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Effect of Hair Dressing Salon Effluent on the Juveniles of African Catfish (*Clarias gariepinus*)

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ABSTRACT: The toxicity of hair dressing salon effluent on *Clarias gariepinus* juveniles was studied using static bioassay method. This study investigated haematological, histopathological and biochemical changes in *Clarias gariepinus* juveniles. The fish samples were distributed randomly into T_1 (800 ml/L), T_2 (600 ml/L), T_3 (400 ml/L), T_4 (200 ml/L), and T_5 control (water alone). All samples were studied in three replicates, in a completely randomized experiment. Mortality increased with increased effluent concentrations; 250.96 ml/L was recorded as LC_{50} for 96 h. Various behavioural changes observed in fishes before death include: odd swimming patterns, jerk movements, bottom resting and loss of appetite. Histopathological changes were observed in treated fishes while the livers and gills of the control fishes showed normal histological appearance. It was concluded that hair dressing salon effluents had some negative effect on histopathology and behavioural responses of juvenile *Clarias gariepinus*. Therefore, it is recommended that the effluent should be properly treated before being discharged into the environment.

Keywords: Environmental pollution, Aquaculture, Juveniles, Salon effluent.

Introduction

Environmental pollution has become one of the most important problems of the world (Chandran *et al*., 2005). Improper management, inadequate treatment alongside its flow of vast amount of wastes generated by various anthropogenic activities (Fakayode, 2005). Nigeria alone isn't confined with this problem as this is also a major problem in most countries that are undergoing the process of development, Wastes from these salons have therefore become a very common source of pollution to the aquatic environment especially in Sub-Saharan Africa; where there is little or no prohibition for violators (Chude and Ekpo, 2010) and who do not treat waste water management as a priority on their list.. Interestingly, an average hair beauty salon generates wastewater each day. Water generated after the use of freshwater, raw water, drinking water or saline water in a variety of deliberate applications or processes is termed waste water. The diversity of biological and chemical sources in the waste water could mean a lot of chemical and biological components in their assorted products are washed into the soil or poured down the drain.

Some of these chemicals are toxic and prohibited from use as ingredients because they have the potentials to cause cancer, mutation, reproductive toxicity and endocrine disruption (Amasa *et al.*, 2012). Trace elements whose toxicity are well documented (Adepoju-Bello *et al.*, 2012; Popoola *et al.*, 2013; Ramakant *et al.*, 2014; Zulaikha *et al.*, 2015) are also incorporated into beauty products for many purposes. Biomarkers (biological responses elicited in living organisms as a results of exposure to or effects of toxicants) such as antioxidant enzymes activity, lipid peroxidation which indicates oxidative stress, histological alterations in tissues of animals have been used to evaluate the potential effects of effluents on model organisms in the receiving aquatic ecosystems (Adeogun *et al*., 2012; Adeogun and Chukwuka, 2012; Sogbanmu and Otitoloju, 2014; Sogbanmu *et al*., 2018).

The African catfish (*Clarias gariepinus*) is of importance commercially and ecologically in Nigeria. It is a freshwater fish and forms the diet of most individuals due to their relative ease of culture and high protein content (Adeogun and Chukwuka, 2012). Ecologically, they are components of the food web in freshwater ecosystems. The aim of this study was to investigate the the potential effect of hair salon effluent on juvenile catfish (*Clarias gariepinus*).

