Research Article

Effects of Palm Oil Mill Effluent on the Histology of Gill, Liver, Kidney, Heart and Muscle and its Heamatological Effects on African Cat Fish, *Clarias gariepinus*

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Abstract

Palm oil mill effluent (POME) is one of the major sources of pollutant produced during oil palm processing. POME in water significantly affects the ability of fishes to detect and respond to chemical stimulus. The aim of this study isto examine the toxicological effects of POME with emphasis on the histopathology of the gills, liver, kidney, heart and muscle as well as the heamatological effects. African catfish, *Clarias gariepinus* of mean weight 300g and standard length 18-21cm were exposed to varying concentrations 0.01, 0.03, 0.05, 0.07 and 0.09 ml/l of POME in duplicates for 14 days to determine the toxicity of POME. 110 healthy fishes were randomly placed into eleven rearing tanks of 100 litres capacity (dimension 50 cm x 25 cm x 25 cm) and filled with 40 litres non-chlorinated water at 10 fishes per tank (one tank for control and two tanks each for each concentration) Histopathological examination of the liver after 14 days of exposure revealed lesions which ranged from necrosis, vacuolation and diffused congestion of cells that deteriorate more with increase in POME effluent concentration. Toxic reactions were observed during the experiment. Haematological values of RBC, PCV and Hb decreases with increase in POME concentration while WBC registered an initial increase before declining at concentrations 0.07 and 0.09 ml/l. POME is harmful to aquatic organisms, according to the findings of this study, hence its discharge into bodies of water without sufficient treatment should be avoided.

Keywords: *Clarias gariepinus*; Heamatology; Histopathology; Toxicity; Lethal test; POME; Sublethal test.

Introduction

The incorrect handling of huge amounts of trash generated by diverse anthropogenic activities is one of the most serious problems faced by emerging countries. The hazardous disposal of these pollutants into the environment is even more difficult. Freshwater reservoirs are particularly vulnerable making these natural resources more frequently inappropriate for both main and secondary use [1]. River systems are the principal means of removing pollution from nearby industry, particularly effluents. These industrial effluents have a significant impact on water pollution; they can change the physical, chemical, and biological qualities of the water body [2]. Pollution stress on surface waters has increased as a result of increased industrial activity, as well as pollution from agricultural and home sources [3]. Palm oil is made from the oil palm *Elaeis guineensis*, which originated in West Africa but has since spread to other tropical lowland areas like Malaysia. The African oil palm, *Elaeis guineensis* belongs to the Palmae family. It's specific

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epithet *guineensis* indicates that the first specimen identified was taken in Guinea, West Africa. Palm oil is now one of the most widely traded vegetable oils, accounting for about half of the world's 36 million metric tons of trade [4]. Oil palm is processed both locally and industrially in Nigeria's oil palm belt, which stretches from South-South region of the country to the South- Western states. Palm oil mill effluent (POME), a by-product of palm oil production, is capable of harming the aquatic environment, particularly aquatic life [5]. After fishes have been exposed to water containing a range of noxious substances, such as pesticides, phenol, and heavy metals, reported histopathological changes in the gills are hyperplasia and hypertrophy, epithelial lifting, aneurysm, and increased mucus secretion [6]. Also, since liver is an essential orga n for breaking down toxins, the cells of the liver are frequently part of those that are harmed by hazardous chemicals. [7] studied the impacts of glyphosate herbicide on Tilapia fish, finding filament cell proliferation, lamellar fusion, lamellar cell hyperplasia, and epithelial lifting. The most evident effects on the gills were oedema, epithelial lifting, thickening of the primary lamellar epithelium, and fusion of secondary lamellae. In the gills of *Oreochromis niloticus* fingerlings exposed to POME, [8] reported lamellar degeneration and cell hypertrophy. [9] reported heamatological changes due to exposure of *Tilapia guineensis* to different levels of effluent under a static condition showed a significant different in all the parameters of exposed fish. He reported that there was a significant increase in the values of white blood cells, mean corpuscular volume (MCV), neutrophils, monocytes and glucose compared to the control value. Therefore, the aim of this study is to examine the toxic effect of POME on Clarias gariepinus, with an emphasis on histopathology and haematology, due to the need for understanding of POME's aquatic adverse effects

Research Methodology

Test Organism Source And Collection

Kazeem farm, a private farm in Igbole, Surulere Local Government of Ogbomoso, Oyo state, Nigeria provided *Clarias gariepinus* with an average weight of 300 g and a standard length of 18-21 cm. Clarias gariepinus was chosen because of its capacity to withstand stress and its great commercial worth in Nigeria influenced the decision.

