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HAEMATOLOGICAL AND HISTOPATHOLOGICAL EXAMINATIONS OF AFRICAN MUD CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO PETROLEUM WASTEWATER

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ABSTRACT

Effects of petroleum refinery wastewater on Clarias gariepinus juvenile were investigated. Commercially obtained C. gariepinus fingerlings were acclimatized in a plastic tank (100 L capacity) of de-chlorinated tap water at 25±2°C for 14 days and fed with commercial feed pellet at 2% body weight of the fingerlings. Bioassay tests were carried out in four transparent plastics tank with nominal concentrations of 100 ml, 200 ml, 300 ml of the wastewater added to 40L of de-chlorinated tap water and only de-chlorinated tap water as control. Each tank contains twenty fish samples, while the assay was replicated three times concurrently. Following standard procedures, behavioural response, growth changes, haematological and histopathological tests were carried out on the samples. Significant reduction in the weight was observed in the fingerlings cultured with the wastewaters, while no significant difference occurred in the control fish. Highest values of Packed Cell Volume (PCV) (22), Haemoglobin (HB) (7.0), Red Blood Cell (RBC) (1.62) and endocochlear potential (EP) (5) were recorded for the control fish than exposed fish. On the other hand, Haptoglobin (HP), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were higher in the blood of exposed fish than in control. Histopathologically, exposed fishes showed no visible lesion in gills except from the thickening of the lamellae as the concentration of wastewater increases, indicating an increase in tissue disintegration. Similarly, gross tissue disintegration was observed in those fish exposed to 200ml wastewater as evidenced by the presence of large open spaces (hepatocytes) in the liver.

Keywords: Aquatic Biota, Corpuscular Haemoglobin, Gill lamellae, Hepatocytes, Histology, Lesion

INTRODUCTION

Pollution of aquaculture habitats is an inevitable problem that an aquaculturist encountered. Various pollutants affect survival, growth and reproduction of organisms, particularly those of economic importance (Ramesh, 2018). Information on long-term effects of exposure to toxic materials on physiological functions such as growth, reproduction and metabolism are useful to establish safe levels of wastewater discharges (Ramesh, 2011). Mining has a poor environmental track record. USEPA (2000) reported mining to contaminate portions of the headwaters of over 40% of watersheds in the western continental. Oil spills can have devastating effects being toxic to marine life, while

polycyclic aromatic hydrocarbons (PAHs) found in crude oil are very difficult to clean up, and last for years in the sediment and marine environment (Panetta, 2003).

Aquatic environments are made up of complex interrelations of living and non-living materials including physical environment. Harm to the physical environment will often lead to the death of one or more species in a food chain, which may lead to damage of other species along the chain (Brown and Weiss, 2008). In shallow waters, oil may harm sea grasses and kelp beds which are used for food, shelter and nesting sites by many different species (Carls *et al.*, 2009). Aquatic lives may also be affected by clean up operations or indirectly through physical damage to the habitats in which plants and animals live (Hill *et al.*, 2000; Hobson and Tiratson, 2005).

Crude oil is made up of petroleum and natural gas which occurs as a dark, sticky and viscous liquid. It is a mixture of gaseous, liquid and solid alkanes, alkenes, cycloalkanes, aromatic hydrocarbon and others. Natural gas consists of mainly of methane (Hobson and Tiratson, 2005). Crude oil is a complex mixture of hydrocarbons from which various petroleum products such as gasoline, kerosene, propane, fuel oil, lubricating oil, wax and asphalt are derived. Refined petroleum products consist largely of hydrocarbons, which are chemicals composed solely of hydrogen and carbon in various molecular arrangements (Holden and Baker, 2000). It also contains other organic and inorganic substances including atoms of sulphur, nitrogen and oxygen, as well as metals such as iron, vanadium, nickel and chromium (Williams *et al*., 2004).

Oil spillage is the discharge of oil fractions into water bodies which its movement result to alteration of the physico-chemical properties of water (Katsouros, 2002). Thus, wide range of aquatic biota which is linked in a complex food chain may be threatened (Katwijk van *et al.*, 2009). Upon the release of oil, less volatile and heavier fractions are left behind while light fractions vaporize. The heavier fractions like gasoline comprises of relatively high proportions of toxic and volatile hydrocarbons such as benzene, which causes cancer in human (Seymour and Geyer, 2002, Ki-Hyun *et al.,* 2013). Also, crude oil and their semi refined products such as diesel and bunkering oil contains cancer causing polycyclic aromatic hydrocarbons (PAHs) and other toxic substances (Ghosal *et al.,* 2016). However, the high flammability of released gasoline and kerosene makes them to be exceptionally hazardous (Smith *et al.*, 2001).