Materials and methods

Study area: This work was carried out in the laboratory of Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike between May and August, 2018.
Collection of salon effluent and physico-chemical analysis: Raw effluents were obtained from hair dressing salon in Umuariaga, Ikwuano L.G.A, Abia State, Nigeria. The effluent was collected in a ten (10) L plastic keg, transported to the laboratory and refrigerated (at 4°C) until use. They were collected from the discharge points. The effluent was analyzed at the Zoology and Environmental Biology Department laboratory, Michael Okpara University of Agriculture, Umudike, Abia State for the following physico-chemical parameters; pH, total dissolved solids (TDS), electrical conductivity, dissolved oxygen (DO), according to (Ajuzie and Osaghae, 2011).
Ethics statement: This study followed the principles in the Declaration of Helsinki on the humane treatment of animals used in research (http://www.wma.net/en/30publications/10policies/a18/).
Collection and acclimatization of test animals: Healthy and active juvenile *Clarias gariepinus* of unspecified sexes (weight range: 15 - 25 g and length range: 16.5 - 18.7 cm) were procured from the fish farm of Michael Okpara University of Agriculture, Umudike and transported to the Department of Zoology and Environmental Biology laboratory in oxygenated polyethylene bags. Fish were stocked in 50 L plastic tanks that was three-quarter filled with dechlorinated tap water. The fish were allowed to acclimatize to laboratory conditions (temperature: 28 ± 2 °C; relative humidity: 78 ± 4 %) for a period of 7 days prior to the experiments. The fishes were fed with Coppens fish feed and mortality was assessed daily. A fish was considered dead if it failed to respond to mechanical stimuli (Arun *et al.*, 2011). Dead fish were removed from stock tanks to prevent contamination.
Acute toxicity studies with salon hair dressing effluent: A range finding test was conducted to establish the dose range of the effluent for the experiment. A static bioassay protocol (in which the toxicant was not renewed over the period of 96 hours) was employed for the acute toxicity test of the effluents against juvenils of *C. gariepinus* according to OECD (1992). Acute toxicity tests were carried out in duplicates. Fifteen plastic rectangular tanks were labelled A-E in 3 replicates. The test solution (Effluent) was thoroughly mixed by pouring into a big plastic container before exposing the test organisms into the labelled tank. The varying concentrations used were T_1=800 ml/l, T_2=600 ml/l, T_3=400 ml/l, T_4=200 ml/l and T_5=control (water alone). Seven (7) fishes were exposed to each concentration including the replicates.
The experiment was monitored for 96 hours during which the following parameters were evaluated: behavioural responses, total number of death (mortality) after 96 hours. The percentage mortality at 96 hours, calculation of the LC_{50} which is the concentration at which half or 50% of the test organism died on exposure using probit analysis Sogbanmu and Otitoloju (2014).
Histopathological analysis: After 96 hours, fish from the experimental groups as well as control were randomly selected for histological studies. The selected fishes were euthanized, the gills and livers were excised and fixed in 10 % formosaline (Amasa *et al.*, 2012). They were processed routinely, embedded in paraffin wax, sectioned at 4-5 µm thickness, stained with haematoxylin and eosin (H and E) (Sogbanmu *et al.*, 2018). Permanent slides were prepared with tissue sections, examined with the aid of a light microscope (Nikon TE 3000) and photomicrographs taken with a digital camera (Nikon 9000).
Statistical analysis: The physico-chemical parameters of the samples were analysed using SPSS version 20.0. The 96 hLC50 value of acute toxicity was determined by probit analysis (using SPSS version 20.0.) Data obtained for sub-lethal toxicity tests were subjected to One-way analysis of variance (ANOVA) test to test for the significance between the treatment means. Significant means (at $p<0.05$) were separated with Least Significant Difference (LSD) using SPSS version 20.

Results

The physicochemical characteristics of hair dressing salon effluent is presented in Table 1.

The physicochemical analysis of the effluent showed that conductivity, total dissolved solids were higher than Federal Ministry of Environment (FMEVN) set limits and do not conform to the standard set for effluent discharge.

Table 1: Physicochemical characteristics of hair dressing salon effluent

Parameters	**Experimental Values**				
	T_1	**T_2**	**T_3**	**T_4**	**FMEVN (2011)**
pH	3.83	4.73	5.73	6.23	6.50-8.50
Dissolved Oxygen DO (mg/L)	7.23	7.16	6.86	6.63	N.S
Total Dissolved Solids TDS (mg/L)	208.66	172.66	131.66	89.00	2000.0
Electrical Conductivity EC (µS/cm)	416.33	338.33	263.66	181.33	ND

DO=Dissolved oxygen, TDS=Total dissolved solid ND=Not detected, EC=Electrical conductivity. NS=Not Specific.

Behavioural response of test organism: *Clarias gariepinus* exhibited distress behavioral response due to the effect of the hair dressing saloon effluent. This was noticed by the sudden change in the organism response to the environment such as erratic swimming, occasional gasping for breath, a frequent surfacing which. As the experiment progressed, some of the test organisms were seen to get weaker evident by reduction in movement, their ventral surface were subsequently turned upwards while those that could not tolerate the concentration any longer went into a state of motionlessness. Normal behaviour was however observed in the control (Table 2).

Table 2: Behavioural response of *Clarias gariepinus* during exposure to acute concentrations of hair dressing salon effluent.