Fish acclimatization

Fishes were kept in non-chlorinated water in glass tanks measuring 100 cm × 25 cm x 25 cm. They were given 14 days to acclimatize in the lab and fed twice a day with commercial feed so that they can adjust to the experimental circumstances. Waters were changed at two days' interval to remove feaces and uneaten feed and the fishes were fed, during acclimatization

Test Effluents

(POME) was collected in a 25-litres keg from Oke-awon palm oil mill in Surulere Local Government of Ogbomoso, Oyo State.

Experimental Set Up

POME sublethal concentrations were discovered using 5 different concentrations within the range obtained during presumptive test. 0.01, 0.03, 0.05, 0.07 0.09 mg/L of POME preparations were used. Each of the prepared concentrations was given to ten healthy fishes. Every 48 hours, the solutions were replaced. The test lasted for 14 days.

Histopathological Studies

The fishes were killed and sacrificed by a blow to the head with a hammer on conclusion of the experiments, and the liver, gill, heart and kidney components were removed. Then the organs were put in 10% formalin for 3 days, then they were subjected to dehydration in periodic acid Schiff's reagent (PAS) within 3 days in alcohol, according to [10] so that the paraffin wax will permeate the tissue while embedded. After that, melted wax was used to encapsulate the organs. Using a rotator microtome, tissue was sectioned into thin sections (5-7 m), was allowed to dehydrate, and then stained using Harris haematoxyllin-Eosin (H&E) stain. Bancroff and a microtone were then used to clear each portion, which was then placed in water that is warm (380 °C), clean slide was used to pick it and then oven dried at 580 °C for half an hour to get the wax melted for five minutes, the segment was stained with haematoxyline Eosin. A light microscope was then used to examine the stained slides with the aid of an Olympus BH2 microscope with a photographic attachment, portions were inspected and photographed at 400 magnifications (Olympus PM C35 AD4).

Heamatology

Survivors of the sublethal assay were decapitated and bled by cardiac puncture using 2 ml sterile plastic disposable syringe fitted with 0.8 mm x 38 mm hypodermic needles. The blood samples were rocked gently in the vial to allow thorough mixing of its contents. Three capillary tubes were filled with well-mixed anticoagulant-treated blood from each specimen and centrifuged for 5 minutes at 10,500 revolutions per minute (rev/min). Pack Cell Volume (PCV) was determined by filling three capillary tubes with well-mixed anticoagulant-treated blood from each specimen and centrifuged for 5 minutes at 10,500 revolutions per minute (rev/min). PCV was measured by the average obtained from three tubes and was recorded for each specimen at various concentrations. Heamoglobin concentration was measured using a Cyanmethaematocrit method. Three microhaematocrit tubes were filled with blood after sealing one end of the capillary tube and allowed to stand for 10 minutes; this gave sufficient time for complete conversion of haemoglobin (Hb)

