RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v19i1.4

Audu *et al*. /*Sokoto Journal of Veterinary Sciences*, **19**(1): 26 - 33.

Histopathological changes in gills and liver of *Clarias gariepinus* fingerlings exposed to acute concentrations of dry cell battery

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Publication History:
Received: 23-06-2020
Revised: 02-12-2020
Accepted: 04-12-2020

Abstract

Waste dry cell batteries are frequently improperly disposed and subsequently washed into water bodies-- causing deleterious effects on fish particularly *Clarias gariepinus* which inhabits diverse freshwater habitats. Acute toxicity of water-soluble fractions of waste dry cell batteries was investigated on *C. gariepinus* fingerlings under laboratory conditions in 96 hours. Ten (10) *C. gariepinus* fingerlings were exposed to acute concentrations (0.31, 0.63, 1.25, 2.50, and 5.00 g/L) of waste dry cell batteries and a control (0.00 g/L), each duplicate replicated. Histopathological alterations evident in the gills were lamellar fusion, hyperplasia, inter-lamella space occlusion, hypertrophy and erosion of secondary lamellae. The liver showed nuclear and hepatocytes degeneration, vacuolation and portal congestion. Acute concentrations of water-soluble fractions of waste dry cell batteries caused significant ($P<0.05$) changes in the histomorphology of the gills and liver of *C. gariepinus* fingerlings, therefore indiscriminate disposal of waste dry cell batteries around riparian ecosystem should be safeguarded to reduce the declining diversity and abundance of freshwater fish species.

Keywords: African catfish, 96 hr.LC_{50}, Fingerlings, Histopathology Zinc-carbon battery

Introduction

The presence of heavy metals in the aquatic environment is reputed to have a long biological half-life (Srivastava & Punia, 2011). Owing to this property, heavy metals are regarded as the most hazardous of all environmental pollutants because they readily bioaccumulate in fish organs (Al-Attar, 2005; El-Nagger *et al*., 2009) leading to bioconcentration and finally biomagnify with attendant consequences on attainment of threshold (Audu *et al*., 2020). Fish organs are regarded as good biomarkers of aquatic metal contamination (Thangam *et al*., 2014) either as soluble or suspended metals in water and are reported to be readily absorbed through gills and skin to elicit toxicity in fish (Abalaka, 2015). In addition, high concentration of heavy metals in aquatic bodies do

not spare the biochemical system of fish and portends long term ecotoxicological effects on the organisms that eat them (Strmack & Braunbeck, 2000). With the important roles of fish in human diets and in animals' rations, it is pertinent to mention that fish sourced from heavy metals-contaminated waters may have great impact on individual and public health. Chemically, waste dry cell batteries (WDCB) contain heavy metals such as Zinc (Zn), Lead (Pb) and Manganese (Mn) (Al-Mahbub *et al*., 2015). These groups of metals have non-essential role in the tissues of living organisms and are also capable of precipitating debilitating health conditions especially at prolonged low-dose exposure (Jarup, 2003; Rajeshkumar & Li, 2018) This feature accounts for WDCB becoming a serious environmental pollutant particularly when they are indiscriminately discarded from house-hold refuse, industrial wastes and electronic/automobile mechanic workshops thereby allowing them to be washed into water bodies (Audu *et al*., 2020). The major pathological effect of the most important constituent of dry cell battery, the zinc, is in its ability to cause gill damage; an organ with the prime osmoregulatory function in fish (Andres *et al*., 2000; Papagianus *et al*., 2004).

The gills of fish remain the organ that is constantly in contact with the external environment and participates in many key physiological roles of respiration and osmoregulation thereby making it to be sensitive to disruption in the water quality that could hamper its function (Camargo & Martinez, 2007). Previous reports on the morphophysiological alterations in the gill were found to be restricted to gills lamellar epithelium and surrounding blood vessels (Hinton & Lauren, 1990). The notable lesions observed in the gill parenchyma of fish exposed to heavy metals laden aquatic environment include; cellular hypertrophy, hyperplasia, epithelial lifting, interstitial edema, blood congestion and fusion of secondary lamellae (Hadi & Alwan, 2012; Abalaka, 2015).