Behaviour	**Concentration**				
	0.00	**200 ml/l**	**400 ml/l**	**600 ml/l**	**800 ml/l**
Erratic swimming	-	-	+	+	+
Gasping for breath	-	+	+	+	+
Loss of reflex	-	+	+	+	+
Opercula beating	-	-	-	+	+
Frequent surfacing	-	-	+	+	+
Motionlessness	-	+	+	+	+
Hyperventilation	-	-	+	+	+
Light	-	-	-	+	+

Legend: Absent (-), Present (+)

Table 3: Mortality rates of *C. gariepinus* exposed to varying concentrations of hair dressing salon effluent

Exposure	**Control**	**800 ml/l**	**600 ml/l**	**400 ml/l**	**200 ml/l**
Period (h)	**$R_1R_2R_3$**	**$R_1R_2R_3$**	**$R_1R_2R_3$**	**$R_1R_2R_3$**	**$R_1R_2R_3$**
12	- - -	- - -	- - -	- - -	- - -
24	- - -	2 1 2	- - -	- - -	- - -
48	- - -	4 2 1	3 2 1	- - -	- - -
72	- - -	1 2 4	4 4 6	- - -	- - -
96	- - -	- 2 -	- 1 -	- - 1	- - -

Table 4: LC_{50} values for hair dressing effluent (with 95% confidence limit) estimated by Probit analysis

Time (hr)	**LC_{50} Values (ml/L)**	**Lower limit (ml/L)**	**Upper limit (ml/L)**
48	380.344	338.24	483.50
72	250.96	299.52	272.31
96	250.96	229.52	272.31

At 95% confidence level, the LC_{50} for the test substance was 380.34 ml/l, 250.96 ml/l and 250.96 ml/l at 48.72 and 96 h respectively (Table 4).

Histological examinations: The results of the histological examinations are shown in Plates 1-6. The liver histology are shown in Plates 1-3 while gill histology are shown in Plates 4-6.

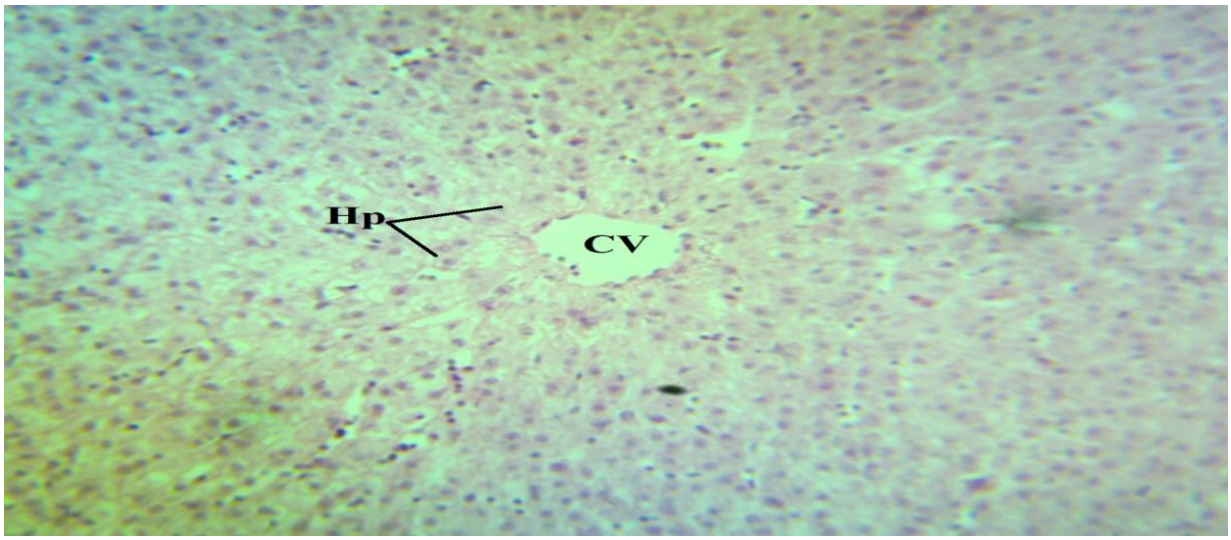

Plate 1: Photomicrograph of liver showing normal histologic architecture in control treatment: central vein (CV) and hepatocytes (HP). X400.