into cyanmet haemoglobin. Transmittance was read from a corning colorimeter 253 at wavelength of 540 mm. This was then converted to haemoglobin concentration in g/dl-1 by using the commercially available cyanmet haemoglobin standards. Erythrocyte (RBC) and Leucocyte (WBC) counts were determined using an electronic cell counter (Coulter Electronic Ltd, England) standardized by an improved Neubauer haemocytometer after the blood had been diluted with dacie's fluid and stained with brilliant cresyl blue. For R.B.C count a dilution of 1:2000 was used while for WBC a dilution of 1:1500 was used.
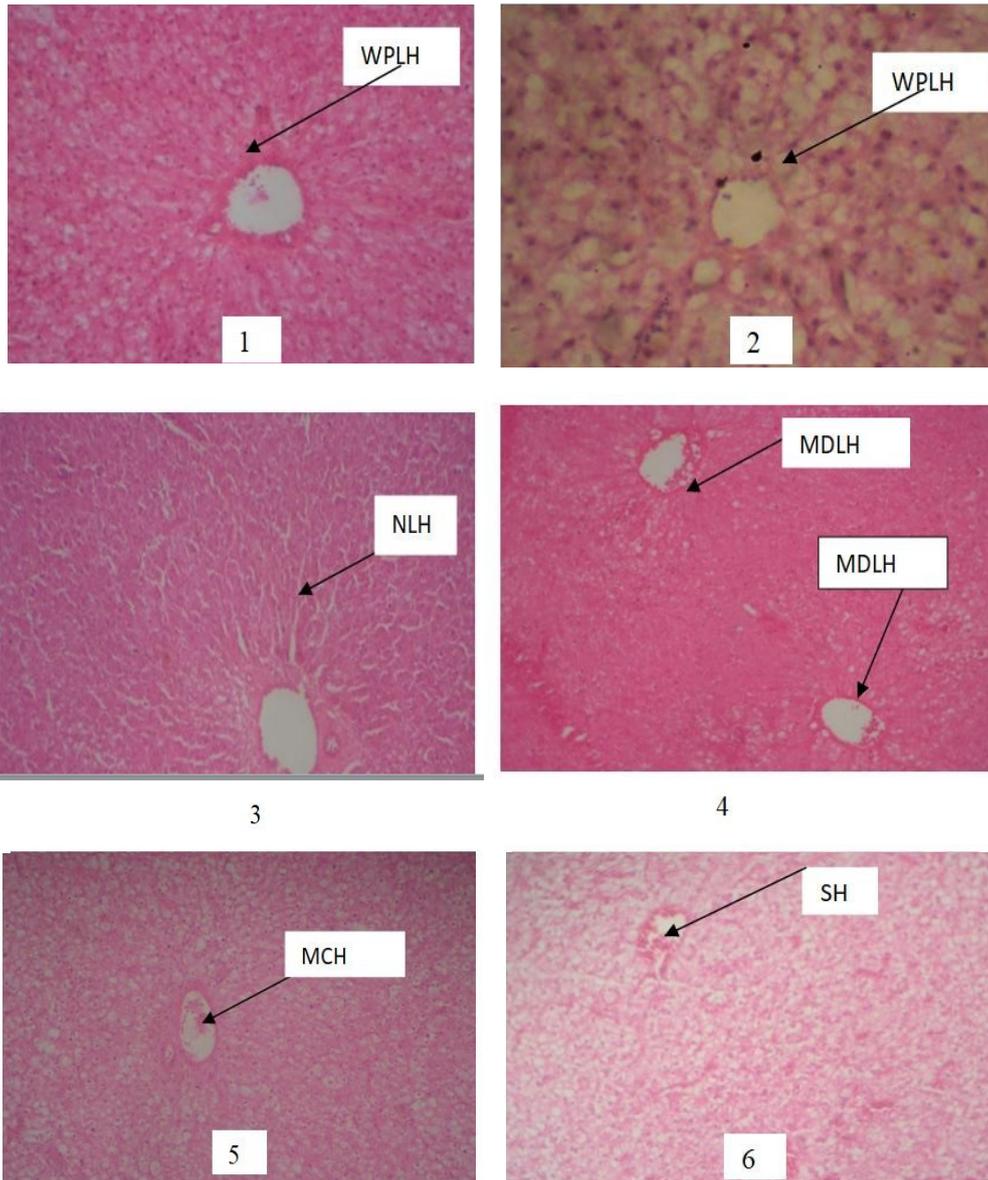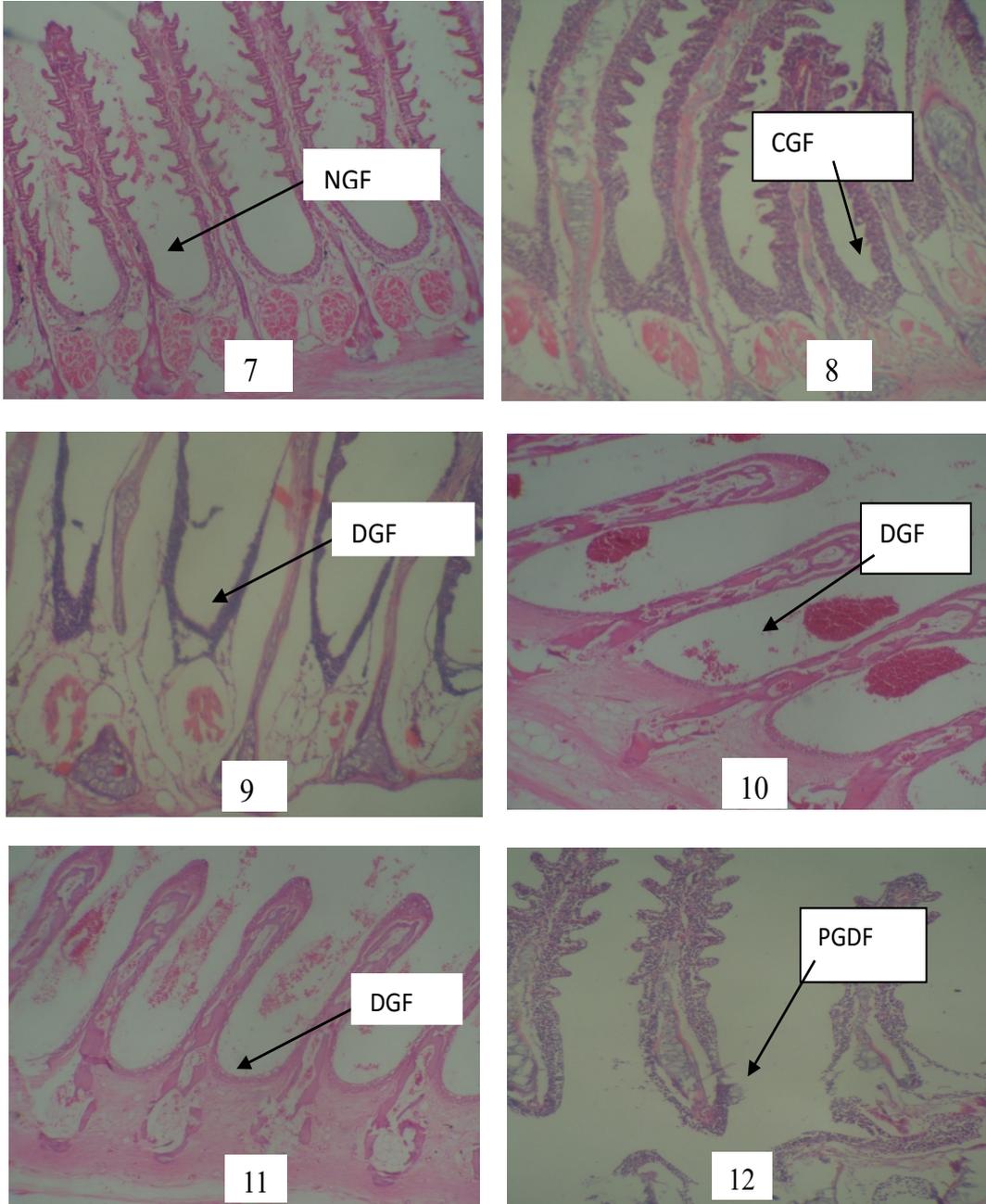
Results

