



Acute Toxicity and Histopathological Studies of Textile and Dyeing Effluents From Kano Metropolis on African Catfish(*Clarias gariepinus*)

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Abstract

*Compromise the processing standard during dyeing and textile activities coupled with incomplete sewage treatment system lead to indiscriminate discharge of large amount of dyestuff into water bodies mostly used for irrigation and fishing. These wastes are potentially toxic, mutagenic and some are even carcinogenic. In this research, acute toxicity test as well as histopathological studies using African catfish (*Clarias gariepinus*) juveniles exposed to different concentrations of textile and dyeing effluents in Kano, Nigeria. The results of acute toxicity test revealed an increase in mortality of the fish with increase in concentrations of the treatment after 72hrs. of exposure, while no such mortality were observed in control group. The lethal concentrations causing 50% mortality (LC_{50}) were determined as 14.8%, 22.4% and 14.45% for ATM, KofarNaisa and Kofar Mata dyeing points respectively, while, the safe concentrations were 1.48%, 2.24% and 1.445% for the above three sites respectively. Histopathological test showed various degree of effects including distortion in skins and muscles as well as mutilations of gills in exposed groups with none such effects in control group. Among these are slight to severe degeneration of skin tissues in 25% and 75% concentrations of textile effluents. In addition, there are localized lacerations in different concentrations of dyeing effluents. The observed histopathological effects on gills ranges from slight mutilation of secondary lamella of the gills rakers to severely injured secondary lamella for textile effluents, the peak being complete deterioration of central core hyaline cartilage in 75% and 100% dyeing effluents. It was concluded that, there were histopathological anomalies that serves as bioindicators of environmental pollution. The study therefore, provides warning of potential adverse environmental effects of textile and dyeing effluents on the receiving aquatic ecosystem in Kano metropolis.*

Keywords: Toxicity; Histopathology; Textile and Dyeing; Kano; Catfish(*Clarias gariepinus*)

Introduction

Kano, right from the ancient time was known as one of the important centre for textiles and dyeing activities, and the resultant effluents and waste of these activities get into water ways that linked into streams, ponds and rivers of the catchment area not taking into account the toxic effect to the aquatic biota. Textiles/dyeing industry act as the most important cause of environmental pollution among all industrial sectors, because they produces wastewater which is diverse in chemical composition (Bharai *et al.*, 2013; Ali *et al.*, 2008). Insufficiencies in dyeing during textile processing always results in large amount of dyestuff being directly lost to the wastewater, which unavoidably find its ways into water bodies mostly used for irrigation and fishing (McMullan *et al.*, 2001). These dyes undergo chemical changes under certain environmental conditions and the transformed products may even be more toxic and carcinogenic than the parent molecules (Weber and Adams, 1995; Ratna and Padhi, 2012). Approximately 10-15% of the dyes used during dyeing process are released into the environment making the effluent highly coloured and aesthetically unpleasant. The effluents from textile/dyeing industries carry a large number of dyes and other additives which are difficult to remove in conventional water treatment procedures. Due to the reported mutagenicity of some synthetic dyes, disposal of untreated textile/dyeing wastewater (especially from our local or handmade dyeing industries that have less knowledge of effluent treatment) has raised concern about the safety of the aquatic environment (Sumathiet *et al.*, 2001). On these ground, it becomes reasonable to monitor aquatic environments on routine basis through toxicity



assessment study of the effluents released by industries especially the textile and dyeing industries, so as to safeguard the environment from total deterioration.