Another organ of importance in heavy metal studies with fish that received impressive interest from several workers (Abou El-Naga *et al*., 2005; Alwan *et al*., 2009; Srivastava & Punia, 2011; Hadi & Alwan, 2012; Thangam *et al*., 2014; Odedeyi & Odo, 2017) is the liver. The liver is known for performing vital functions such as detoxification, synthesis of several components of blood plasma, glycogen storage and release of glucose to the blood (Ortiz *et al*., 2002; Hadi & Alwan, 2012). These workers reported that hepatic lesions, vacuolization of the hepatocytes, rupture of hepatic veins and hemorrhages in the liver are associated with the fish exposed to heavy metals.

There is paucity of information on the effect of water-soluble fractions (WSFs) of WDCB on the morphophysiology of the gills and liver of *C. gariepinus*. This study was designed to evaluate the effects of acute toxicity of WSFs of WDCB on the histopathology of the gills and liver of *C. gariepinus* fingerlings.

Materials and Methods

Collection and preparation of WSFs of WDCB

Waste dry Cell Batteries (Tiger head brand®) were collected from a riparian dump site along River Dilimi Jos, Plateau State, Nigeria and transported to the Applied Hydrobiology and Fisheries postgraduate laboratory of University of Jos, Nigeria. The transparent plastic wrappings were carefully removed and the spent dry cell batteries were exposed to decay on surgical tray for a period of two weeks and signs of Ammonium Chloride deposits on the surface confirmed decomposed state of the WDCB (Audu *et al*., 2020). The WDCB were collected, weighed (50 g) and crushed (whole mount) into fine powder (90 µm) using ceramic mortar and pestle (Audu *et al*., 2020).

Preparation of WSFs of WDCB for acute toxicity test

The procedure for the preparation of WSFs of WDCB for the 96 hr. toxicity experiment has been reported by Audu *et al*. (2020). According to the authors, a pilot test was conducted to determine the concentration of the WSFs of WDCB that would kill 50% of the test fish after 96 hours (4 days). The test was conducted on *C. gariepinus* fingerlings (mean weight 9.77±0.42 g) in a non- renewable static bioassay. Waste dry cell battery powder (90µm mesh) was weighed using Metler digital balance into five test concentrations namely: 3.13, 6.25, 12.50, 25.00 and 50.00 g/L and each of the concentration was dissolved in one 1L of distilled water and allowed to macerate (25 ℃) for 24 hours. The mixture was filtered through a funnel choked with non-absorbent cotton wool. The individual filtrate concentrations (3.13, 6.25, 12.50, 25.00 and 50.00 g/L) were diluted by adding nine litres (9L) of municipal water into each of the five filtrate concentrations in five designated rectangular (40x25x23cm) glass tanks resulting to definitive concentrations of 0.31, 0.63, 1.25, 2.50 and 5.00 g/L respectively (Audu *et al.*, 2020).

Acute toxicity bioassay

After the range finding test (RFT), a total of one hundred and twenty (120) *C. gariepinus* fingerlings;

(mean weight 9.77 ±0.42 g, same cohort, mixed sex), were exposed to five (5) definitive test concentrations (0.31, 0.63, 1.25, 2.50 and 5.00 g/L) and a control (0.00 g/L) experiment with no test material; all arranged in a randomized block design in duplicate replication. The fingerlings were kept under natural light regime (12 Light: 12 Dark); starved for 24hr prior to the acute bioassay and subsequently not fed throughout the 96 hours toxicity test (Audu *et al*., 2020). The acute toxicity test was conducted according to the standard methods of OECD (1992).

Determination of water quality parameters of the experimental tanks

Water quality parameters such as Temperature (℃), pH, Dissolved Oxygen (DO), Carbon (IV) oxide (CO_2), Nitrite and Total Ammonia (NH_3) as well as mortality rate of *C. gariepinus* fingerlings exposed to acute concentrations of WSFs of WDCB were earlier determined by Audu *et al*. (2020).

Preparation of gills and liver for histological analyses

At the end of the experiment, fish were euthanized using 0.1 g/L of benzocaine and then sacrificed by cervical dislocation. Thereafter, they were dissected carefully to excise the gills and liver. The excised tissues were fixed in 10% formalin and subsequently subjected to routine tissue processing by adopting the method earlier described by Audu *et al*. (2017). Briefly, fixed tissues were dehydrated in grades of ascending alcohol concentrations, cleared in xylene, infiltrated in paraffin wax and sectioned at 5 µm for gills and liver tissues. The sectioned tissues were stained with Haematoxylin-Eosin (HE) and viewed under light microscope (Olympus, China).