Liver

Plate 1 (control) and 2 showing WPLH: Well Preserved Liver Hepatocytes Photomicrograph of the liver showing well preserved architecture of Liver cells with no areas of inflammatory changes. H/E. Plate 3 NLH: Normal Liver Hepatocytes Photomicrograph of the liver showing normal architecture of liver hepatocytes with no areas of inflammatory changes when compared with the control (Plate 1). SDLH: Slightly Distorted Liver Hepatocytes Photomicrograph of the liver exposed to 0.05mg/L showing slightly distorted architecture of liver hepatocytes. Plate 4 MDLH: Mildly Distorted Liver Hepatocytes(0.07mg/L) Photomicrograph of the liver showing mild distortion of the liver Hepatocytes architecture when compare with the control (Plate 1). Plate 5 MCH: Mildly Congested Hepatocytes Photomicrograph of the liver showing mild congestion of central vein when compared with the control (Plate 1). Plate 6 SH: Shrink Hepatocytes Photomicrograph of the liver showing pronounced distortion of liver hepatocytes architecture when compared with the control (Plate 1)

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Gills

NGF: Normal Gill Filament PLATE 7(control) NGF: A photomicrograph of gill showing normal and well vascularised gill Filaments. Gill filaments architecture is well preserved. Plate 8 CGF: Curl Gill Filament:photomicrograph of gill showing well vascularised and slightly distorted gill filaments after exposure to 0.01mg/L of POME. Plates 9, 10 and 11 DGF: Distorted Gill Filament A photomicrograph of gill showing vascularised and distorted gill Filaments after exposure to 0.03, 0.05 and 0.07mg/L POME concentrations respectively. Plate 12 PDGF: Poorly Degenerated Gill Filament A photomicrograph of gill (E) showing poorly vascularized and grossly degenerated gill filaments after exposure to 0.09mg/L POME.
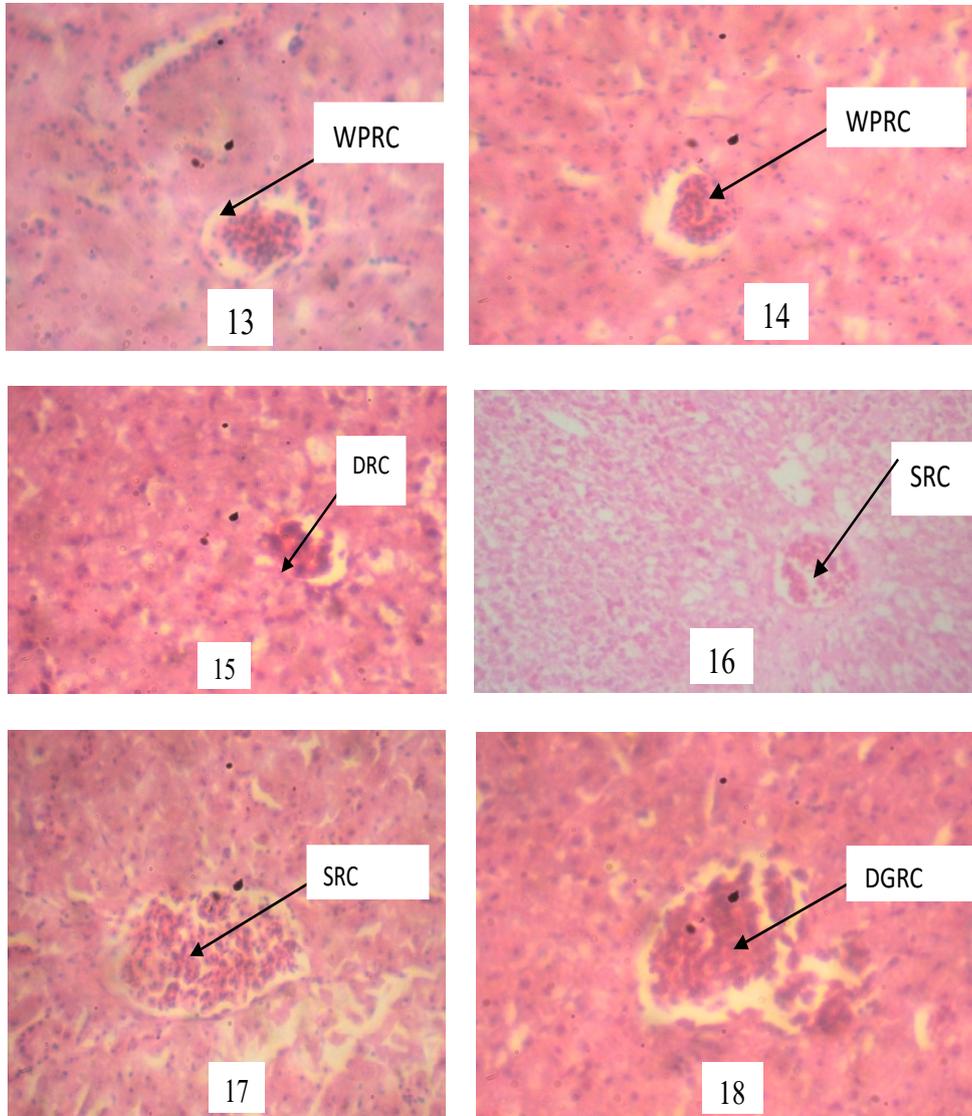
Kidney