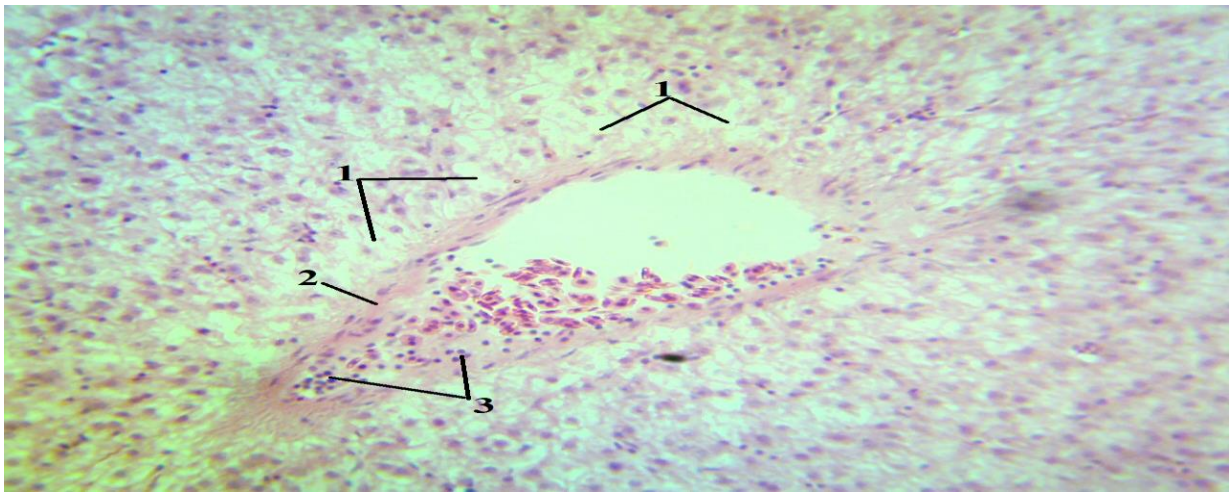

Plate 2: Photomicrograph of liver showing hydrophobic degeneration of hepatocytes (swelling of cells) (1), thick fibrous connective tissue (fibrosis) lining the central vein (2), moderate congestion and mononuclear cell infiltrations (3), in 200 ml/L of salon hair effluent. H&E, X400.

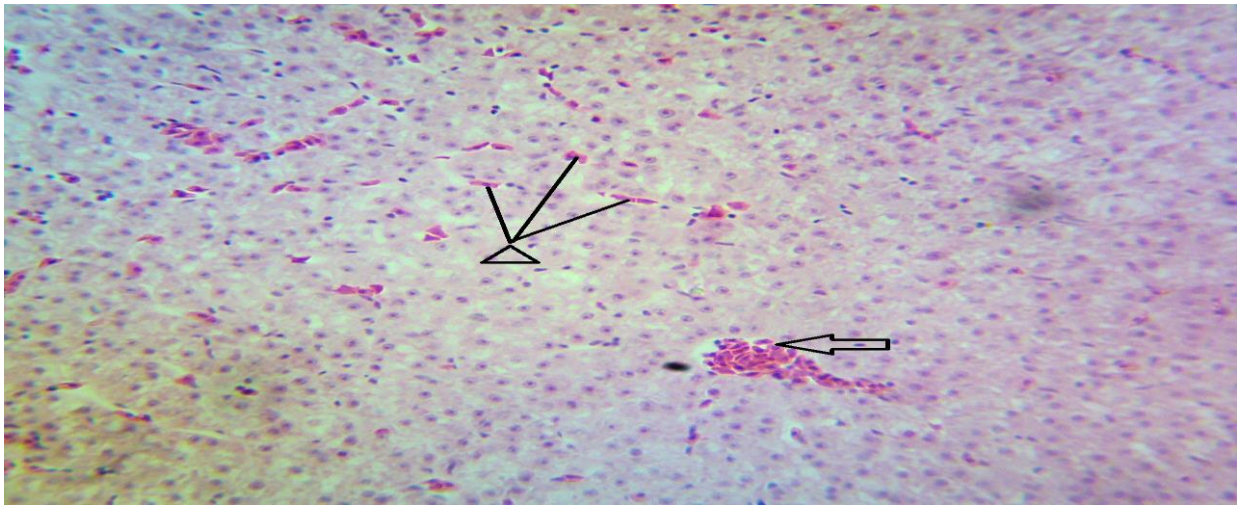

Plate 3: Photomicrograph of liver showing diffused marked congestion of central vein (arrow) and sinusoids (arrow head), in 400 ml/L of salon hair effluent. H&E, X400.