The complexity of industrial effluents makes it impossible to carry out hazard assessment study based on physical and chemical analysis only. Thus, the use of aquatic organisms such as fishes in environmental monitoring and toxicity screening is important, since the ultimate destination of almost all the industrial waste is aquatic environment (Talapatra *et al.*, 2006). Fishes serve as chief source of protein and other vital nutrients in the diet of man, mutagenicity in fishes therefore, can serve as early warning of potential risks both to fishes and to human and non-human consumers of contaminated fish (Iji and Adeogun, 2014). The selection of fish as a model in eco-toxicity studies could be valuable as fishes serve as a very sensitive bio-indicator of aquatic contamination in tropical regions (Mdegela *et al.*, 2006). Moreover, fish play important role in tropic web, as they bioaccumulate or biomagnify certain environmental pollutants and bio-transform some of them to less or more toxic metabolites (Goksoyr, 1991). African catfish (*Clarias gariepinus*) is one of the most important tropical cultured fish due to its high growth rate, high stocking-density capacity, high consumer acceptability and high resistance to poor water quality and oxygen depletion (Osman *et al.*, 2007; Karamiet *et al.*, 2010). Based on these reasons, it is being used in fundamental researches and therefore selected and used in evaluating the impact of industrial effluents (particularly textiles and dyeing) to the quality of the environment as described by Yogita *et al.*, 2014 and hence considered as an excellent model for this studies.

Materials and Methods

Study Area

Kano, is positioned at 11°59'18''N and 08°32'06''E about 418 metres above the sea level and occupies the central position in Northern Nigeria. It is one of the most populous and developed cities in Nigeria with dyeing and textile being among the dominating activities in the city (Akan *et al.*, 2007). Within Kano metropolis, African Textile Manufacturers (ATM) (11°53'15''N; 08°28'96''E) encoded as E1, Kofar Naisahand-made dyeing point (11°58'95''N; 08°30'86''E) as E2 and Kofar Mata local dyeing point (11°39'95''N; 08°31'41''E) as E3 were selected for the study.

Effluents Sample Collection

Effluent Samples were collected from the above mentioned three (3) points, in the early morning hours using five (5) litre capacity jerry-can as described by Maiti (2004).

Fish Sample Collection

Sixty (60) healthy juveniles catfish (*C. gariepinus*) per site making the total of 180 for the three sites and five (5) fishes as a control (irrespective their sex) were procured from Rumbunkifi fish-hatchery centre, opposite Bayero University, Kano Old site, along Dorayi road, Kano. The fish samples were transported to the aquarium using standard fish transportation procedure. These fishes were acclimatized to laboratory condition using dechlorinated tap water for 1 week in glass aquaria (Maiti, 2004). The fish used have the average standard length of 8-10cm and average weight of 7-9g. These fish samples were used for acute toxicity test as described in (Iji and Adeogun, 2014; APHA, 2005) and for histopathological study using biopsy of some tissues (skin, muscles and gills) of *Clarias gariepinus* juveniles fish (Auwioro, 2010).

Acute Toxicity Test Using *C. gariepinus*

Acute toxicity test provide an estimate of the exposure concentration causing 50% mortality (LC₅₀) i.e. lethal concentration causing 50% mortality of the subject during specific period of time. This test was done following the etiquette of APHA (2005). In this technique, four (4) different concentrations of the effluent were prepared using dechlorinated tap water. A total of 185 fishes (*C. gariepinus* juveniles) were exposed to these effluent concentrations in triplicate including the control, that is 5 fishes per replicate per treatment. The four



concentrations of the effluent samples used were 25%, 50%, 75% and 100% for both sites (E1, E2 and E3) and control. The control was dechlorinated water only. Observation was made after 24hrs., 48hrs. and 72hrs. The status and behaviour of the fish was monitored throughout the experiment, in fact the differences between fishes exposed to treatments and those in control set-up in terms of these behaviours were observed, and the number of dead fishes (mortality) within the 3 days post exposure were recorded (APHA, 2005).

Determination of LC₅₀

LC₅₀ was determined by Probit Analysis using MS- Excel (Rank, 2006). Safe concentration was calculated by multiplying the lethal concentration values (LC₅₀) with an application factor of 0.1 (Bobmanuelet al., 2006).

Histopathological Study

After three days post exposure, the biopsy (removal and examination of a sample of tissue from the body) of fish i.e. skin, muscles and gills were made and prepared for probable histopathological degradation study. These tissues were fixed in 10% normal saline for 12hrs (Agboola and Fawole, 2012). The tissues were later dehydrated with ascending grade of alcohol, cleared with toluene and infiltrated with molten paraffin wax.