Histopathological scoring technique

The histopathological lesions in the liver and gills of *Clarias gariepinus* exposed to graded concentrations of water-soluble fractions of dry waste battery were scored using the ordinal scoring approach described by Gibson-Corley *et al*. (2013). Briefly, the histopathological lesions were observed at ten field of microscopic slide view per fish with a total of ten fish per test concentration. The mean values obtained were ranked based on the ordinal histopathological scoring technique as follows: For the liver; 0= normal, 1-4= mild, 4-8= moderate, while 8-20= severe for hepatic lesions HND and LHO. Hepatic lesions HCV and PC had, O= normal, 1-2= mild, 2-4= moderate while values of 4-7= severe in that category.

Similarly, the category of gill lesions scoring is as follows: 0= normal, 1-5= mild, 5-10 moderate and 10-30 severe for observed histopathological lesions of SLF, OILS and LH respectively.

Statistical analyses

Data obtained from histopathological scoring was subjected to analyses using R Console software version 3.2.2. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare the difference in the mean histopathological scores.

Results

Gills histopathology

The results of the histopathological alterations in the gills of *C. gariepinus* exposed to acute concentrations of WSFs of WDCB are presented in Plate 1 A-F. The *C. gariepinus* in the control group (Plate 1A) displayed normal gill parenchyma histoarchitecture as evidenced by the presence of primary (black arrow) and secondary (red arrow) lamellae and patent interlamellar space (asterisk). However, the gills of *C. gariepinus* exposed to various concentrations of WSFs of WDCB showed dose-dependent increase in severity of histopathological changes such as; the hyperplasia of the (secondary and primary) lamellar epithelium (arrow), the erosion and fusion of the secondary lamella (brace) and occlusion of the interlamellar space (star) (Plate 1 B-F) when compared to the control. The gill lesions of the fish exposed to the higher concentrations of the WDCB seems to be more severe relative to others.

Liver histopathology

With regard to the histopathological changes in the liver, the control fish showed normal hepatocyte architecture with a conspicuous central vein (cv), polygonal hepatocytes with prominent nuclei (black arrow) within substantial cytoplasmic volume (Plate 2A). Conversely, the liver of *C. gariepinus* exposed to graded concentrations of WSFs of WDCB exhibited similar pattern of dose-dependent severities observed for the gill that includes; hepatocyte nuclear degeneration (red arrow), cytoplasmic vacuolation (black arrow) and portal congestion (star) compared to the control (Plate 2B-F). The severity of the hepatic damage appeared to be more conspicuous in the fish exposed to the higher concentrations of the WSFs of WDCB.

Hepatic and gill histopathological lesions

Hepatic lesion: The prominent hepatic lesions such as hepatocytes nuclear degeneration (HND), loss of

hepatocyte outline (LHO), hepatocyte cytoplasmic vacoulation (LHO), portal congestion (PC) induced by the exposure of *C. gariepinus* to concentrated grades of WSTs of WDCB were significantly higher ($p<0.05$) in the fish exposed to higher concentrations (1.25, 2.50 and 5.00 g/L) of waste dry cell battery compared to others (Table 1). There is a dose-dependent increase in the severity of the various types of the hepatic lesions with marked display of histopathological lesion severity in the fish exposed to the highest concentration (5 g/L) of the dry cell battery. There was no significant difference ($p>0.05$) in the value of PC lesion in the liver of *C. gariepinus* exposed to 0.31 g/L and the control (Table 1).

Gill lesion: The gill histopathological lesions pattern in the gills of *C. gariepinus* exposed to graded concentrations of WSFs of WDCB showed a significant ($p<0.05$) dose-related increase in the severity of gill lesions such as secondary lamellar fusion (SLF), occluded inter-lamellar space (OILS), and lamellar hypertrophy (LH), with prominently severe lesions seen in the gill of cat fish exposed to the highest concentration (5 g/L) of the WDCB (Table 2). There was no significant ($p>0.05$) lamellar hypertrophy in the gills of the fish intoxicated with the 0.31 g/L of dry cell battery and the control (Table 2).