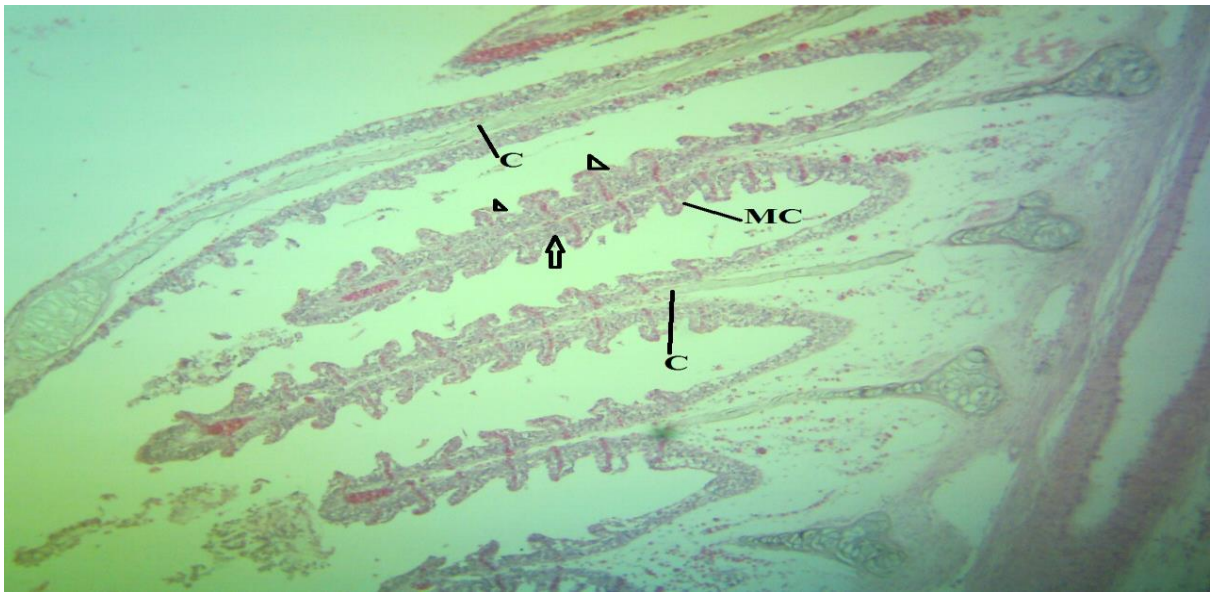

Plate 4: Photomicrograph of gill showing normal histologic architecture in Control group: Primary lamella (PL) secondary lamella with the epithelial covering (arrow head) and mucous cell (MC). In the central core is a mass of hyaline cartilage (C) and blood vessels and is covered with stratified epithelium. H&E, X400.