Microtome sections were made and stained with heamatoxylin followed by eosin (Stevens, 1982; Llewellyn, 2009; Auwioro, 2010; Chan, 2014). Each section of the gills, muscles and/or skin was then used to make slides for examination.

Microscopy

Slides were examined using Leica DM-150 microscope (manufacturer: Leica Microsystems GmbH) for proper description of histological structures, appearance, cell arrangement and some effect such as mutilation of tissues, degeneration of cell layers and rapture of gill rakers. Photomicrographs were taken with Leica ICC 50 Hi camera (Auwioro, 2010).

Results and Discussion

Table 1 below shows the results for acute toxicity effect of the textile and dyeing effluents on *C. gariepinus* where total death, percentage death, LC₅₀ and safe concentrations were all shown in the table.

Table 1: Results of Acute Toxicity Test for Textile and Dyeing Effluents on *Clarias gariepinus* Juveniles

Effluents Conc. (%)	No. of Fish Used per Dilution	Aggregate Mortality After 72hrs. in 3-Replicates for Each Concentration					
		E1		E2		E3	
		Total Death	% Death	Total Death	% Death	Total Death	% Death
25	15	7	47	5	33	6	40
50	15	9	60	6	40	10	67
75	15	11	73	8	53	12	80
100	15	14	93	12	80	13	87
LC ₅₀			14.8		22.4		14.45
Safe Conc.			1.48*		2.24*		1.445*

NB: No mortality was observed in control

*Bobmanuelet al. (2006)

Mortality of fishes was observed at 24hrs., 48hrs. and 72hrs. and the overall percentage mortality after 72hrs of exposure was 47%, 60%, 73% and 93% for the four concentrations of the effluent in site E1. In site E2 however, there were 33%, 40%, 53% and 80% mortality of the fishes in 25%, 50%, 75% and 100% concentrations respectively.

Moreover, 40% was the lowest percentage mortality of fish observed in 25% concentration in site E3 while 87% mortality was observed in 100% concentration Table 1. No mortality was observed in the control set-up throughout the duration of the experiment which were set and maintained under identical experimental conditions with the treated set-up.

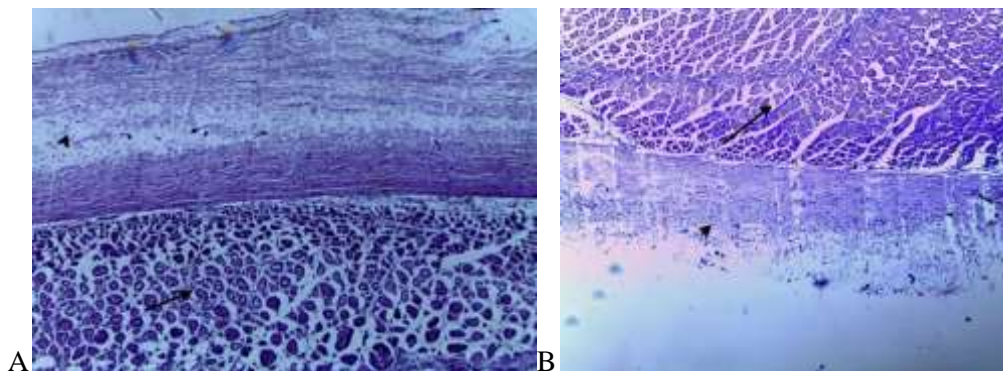
The percentage mortality rate of *C. gariepinus* in this study was found to be directly proportional to the concentrations of exposure, thus higher mortality were observed at higher concentrations and *vice-versa*. This result was similar to the work done by Roopadevi and Somasheker (2012) who reported similar result in *Cyprinus carpio* fish where they assessed the toxicity of wastewater from Sri-Srinivasa textile industry in Bangalore, India. It is also in line with the findings of Gabriel and Okey (2009) and Pool *et al.* (2009) who worked on hybrid of catfish.