Discussion

The gills of fish represent the greatest surface area in contact with their external environment and are crucial in physiological roles that includes respiration, osmoregulation, acid–base balance, and in nitrogenous waste excretion (Simonato *et al*., 2008). Therefore, the observed dose-dependent histo-architectural disruptions in the gill parenchyma of fish exposed to WSFs of WDCB are morphological alterations that might be accompanied with serious functional compromise. The direct functional

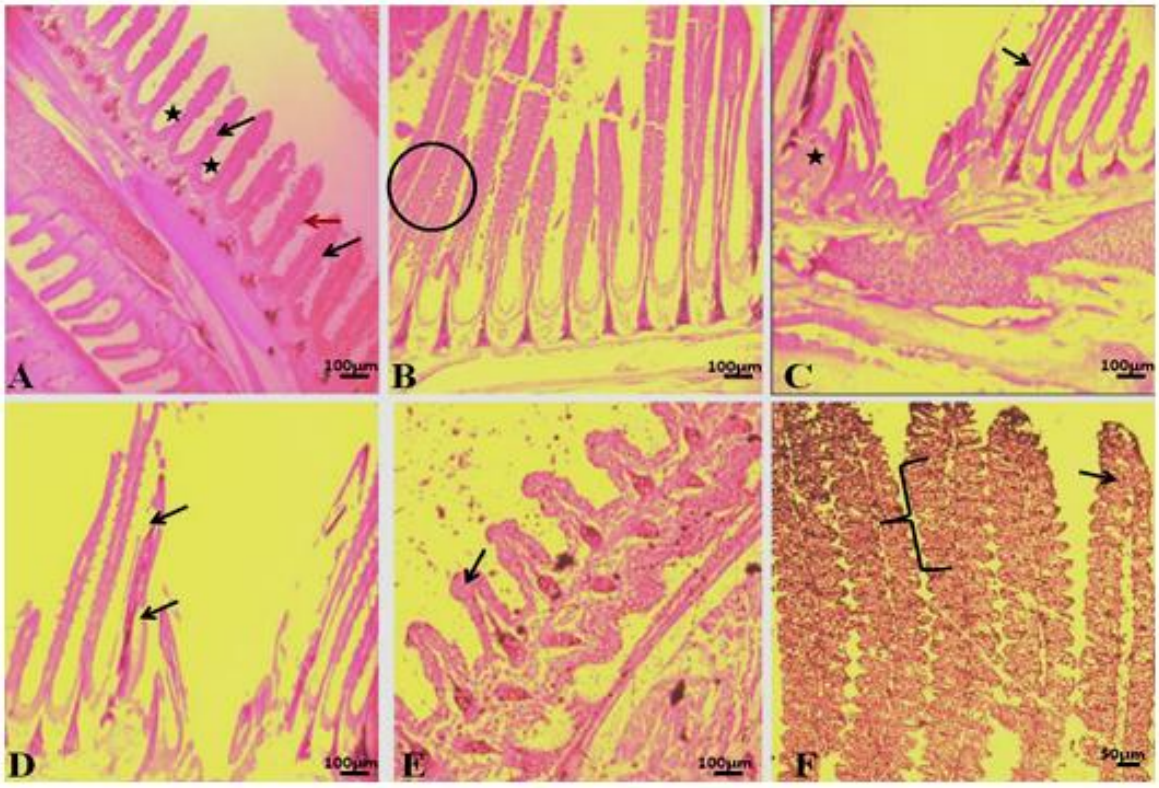

Plate I: Photomicrographs of the gill of *C. gariepinus* exposed to 96 hr. acute concentrations of water-soluble fractions of waste dry cell battery. A Control (0.00 g/L): The gill has normal histoarchitecture as revealed by the presence of primary (black arrow) and secondary (red arrow) lamellae and patent interlamellar space (star). B. 0.31 g/L: There is mild lamellar fusion (circle outline) C. 0.63 g/L: Showed moderate secondary lamellae erosion (arrow) and interlamellar space occlusion (star) D. 1.25 g/L: There is moderate erosion of secondary Lamella (arrow) E. 2.50 g/L: There is marked hypertrophy of both primary and secondary lamellae (arrow) F. 5.00 g/L: There is severe hypertrophy of the primary and secondary lamellae (arrow) with conspicuous fusion of secondary lamella (brace). Stain: HE; Scale bars: 50 µm and 100 µm.

