safe concentration of both experimented organisms.

Table 2: Mortality Details of *C. gariepinus* During Acute Exposure to Varying Concentrations of Tannery Effluent (n=6)

Concentration (%)	12hr			24hr			48hr			72hr			96hr		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
25.0	6	5	6	0	1	0	0	0	0	0	0	0	0	0	0
12.5	1	0	2	2	1	2	3	3	2	0	2	0	0	0	0
9.0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
6.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
3.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

R= Replicates

Table 3: Mortality Details of *A. salina* During Acute Exposure to Varying Concentrations of Tannery Effluent (n= 10)

Concentration (%)	24hr			48hr		
	R1	R2	R3	R1	R2	R3
100	10	10	10	0	0	0
50	10	9	10	0	1	0
25.0	10	9	10	0	1	0
12.5	8	5	8	2	4	2
6.25	7	5	4	3	4	4
3.16	4	3	2	5	5	5
1.56	2	1	3	6	6	4
0.00	1	0	0	1	2	2

R= Replicates

Acute toxicity with *A. salina* (Table 3) was also observed to have dose-and time-dependent relationship with mortality most pronounced at the highest concentration (100%). During a trial test, more *A. salina* were counted alive, this is an indication of an error in counting. Moreover, it may also be from an incorrect technique of hatching the cysts and manipulation of nauplii during experiments. Hence the transferring of unhatched cyst to experiment chamber which

hatches later during experiment. This is in line with the findings of Norsworthy (2000) when studying effects of selenium on death of brine shrimps.

The second trial recorded mortality even in control to about 20% after 48 hours. This could be associated with lack of food (Norsworthy, 2000) or the high sensitiveness of larval bioassay than egg (Krishnakumar *et al.*, 2007).

Table 4: Summary of Acute Toxicity of Both *C. gariepinus* and *A. salina*

Test organisms	24hrs LC ₅₀ (%)	24hrs TUa	Safe Conc. (%)	48hrs LC ₅₀ (%)	48hrs TUa	Safe Conc. (%)	96hrs LC ₅₀ (%)	96hrs TUa	Safe Conc. (%)
<i>C. gariepinus</i>	-	-	-	-	-	-	9.95	10.05	0.995
<i>A. salina</i>	4.63	21.60	0.46	0.73	136.9	0.073	-	-	-

Key TUa= Acute toxic unit, safe concentration

The 96 hours LC₅₀ of acute toxicity with *C. gariepinus* was estimated (Table 4) to be 9.95%. Navaraj and Yasmin (2012) in India, recorded LC₅₀ value of *O. mossambicus* on exposure to tannery effluent as 7%. In a similar study conducted, Dasgupta and Panigrahi (2015) used *Labeo rohita* and registered concentration even at 3.53% as highly toxic. These variations in LC₅₀ and behavioural responses values may be linked to differences in the sensitivity of a particular species to pollutant, age, sex and environmental conditions (Olayinka, 2013; Olorunfemi, 2014).

The estimated LC₅₀ for *A. salina* after 24 hours and 48 hours is 4.63% and 0.73% as shown in Table 4 is similar to the finding reported in Chile, by Cooman *et al.* (2003) of 0.36%-3.61% (considered extremely toxic) on testing different tannery processes effluent using *Daphnia magna* and *D. pulex*. Baniamam (2014) studied vanadium toxicity using *Artemia urmiana* and *A. Franciscana* recording LC₅₀ in 24 hours as 0.0107 and 0.011 mg/L. Pimentel *et al.* (2009) found LC₅₀ values for crude cashew nut industrial effluents 1.38% and 0.60 % after 24 and 48 hours exposures, respectively, and were 2.16% and 0.88 % for treated effluent. Further findings base on some authors across the globe recorded acute toxicity (LC₅₀) with *A. salina* on exposure to different raw effluents as; 2.73%-35.5% for Chemical plant (Guerra, 2001), 4.5% for Olive

oil mill (Aggelis *et al.*, 2003), 1.2 % for Oilfield (Campos *et al.*, 2002), 70%-80% for Landfill leachate (Svensson *et al.*, 2005), 55% for Textile (Souza *et al.*, 2007).

Toxic unit estimated in Table 4 for both experimented organisms were far above 0.3TUa, a value set by NYS DEC (2007) as SPDES permit program guidance for protection and survival of aquatic organisms. Classification of toxicity for wastewater discharged of Ross (1993) cited in Libralato *et al.* (2010) ranked the estimated LC₅₀ of the effluent as class I (highly toxic). In contrast, Personne *et al.* (2003) cited in Libralato *et al.* (2010) categorise calculated toxic unit of the 24 hours and 48 hours of acute *A. salina* toxicity to class II (slight acute toxicity) and class I (no acute toxicity) while 96 hours acute of *C. gariepinus* toxicity as class IV (high acute toxicity).

CONCLUSION

C. gariepinus subjected to varying concentrations of tannery effluent showed respiratory disturbance and sudden death. In the same regard, *A. salina* was recorded to have a high mortality. The toxicity of the two tested organisms varies greatly with increase in concentration of effluent. Both of the tested organisms in terms of LC₅₀ revealed effluent as highly toxic with estimation of 9.95%, 4.63% and 0.73% respectively.

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Special Conference Edition, November, 2019

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