The results of the mortality analysis of different concentrations of effluent against *C. gariepinus* in this study yielded toxicity indices values (LC_{50}) of 14.8% (v/v) for E1, 22.4% (v/v) for E2 and 14.45% (v/v) in E3. The LC_{50} values obtained for E1 and E3 fall within range of 10-15% obtained by Agboola and Fawole (2010) who worked on chronic toxicity effect of pharmaceutical effluents on *C. gariepinus*.

The safe concentrations obtained for the effluents were 1.48%, 2.24% and 1.44% for site E1, E2 and E3 respectively following the protocol of Bobmanue *et al.* (2006). These safe concentrations are practically useful in establishing the dilution limits to which discharge of wastewater of textile and dyeing origin into aquatic ecosystem cannot harm the biota there-in even during prolong period of exposure (Roopedevi and Samasheker, 2012). Hence, boundary of values for the discharge of textile and dyeing effluents into aquatic ecosystem in Kano as obtained by this research may be between 1.44 to 2.24%.

Overall, the result of toxicity index (LC_{50}) obtained as well as the safe concentrations agreed with the findings of Agboola and Fawole (2010) and Roopedevi and Samasheker (2012).

Plate I A below was the photomicrograph sections of skin and muscles of the catfish (*C. gariepinus*) in the control group while B to F showed the histopathological effects of textiles and dyeing effluents on skin and muscles of *C. gariepinus* at different concentrations.