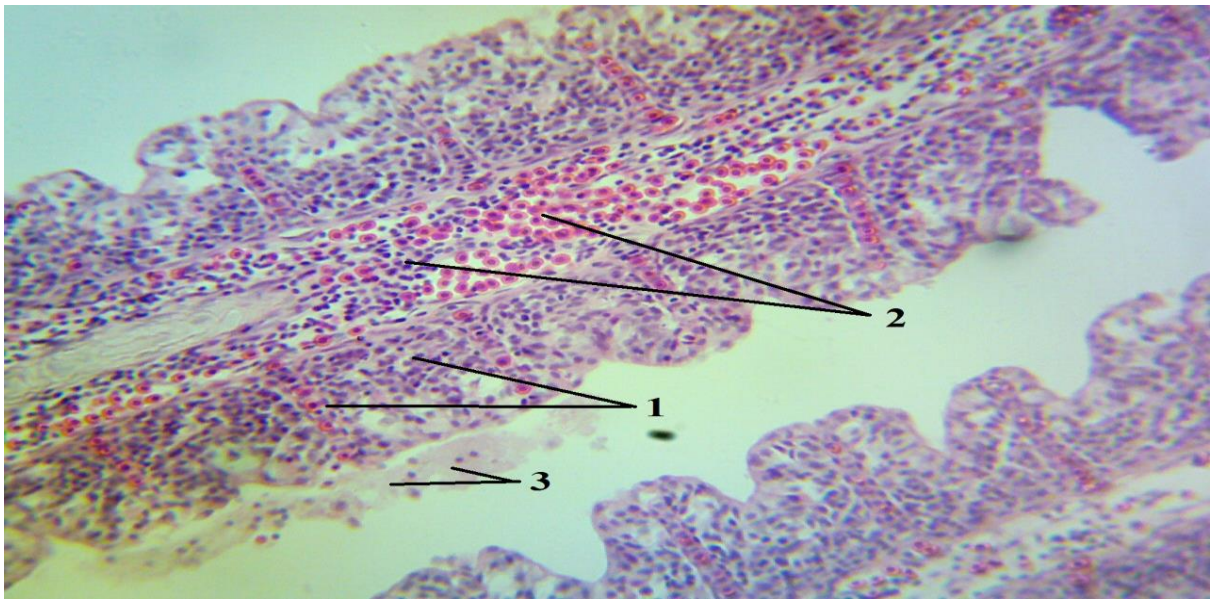

Plate 5: Photomicrograph of gill showing swelling and fusion of the epithelial cells with necrosis and adhesions of gill lamellae(1), sloughing of epithelial cells (arrowhead), marked proliferation of mucous cells (2), congestion of blood vessels (arrow) and haemorrhages (H)and infiltration of mononuclear inflammatory cells, in 200 ml/L of salon hair effluent. H&E, X400.

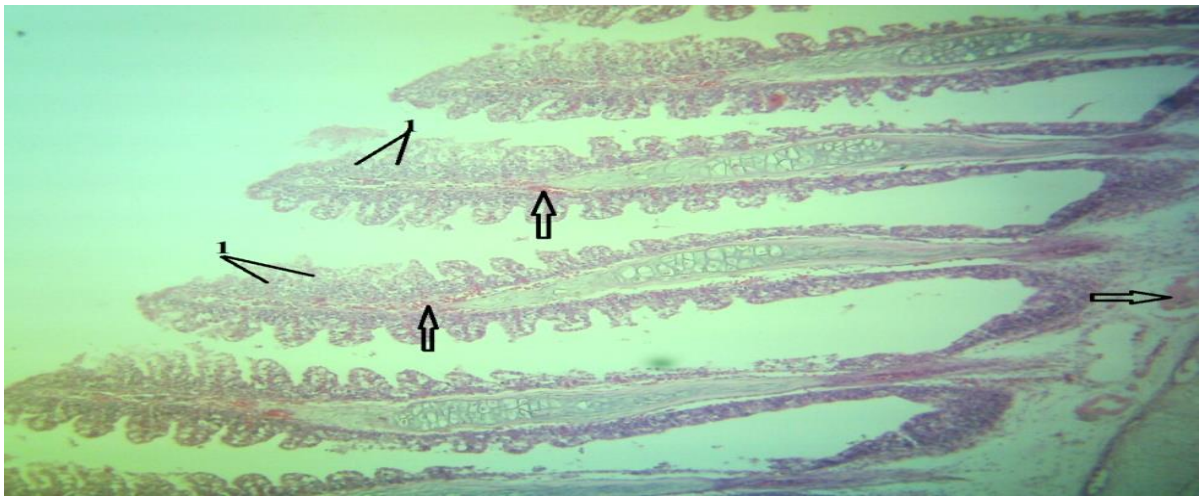

Plate 6: Photomicrograph of gill showing swelling of epithelial cells and adhesions of gill lamellae (1), moderate proliferation of mucous cells, congestion of blood vessels and infiltration of mononuclear inflammatory cells (2), and excessive production of mucus (3). H&E, X400.

Discussion

The physico-chemical parameters of the effluent in this study showed risk of contamination (Bautista *et al.,* 2007).The abnormalities (gasping for breath and frequent surfacing) observed prior to mortality are indications of depleted oxygen content (hypoxias) due to higher demand for oxygen. The erratic swimming, restlessness, increase in opercular beating, gasping for air as well as accumulation of mucus on the fish body is as a result of skin irritation, respiration rate impairment or a response to altered locomotion activity, an indication of the effect of toxicant on the nervous system.