Oil spillage also caused destruction of food resources (Percival and Evans, 2007). Animal species that are not directly in contact with the oil spillage can also be harmed via the food web. Predators that consumed contaminated marine preys can be exposed to the spilled oil through ingestion of the prey (Parvathi *et al.*, 2011). More so, man may avoid the consumption of contaminated aquatic animals due to the unpleasant taste and smell induced by the spilled oil, thus resulting to starvation.

Discharge of cargo residues from bulk carriers can pollute ports, waterways and oceans. It has been estimated that container

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ships lose over 10,000 containers at sea each year (Janice, 2001). Removal of parts of the sea floor will result in disturbances of benthic layer, increased toxicity of the water column and sediment plumes from tailings (Halfar *et al*., 2007). Removing parts of the sea floor disturbs the habitat of benthic organisms, possibly, depending on the type of mining and location, causing permanent disturbances (Ahnert and Borowski, 2000). Aside from direct impact of mining the area, leakage, spills and corrosion would alter the mining area's chemical makeup.

In Nigeria, cases of oil spill and contamination of the intertidal mangrove swamps resulted in high mortalities of crabs and fish, including their intertidal eggs which the effects persisted for several months (Akpofure *et al.*, 2000; Aguigwo, 2002). Other effects on aquatic environments include direct lethal toxicity, alteration of biological habitats, high rate of mortality of fish eggs and sub-lethal disruption of physiological and behavioural activities, which can lead to death owing to interference with feeding and reproduction. This work was aimed to determine the sublethal effects of petroleum refinery wastewater on *Clarias gariepinus* in relation to their behavioural responses and effects on their tissue, organs and haematological indices.

MATERIALS AND METHODS

Samples Collection and Preparation

Petroleum refinery wastewater was collected after distillation process from Tarkwa Bay opened to the Lagos Lagoon. They were immediately transported to laboratory for further analysis. The physical and chemical properties of the wastewater were determined in accordance with APHA, (2005) standard methods. Commercially obtained C*larias gariepinus* fingerlings from Jamsky fisheries, Okonirugba, Ijebu Ode were acclimatized in a plastic tank (100 L capacity) containing de-chlorinated tap water at 25±2°C for 14 days and fed with commercial fish pellet.

Bioassay tests were carried out in a four transparent plastics tanks with size 30.5 x 30.5 by 92.5cm each and appropriate nominal concentration of the wastewater (100 ml, 200 ml and 300 ml) was diluted with de-chlorinated water to mix to a final volume of 20 L. Control experiment was set-up with purely de-chlorinated water with no wastewater. The nominal concentrations of wastewater used were: 0 ml (0.0 L wastewater and 20.0 L dilution water as control), 100 ml, 200 ml, and 300 ml. The mixture of toxicant and dilution water was allowed to stand for 30 minutes before the introduction of the test fishes. The test solution (mixture of effluent and dilution water) was renewed every 48 hours during the experimental period.

Each tank contained 8 fish and each bioassay test was repeated simultaneously to determine reproductability. The test solution (mixture of effluent and dilution water) was renewed every 48 hours during each series of test for 6 weeks. Fish bodyweight were taken before and during each test for the period of the study. Behavioural and general conditions of the fish were observed before, during and after each test, while mortality was recorded every 2 hours during exposure. A fish was considered dead when there was lack of opercular movement when prodded with a glass probe.

Laboratory Analysis

Haematological examination was conducted on each fish that survives at every 7 days acute exposure in the various test tanks and control tank. Their blood samples were collected at termination of acute exposure and at the start of experiments from the caudal vein of fish with 1 mL sterile syringe and needle and mixed in a 5 ml heparinised disposable bottles. Total erythrocyte and leukocyte count, packed cell volume, endocochlear potential (EP), Lumbar puncture (LP) and heamoglobin estimation were carried out in accordance to Blaxhall and Daisley (1973) while derived haematological values (MCV, MCH and MCHC) were calculated according to Schalm *et al*. (1975), Jain (1993) and Svoboda *et al*. (2005).

Histopathological examinations were also carried out on the gills, heart and liver of the fish sacrificed every 7 days for the period. They were anaesthetized in chloroform to collect their gills, heart and liver.

Statistical Analysis

SPSS version 20.0 was used to analyse the data obtained from the haematological studies and bioassay tests. Values were accepted not to be significantly different if $p > 0.05$. Duncan multiple range test of variance was used to separate the means and determine the variations due to sampling errors.