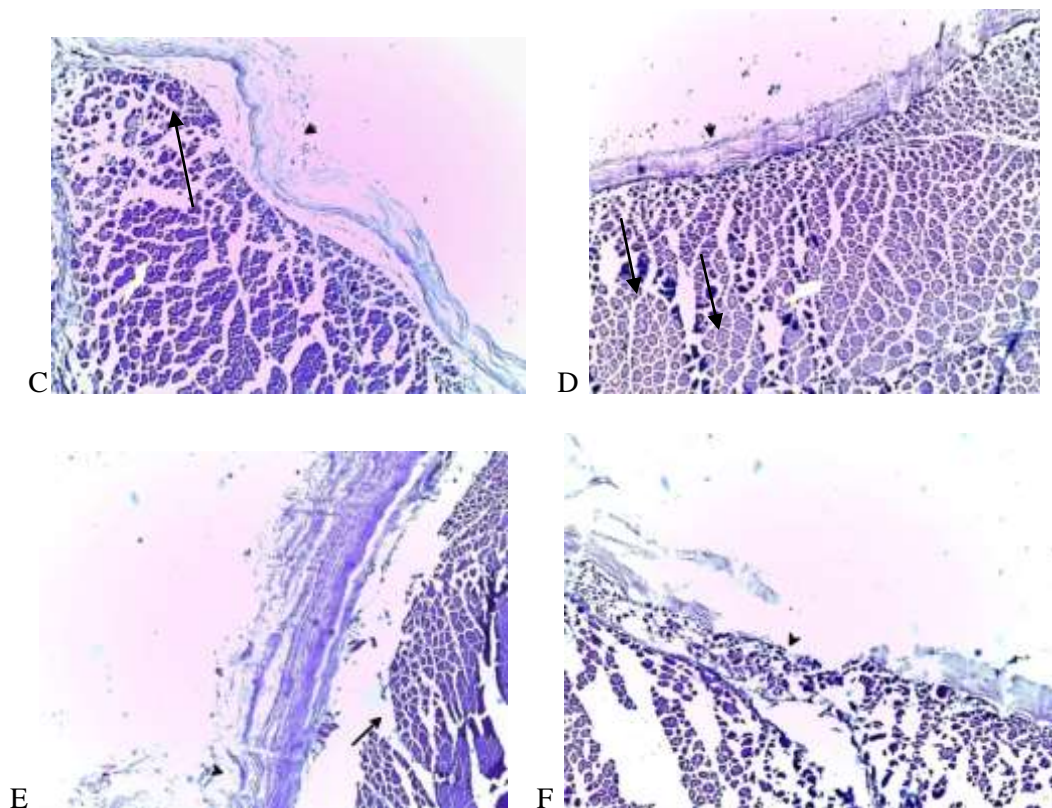


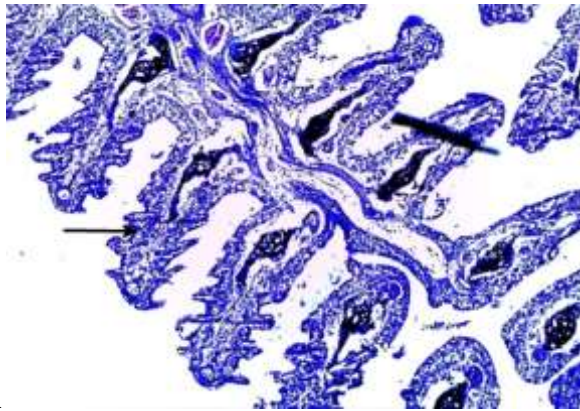
Fig I:(A) Photomicrograph of the Section of Skin and Muscle of *C. gariepinus* Juveniles in Control Group Showing Remarkable Thickness in Skin and Muscle Tissue(Arrow Head) and (Long Arrow). (B) Photomicrograph of the Section of Skin and Muscle Tissues of *C. gariepinus* Juveniles Exposed to 25% concentration of Textile Effluent Showing Slight Degeneration of Peripheral Skin Layer (Arrow head). (C)Section of Skin and Muscle Tissues of *C. gariepinus* Juveniles as Exposed to 75% Concentration of Textile Effluent Showing Area of Severe Degeneration of Skin Tissues (Arrow Head) and Area of Abscess or Eruption of Cells in Muscles Tissue Creating Gap Between the Skin Tissue Layer (Long Arrows).(D) Section Through Skin and Muscle Tissues of *C. gariepinus* Juveniles Exposed to Dyeing Effluent (25% Concentration) Showing Degeneration of Skin Tissues (Arrow Head) and Localized Laceration of Muscle Cells (Long Arrows).(E) Section Through Skin and Muscle Tissues of *C. gariepinus* Juveniles Exposed to Dyeing Effluent (75% Concentration) Showing Severe Degeneration of Both Skin and Muscle Cells (Short and Long Arrow). Noting the Gap Caused by Deterioration of Muscle Cells. (F) Section Through Skin and Muscle Tissues of *C. gariepinus* Exposed to Dyeing Effluent (75% Concentration) Showing Severe Degeneration of Skin Layer (Arrow Head) and Severe Localized Lesion on Muscle Cells (Long Arrows) (H& E, Mag. \times 100)

Plate II A1 on the other hand was the photomicrograph sections of gills of the catfish (*C. gariepinus*) in the control group and B1 to G1 showed the histopathological effects of textiles and dyeing effluents on the gills at different concentrations.

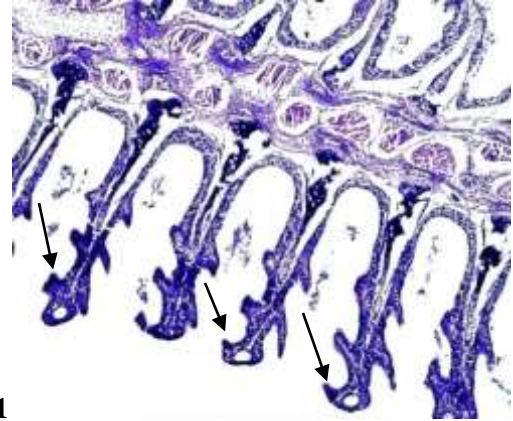
Fig II: (A1) Photomicrograph of the Section of Gills of *C. gariepinus* Juveniles in Control Group Showing Intact Arrangement of Primary and Secondary Lamella of the Gills Rakers with Little Inflammation of the Gill's Tissues (Arrow). (B1) Section of Gills of *C. gariepinus* Juveniles Exposed to Textile Effluent (25% Concentration) Showing Slight Mutilation of Secondary Lamella of the Gills Rakers (Arrows). (C1) Section of