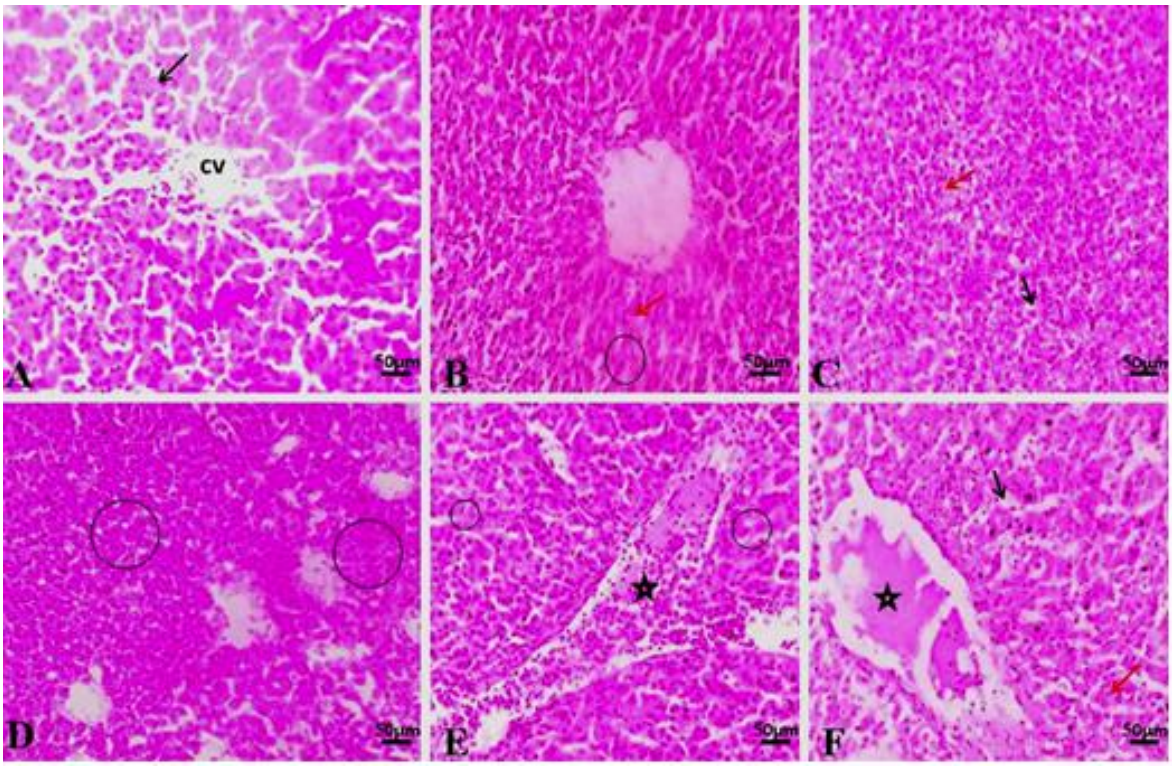

Plate II: Photomicrographs of the liver of *Clarias gariepinus* exposed to acute concentrations of water-soluble fractions of waste dry cell battery. A. Control (0.00 g/L): Normal hepatocyte histoarchitecture as evidenced by a conspicuous central vein (cv), polygonal hepatocytes with prominent nuclei (black arrow) within substantial cytoplasmic volume. B. 0.31 g/L: There is moderate hepatocyte nuclear degeneration (red arrow) with gradual loss of cellular outline (circle outline). C. 0.63 g/L: Displayed mild hepatocyte nuclear degeneration (red arrow), and marked cytoplasmic Vacuolation (black arrow). D. 1.25 g/L: There is marked hepatocyte degeneration (circle outline). E. 2.50 g/L: Showed marked foci of hepatocyte degeneration (circle outline) and portal congestion (star). F. 5.00 g/L: Showed severe hepatocyte nuclear degeneration (red arrow) and cytoplasmic vacuolation (black arrow) as well as moderate portal congestion (star). H&E: 400; Scale bar: 50 µm.

Table 1. Profile of hepatic lesions in *Clarias gariepinus* exposed to acute concentrations of water-soluble fractions of waste dry cell battery.