Plate 13 and 14 WPRC: Well Preserved Renal Corpuscle: Photomicrograph of the kidney showing well preserved architecture of the renal corpuscle with a wide Bowman's space. Plate 15 DRC: Distorted Renal Corpuscle:(0.03mgl-1) Photomicrograph of the kidney showing distortion of the renal corpuscle with a narrow

Bowman's space when compared with the Control. Plate 16: Shrink Renal Corpuscle: (0.05mgl-1) Photomicrograph of the kidney showing distortion of the renal corpuscle with a narrow Bowman's space when compare with the Control. SRC: Shrink Renal Corpuscles (0.07mgl-1) Photomicrograph of the kidney showing inflamed renal corpuscles with narrow Bowman's space when compare with the control. Plate 18 DGRC: Degenerated Renal Corpuscles: (0.09mgl-1) Photomicrograph of the kidney showing degenerated renal corpuscles when compared with the control. H/E Stain.mag. X400.

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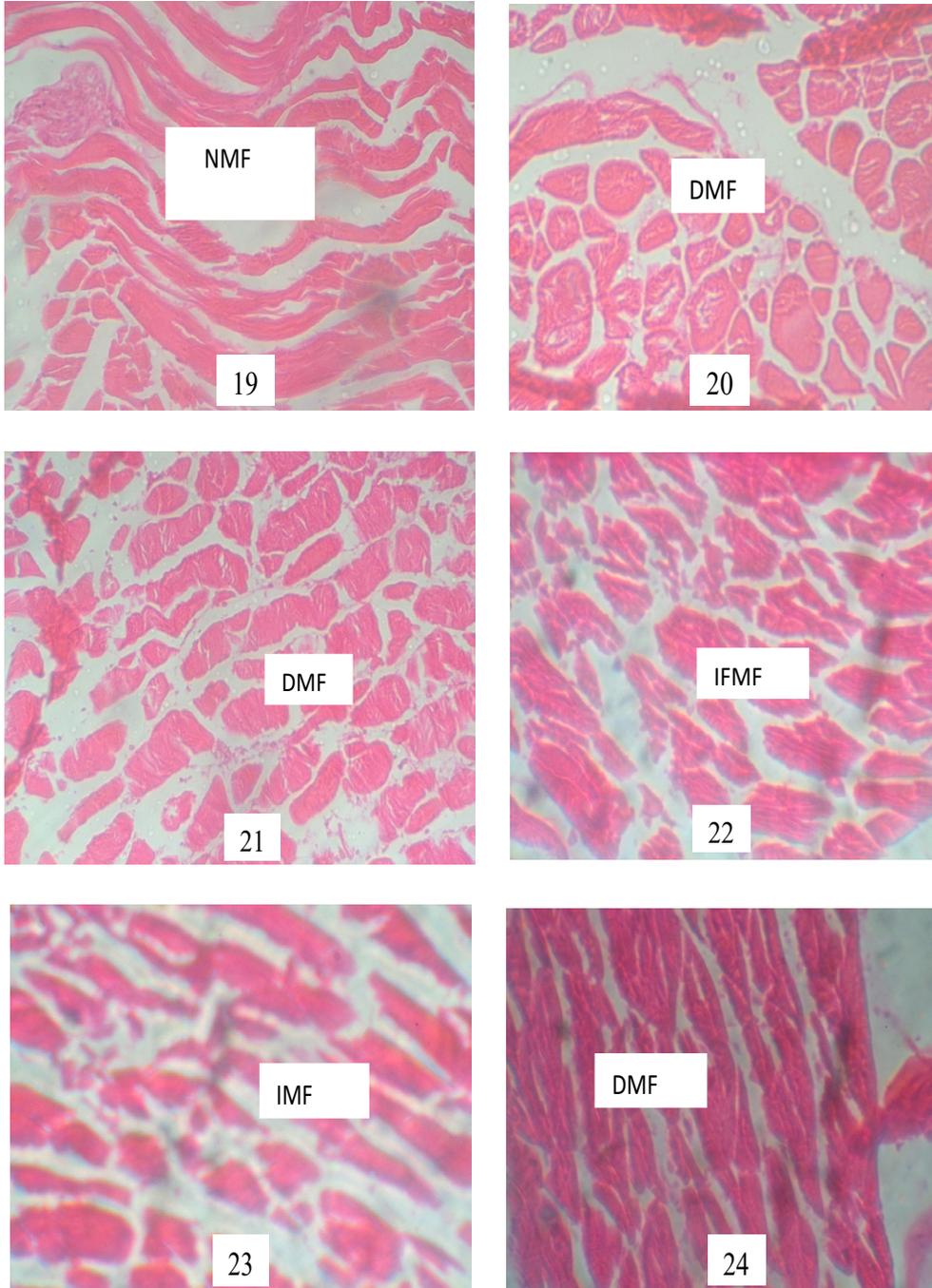
Muscle