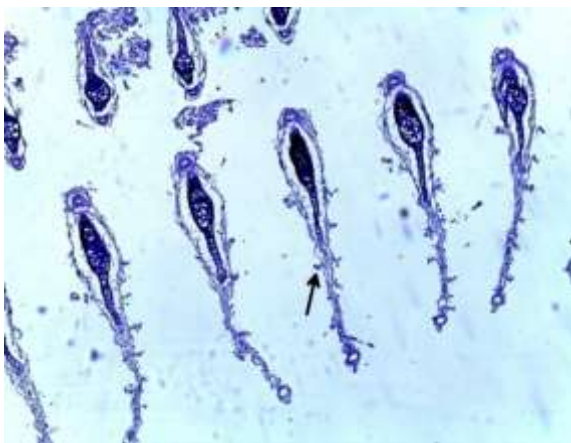
Gills of *C. gariepinus* Juveniles Exposed to Textile Effluent (75% Concentration) Showing Poorly Formed Gills with Severely Injured Secondary Lamella and Deterioration of Gill's Central Hyaline Cartilage.



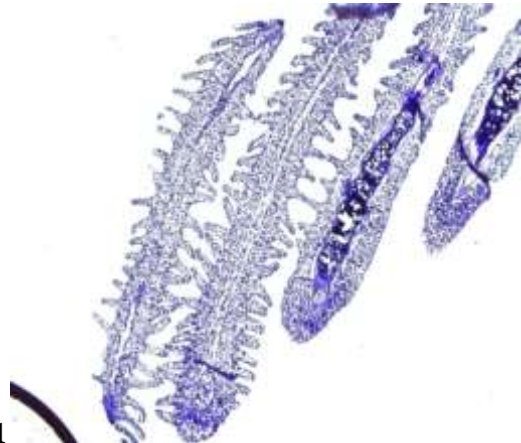
A1



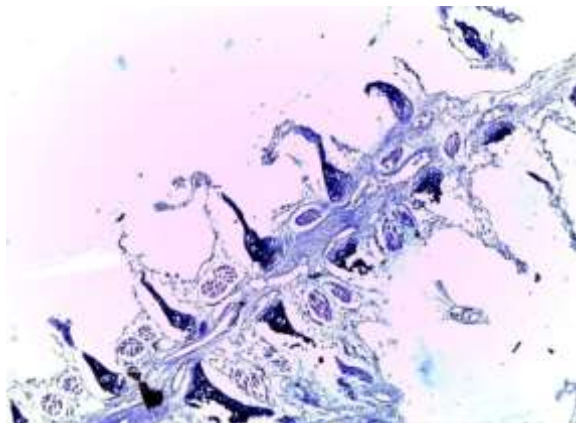
B1



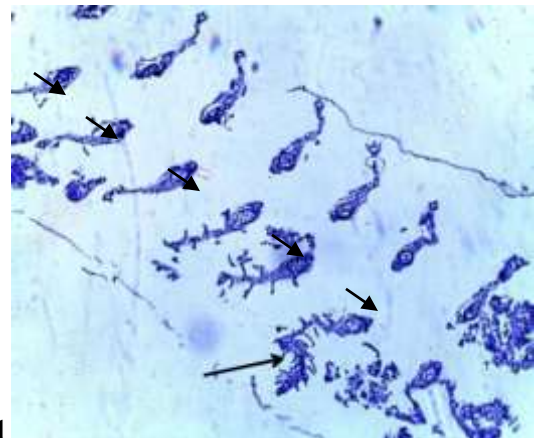
C1



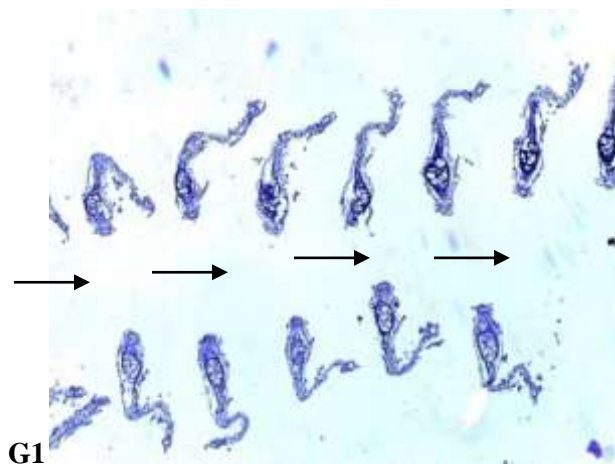
D1



E1



F1



(D1) Section of Gills of *C. gariepinus* Juveniles Exposed to Dyeing Effluent (25% Concentration) Showing Thinning of Secondary Lamella. **(E1)** Section of Gills of *C. gariepinus* Juveniles Exposed to Dyeing Effluent (75% Concentration) Showing Conspicuous Gills Damage. **(F1)** Section of Gills of *C. gariepinus* Juveniles Exposed to Dyeing Effluent (75% Concentration) Showing Severely Injured Gills with Mutilated Lamella (Long Arrow) and Completely Deteriorated Central Core Hyaline Cartilage (Position- Short Arrows). **(G1)** Section of Gills of *C. gariepinus* Juveniles Exposed to Dyeing Effluent (100% Concentration) Showing More Eroded Gills Lamella and eroded Hyaline Cartilage (Long Arrows) (H & E, Mag. $\times 100$)

Conclusion

Although, textile and dyeing activities are promoting phenomena for economic growth in Kano metropolis, but their effluent should be effectively managed before discharge. This is because, the toxic constituents of textile and dyeing effluents (according to the study) caused fish mortality at different concentrations during acute exposure and as well causes histopathological degradation of tissues in fishes as shown by the responses of these tissues to the toxicant present. These degradations of histological origin were detected to the large extent in skin, muscles and gills, because, they are the ones predisposed more to the effluent concentrations during exposure and therefore are sensitive and suitable for eco-toxicity study for textiles and dyeing effluents.