Hepatic Lesions	Acute Concentrations of Water-soluble Fractions of Waste Dry Cell Battery (g/L)					
	0.00	0.31	0.63	1.25	2.50	5.00
HND	0.00 ± 0.00^{a}	4.40 ± 0.51^{b}	4.80 ± 0.66^{b}	8.20 ± 0.58^{c}	10.40 ± 0.50^{c}	15.60 ± 0.93^{d}
LHO	0.00 ± 0.00^{a}	4.80 ± 0.45^{b}	5.20 ± 0.73^{b}	8.60 ± 0.68^{c}	10.00 ± 0.55^{c}	16.20 ± 1.28^{d}
HCV	0.00 ± 0.00^{a}	1.60 ± 0.24^{b}	3.40 ± 0.67^{c}	5.20 ± 1.20^{d}	6.60 ± 0.75^{d}	7.80 ± 0.66^{d}
PC	0.40 ± 0.00^{a}	0.80 ± 0.02^{a}	1.80 ± 0.02^{b}	2.40 ± 0.02^{c}	2.60 ± 0.07^{c}	3.80 ± 0.03^{c}

Values with different alphabet superscripts between the rows differ significantly ($p<0.05$)

HND – Hepatocytes Nuclear Degeneration, LHO – Loss of Hepatocyte Outline, HCV – Hepatocyte Cytoplasmic Vacuolation, PC – Portal Congestion

Table 2. Profile of gill lesions in *Clarias gariepinus* exposed to acute concentrations of water-soluble fractions of waste dry cell battery

Gill Lesions	Acute concentrations of water-soluble fractions of waste dry cell battery (g/L)					
	0.00	0.31	0.63	1.25	2.50	5.00
SLF	0.00 ± 0.00^{a}	1.00 ± 0.05^{b}	3.60 ± 0.07^{c}	7.60 ± 0.01^{d}	8.00 ± 0.17^{d}	30.00 ± 3.69^{e}
OILS	0.00 ± 0.00^{a}	1.60 ± 0.07^{b}	2.20 ± 0.08^{c}	3.60 ± 0.07^{c}	6.20 ± 0.12^{d}	17.60 ± 0.17^{e}
LH	0.00 ± 0.00^{a}	0.0 ± 0.00^{a}	1.00 ± 0.04^{b}	7.80 ± 0.06	10.00 ± 0.10	25.60 ± 1.72

Values with different alphabet superscripts between the rows differ significantly ($p<0.05$)
SLF – Secondary Lamellar Fusion, OILS – Occluded Inter-lamellar Space, LH – Lamellar Hypertrophy

consequence of the alterations in the gill structure highlighted above could manifest as impairment in oxygen uptake which has to be compensated for through transient morphological adaptive mechanisms such as lamellar epithelial hyperplasia to combat the respiratory stress induced by the toxicant on the fish. The gill histopathology observed in this study is similar to those earlier reported for organopesticides intoxication (Fernandes & Mazon, 2003; Khosravi *et al*., 2014; Ghasemzadeh *et al*., 2015) and this could be associated with stressor toxicants in the aquatic environment.

The parenchyma cells of the liver (hepatocytes) primarily partake in the conversion of glucose to glycogen, regulate lipids and deaminate amino acids (Wright & Plummer, 1974). The liver is one of the vital organs that respond to aquatic bodies laden with pollutants (Rodrigues & Fantail, 1998; Hopwood *et al*., 2004). Based on the this background, the increased moderate to severe hepatic lesions (hepatocyte nuclear degeneration, cytoplasmic vacuolation and portal congestion) observed on exposure of *C. gariepinus* to graded concentrations of WSFs of WDCB further affirm the probable toxic potential of spent dry cell battery. The physiological implication of these morphological alterations to the liver of exposed fish is that, the earlier mentioned liver functions would be perturbed. The hepatic damage seen in this study could be attributed to the elemental components of WSFs of WDCB more specifically, the metal zinc. The histological distortions exhibited by fish exposed WSFs of WDCB is similar to those previously reported for fish exposed to pollutants (Fanta *et al*., 2003; Peebua *et al*., 2008; Mohammad *et al.*, 2014).

The water-soluble fraction of WDCB is toxic to *C. gariepinus* fingerlings with resultant mortalities in acute concentrations and severe histopathological alterations in the sub lethal concentrations. The indiscriminate anthropological or industrial disposal of WDCB in riparian areas should be avoided to safeguard aquatic life.

Acknowledgement

We acknowledge the tremendous assistance offered by the technical staffs of the histology units of the National Veterinary Research Institute, Vom, Plateau State, Nigeria and those of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Nigeria. Our unflinching thanks for the provision of experimental bench go to the Department of Zoology, University of Jos, Nigeria.

Conflicts of Interest

The authors declare no conflict of interest.

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