Plate 19 NMF:(control) Photomicrograph showing normal appearance of the muscle fibres with wider interfibre spaces. Plate 20 DMF: Distorted Muscle Fibre: A photomicrograph showing mild morphological distortion of muscle fibres after exposure to 0.01mg/L POME. Plate 21 and 24 DMF: Distorted Muscle Fibre. A photomicrograph showing further distortion of muscle fibre after exposure to 0.03 and 0.05mg/l POME. Plate 22 IFMF: Irregular & Fragmented Muscle Fibre A photomicrograph showing distorted, irregular and fragmented Musclefibres of fish exposed to 0.09mg/L POME concentration. Plate 23 IMF; Irreguar muscle Fibre of fish exposed to 0.07mg/L POM

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Table 1: haematology changes in c. Gariepinus exposed to different concentration of pome for 14 days

Concentrations (mgl⁻¹)	0	0.01	0.03	0.05	0.07	0.09
PCV (%)	49±1.41[a]	48.9±1.41[a]	38.5±0.71[b]	37.0±1.41[c]	34.5±0.71[d]	31.0±1.41[cd]
WBC($\times10^3$mm³)	208.5±0.71[a]	209.0±0.71[b]	250.9±0.42[c]	255.4±0.98[c]	188.2±0.8[cd]	164±0.98[d]
RBC($\times10^6$mm³)	3.87±0.11[a]	3.87±0.11[a]	3.46±0.11[b]	3.27±0.35[c]	3.22±0.35[d]	3.15±0.71[d]
HB (g/dm³)	14.25±0.35[a]	14.20±0.35[a]	12.5±0.35[c]	11.25±0.35[c]	10.5±0.71[bc]	9.25±0.35[d]

Discussion

In fish, the gill, kidney, and liver are in charge of vital activities like respiration, excretion, and the accumulation and biotransformation of xenobiotics. One of the most significant advantages of utilizing histopathological biomarkers in environmental monitoring is the ability to examine these specific target organs [11].

Liver.

Normal cellular pattern, normal central vein, hepatic plate, and hepatocytes were seen in a transverse section across the liver. In the control experiment (Plate 1) and fish subjected to a concentration of POME 0.01 ppt, no lesion, necrosis, pigmentation, malignancy, or inflammation were seen (Plate 2). In the livers exposed to higher POME concentrations, there were areas of mild lesion, necrosis, malignancy, and inflammation in concentrations 0.03 mg/l

0.05 mg/l 0.07 mg/l and 0.09 mg/l (Plate 3, 4, 5 and 6). In POME-treated fish at a concentration of 0.07 mg/l, vacuolation and tissue disarray were observed. Cellular shrinkage and hyperplasia were also found. At the greatest dose of 0.09 mg/l, complete degeneration of tissue was seen. Histopathological alterations in C. gariepinus tissues subjected to sublethal concentrations of POME effluents revealed lesions ranging from necrosis and vacuolation to widespread cell congestion. This supports [12] results that pollutant cause varying degrees of damage to liver cells depending on exposure period and test media concentration. In the investigation of the histological effect of POME on juveniles of Oreochromis niloticus, [8] reported areas of lesions, necrosis, malignancy, and inflammation in the livers. He also said that fish exposed to higher amounts experienced vacuolation, cell shrinkage, and total degeneration.