The physiological changes and the histopathological anomalies serve as bioindicators of environmental pollution. Thus, the study provides warning of potential adverse environmental impact of textile and dyeing effluent to the receiving aquatic ecosystems in Kano. The derived limit of acceptability for discharge of textiles and dyeing effluents as obtained by this research was between 1.45 and 2.24%. This is at least the level of dilution of effluents required for these industries before discharging into aquatic environment.

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Appendix: Mortality Details

As the reviewer request for mortality of experimental animals on exposure after 24hrs. and 48hrs. in each site (If not needed in the main article should please be deleted)

Mortality Detail of *C. gariepinus* Exposed to Different Concentrations of Textile Effluents (E₁)

Conc.(%)	24hrs			48hrs			72hrs		
	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃
25	1	1	1	1	2	1	0	0	0
50	1	1	2	1	1	1	1	0	1
75	2	2	1	1	2	1	1	0	1
100	3	2	3	1	1	1	1	1	1

Mortality Detail of *C. gariepinus* Exposed to Different Concentrations of Dyeing Effluents (E₂)

Conc.(%)	24hrs			48hrs			72hrs		
	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃
25	0	0	1	0	1	0	1	1	1
50	1	1	1	1	0	0	0	1	1
75	1	1	1	1	1	1	1	1	0
100	2	2	2	1	1	1	1	1	1

Mortality Detail of *C. gariepinus* Exposed to Different Concentrations of Dyeing Effluents (E₃)

Conc.(%)	24hrs			48hrs			72hrs		
	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃	R ₁	R ₂	R ₃
25	0	1	0	1	1	1	1	0	1
50	2	1	1	1	1	1	1	1	1
75	2	1	2	1	1	2	1	1	1
100	2	2	2	2	2	2	0	0	1