Fish exposed to very low concentration of toxicant may not react quickly, rather become very adapted to the new environment. This was essentially the case with test organisms in treatments A and B where abnormal behaviours were not observed. However, the stressful and erratic behaviour of the fish in treatment A and B gives a signal to respiratory impairment. This may be a consequence of the high effluent levels or toxicity effect of the gills. This agrees with the work of Ogaga *et al* (2015).

The acute toxicity result supports the observation of Akaniwor *et al.* (2007) that in all toxicants, a threshold is reached above which there is no drastic survival of animals. Below the threshold, an animal is in a tolerance zone, above the tolerance zone is the zone of resistance (Nadal *et al.,* 2004). The established 96 hLC50 value for the acute test was 250.96 mL/L showed that the hair dressing salon effluent was quite toxic to the exposed fish. Although *C. gariepinus* has been proven to be relatively resistant to various toxicants when compared to other species (Patkar, 2008; Onwusah *et al.,* 2015).

The gills were the primary target tissue affected by the air dressing salon effluent. Gills are generally considered good indicator of water quality (Chude, 2008), being models for studies of environmental impact (Chude and Ekpo, 2010; Onwusah *et al.,* 2015), since they are the primary route of entry of most contaminants. After LC_{50} for 96 hours exposure Gill showed swelling and fusion of the epithelial cells with necrosis and adhesions of gill lamellae, sloughing of epithelial cells (arrowhead), marked proliferation of mucous cells, congestion of blood vessels (arrow) and haemorrhages (H) and infiltration of mononuclear inflammatory cells as observed in plate 5 resulted from excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification. The changes in appearance of the secondary lamellae result from the collapse of the pillar cell system and breakdown of vascular integrity with release of large quantities of blood that push the lamellar epithelium outward (Bautista *et al.*, 2007). Necrosis and cell desquamation of the gill epithelium observed may be due to the direct toxic effects of trace metals, while epithelial oedema and partial and total fusion of the lamellae represent a defense response (Ramakant *et al.,* 2014).

The liver is the main organ for detoxification that suffers serious morphological alterations in fish exposed to toxic trace elements (Saliu and Bawa-Allah, 2012). High accumulation of several components of hair dressing salon effluent in the liver is a pointer to the fact that, liver plays a major role in detoxification. (Arun *et al.,* 2011) the bioaccumulation of these trace element in the liver tissue reaches a proportion in which the function of the liver is impeded. Thus, resulting in a progressive degeneration of the liver cells syncytial arrangement. Therefore, necrosis became evident as the concentration increases and this may be due to the inability of the

fishes to regenerate new liver cells and excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, (Shalom *et al.* 2013).

It was also observed that the histopathological changes in the liver caused metabolic problems. An increase in the degree of damages done to the gills of the fishes held in 200 and 100ml/L hair dressing salon effluent was generally related to important degenerative and necrotic processes, this observation was in line with the submission of Pacheco and Santos (2002).

The histological alterations identified within the hepatocytes may have been the results of various biochemical lesions. Irregular shaped central vein and infiltration may be attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to the toxicants, this is in conformity with the submission of Fanta *et al* (2003). Pacheco and Santo (2003) also described increased level of vacuolation of the hepatocytes as a signal to the degenerating process that suggest metabolic change, possibly related to exposure to contaminated water.

Therefore, the histological changes observed in the liver indicated that the fish were responding to the direct and the cumulative effects of the contaminants as much as other effects such as stress. Such information confirmed that histopathological alterations are good biomarkers for both field and laboratory assessment, particularly in tropical areas that are naturally subjected to a multiplicity of environmental variations or depletion due to chemical contamination. Ajani and Awogbade (2012).

Conclusion

In conclusion, exposure of *Clarias gariepinus* juvenile to even low concentrations (100 ml/L) of hair dressing salon effluent could result in change in behavioural pattern, histological degradation and death. In view of the toxicity effect of this effluent, it can be inferred that, indiscriminate discharge of effluents end up depleting the water quality which increases the mortality rate of aquatic organisms. Therefore, there is need for regulatory agencies to identify and monitor indiscriminate discharge of effluents, adoption of proper effluent treatment technology which would ensure proper treatment of industrial effluents prior to their discharge into the environment and endeavour to create adequate awareness. Management strategies should be developed, the populace should be informed about the adverse effects of effluents and the role they play in our immediate environment.

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Conflict of Interest:

No potential conflict of interest was reported by the authors.

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