Gill

Histopathological changes seen in the gill tissues of C. gariepinus experimented on showed a considerable dependence on POME concentration. The gradual and complete vascularization of the gill filaments of C. gariepinus coupled with traces to severe necrosis could be attributed to their ability to act as primary site of absorption of pollutants, which degraded the gill tissues. Similarly, [8] found enlargement of the gill arch and total filament degeneration

in juvenile of Oreochromis niloticus subjected to 50.0 mg/l pome for 96 hours.

Heart

The histological changes observed in the heart tissues of fishes exposed to the effluent showed distorted muscle fibre and congestion of the heart wall. The degree of the alterations could be attributed to the continuous and rapid movements of mouth and the gulping for air which might weaken and impair the heart tissues, as a result of the accumulation of pollutants. This conformed with the submission of [12] that the level of the degeneration of the heart tissue depends on the concentrations of the pollutant and period of exposure.

Kidney

There was a distortion of the renal corpuscle with a narrow Bowman's space from treatments with concentration 0.03 mg/l by POME effluents when compared with treatment experiment. Shrinkage of Bowman's capsule and vacuolation formed around the capsule were also noticed. This also conformed with the work of [13] who reported that the kidney of Channa punctatus revealed varying degree of distortion on exposing the fish to tannery effluent.

Muscle

The observed histological changes in the muscles examined could be attributed to the destructive effects of the varying concentrations of POME effluents and the exposure time. The observed histological changes agreed with the submission of [14] that the higher the concentration of pollutant the more the degree of damages to the tissue. [15] however opined that the alteration in tissues morphology may probably lead to several physiological stresses in fish.

Haematology

The total white blood counts registered an initial significant increase as compared to the control but at higher concentrations of 0.09 mgl-1 and 0.07 mgl-1, there was a decline in the counts. The revealed increase in total white blood counts could be traced to an attempt of the fish to build up defence against stress which resulted to behavioural responses of C. gariepinus to POME effluents. This observation agreed with the submission of [16] and Sowumi (2003) that the increase could be attributed to stress reaction when fish tried

to restore disturbed homeostasis. Similarly, [17] opined that the increase was due to initiation of pathogenic condition, most likely in the form of irritation, injury to the cells and formation of tissue debris and occurrence of secondary infection in the fish body. The decrease was attributed to limited role in exercising protective and immune responses under effluent stress [17]. It was observed that the red blood and haemoglobin (HB) counts were found to decrease with increase in concentration of POME effluents except those exposed to 0.01 mg/l. This may be due to in ability of the C. gariepinus to cope with the metabolic stress as a result of increase in demand for oxygen. Hence, there might be damage counts cells to the tissues which in turn impair the production of erythrocytes and haemoglobin. This conformed with the submission of [18]. [19] noticed the same trend and opined that the decline in red blood cells and haemoglobin counts was indicative of worsening state of health and possibly of aneamia development in the organisms. The values of the derived variables of the packed cell volume (PCV) in the experimental C. gariepinus were lower than the control apart from fish exposed to concentration 0.01 ppt. Generally, there were significant decline in the values of these parameters as concentration of the POME effluent increases with exception to concentration 0.01 ppt. The observed reduction in the derived indices could as well be attributed to the cause of decline in red blood cell counts, that metabolic stresses might lead to aneamic situation as a result of damaged tissues. Similarly, [20] and [19] submitted that high concentrations of pollutant and long-term exposure of fish to pollutant usually result in decrease of the derived indices and that worsens the fish health status and indicates anemia.

Conclusion

POME is hazardous to fish organs and causes histopathological abnormalities in many organs such as the gills and liver, according to the findings of this study. As a result, indiscriminate discharge into water bodies by palm oil producers should be prohibited, particularly inland rivers.

Ethical approval

I hereby declare that this work is original and conducted to ethical standards.

Consent to participate

The authors participated in the research process and give their consent to further questions on this work

Consent to publish

The authors give their consent to publishing this work.

Author's contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Oluniyi Adediran AFOLABI, Solomon Olayinka ADEWOYE and Folakemi Dupe AFOLABI, Oluwasheun Adebayo. The first draft of the manuscript was written by Oluniyi Adediran AFOLABI and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

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