RESULTS

Behavioural Response

The initial and final weights of catfish cultured in various concentration mixtures for 48 hours are presented in Table 1. No significant differences ($p>0.05$) in the initial and final weights of *C. gariepinus* in the control water group. However, a significant reduction ($p<0.05$) in the final weight was observed in various mixtures of treated water. Table 2 presents the behavioural response of *C. gariepinus* under study during exposure to wastewater for 48 hours. The fish displayed different behavioural responses in hours ranges of two (2) hours away from each other. There was no observation made between 16 and 24 hours (11 pm – 5 am) because of access denial to the laboratory in those odd hours, same for 40 – 48 hrs.

Haematological Parameters

The haematological parameters of juvenile catfish exposed to petroleum refinery wastewater are shown in Table 3. Level of PCV was significantly higher in the control group than those exposed to the varying concentrations of the petroleum refinery wastewater. Level of PCV was observed to significantly reduce in the experimental catfish with increase in the concentration of exposed refinery water. This followed the trend; 100 ml > 200 ml > 300 ml respectively. Also, levels of haemoglobin and red blood cell count were significantly higher in the control group.

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Table 1: Initial and final weights of catfish exposed to various mixtures of wastewater polluted water for 48 hrs

Mixtures of crude oil (ml)	Initial weight (g)	Final weight (g)
Control	75.33±3.00[a]	75.32±3.01[a]
100	75.29±2.93[a]	66.28±2.03[b]
200	75.27±3.34[a]	59.70±2.04[c]
300	75.25±3.00[a]	50.23±1.60[d]

Mean Value ± SEM. Row and column values with different superscripts are significantly different ($p<0.05$).

Table 2: Behavioural response of *C. gariepinus* exposed to wastewater

Hours	Low Feeding			Resting			Clustering			Hanging		
	100ml	200ml	300ml	100ml	200ml	300ml	100ml	200ml	300ml	100ml	200ml	300ml
0 hr / 7am	-	-	-	1	1	2	3	-	-	-	4	3
2 hrs / 9am	Yes	Yes	Yes	4	1	-	-	2	3	1	2	2
4 hrs / 11am	-	-	-	2	3	-	-	-	4	2	2	1
6 hrs / 1pm	-	-	-	-	2	1	2	2	3	3	1	1
8 hrs / 3pm	-	-	-	2	3	-	-	-	4	3	2	1
10 hrs / 5pm	-	-	-	3	1	2	-	2	1	2	2	2
12 hrs / 7pm	No	Yes	Yes	1	2	3	4	3	2	-	-	-
14 hrs / 9pm	-	-	-	1	1	2	2	3	3	-	-	-
16 hrs / 11pm	-	-	-	-	-	-	-	-	-	-	-	-
18 hrs / 1am	-	-	-	-	-	-	-	-	-	-	-	-
20 hrs / 3am	-	-	-	-	-	-	-	-	-	-	-	-
22 hrs / 5am	-	-	-	-	-	-	-	-	-	-	-	-
24 hrs / 7am	-	-	-	1	3	1	-	-	-	4	2	4
26 hrs / 9am	No	Yes	Yes	-	4	4	3	-	-	2	1	1
28 hrs / 11am	-	-	-	3	2	5	-	-	-	2	3	-
30 hrs / 1pm	-	-	-	3	3	2	2	-	2	-	2	1
32 hrs / 3pm	-	-	-	2	4	3	2	-	2	1	1	-
34 hrs / 5pm	-	-	-	4	3	2	1	2	2	-	-	1
36 hrs / 7pm	No	Yes	No	2	3	2	2	-	3	-	2	-
38 hrs / 9pm	-	-	-	1	2	3	4	3	2	-	-	-
40 hrs / 11pm	-	-	-	-	-	-	-	-	-	-	-	-
42 hrs / 1am	-	-	-	-	-	-	-	-	-	-	-	-
44 hrs / 3am	-	-	-	-	-	-	-	-	-	-	-	-
46 hrs / 5am	-	-	-	-	-	-	-	-	-	-	-	-
48 hrs / 7am	-	-	-	2	2	2	3	3	2	-	-	1

Table 3: Haematological parameters of juvenile catfish exposed to petroleum refinery wastewater

	Control	**100 ml**	**200 ml**	**300 ml**
PCV	22.00±1.15[a]	15.00±1.73[b]	11.00±2.65[c]	10.33±2.60[c]
HB	7.00±0.58[a]	4.67±0.52[b]	3.30±0.93[b]	3.07±0.84[b]
RBC	1.62±0.01[a]	0.86±0.23[b]	0.72±0.28[b]	0.74±0.24[b]
WBC	10500.0±850.0[c]	40666.7±11078.9[a]	29850.0±4532.2[b]	19583.3±1533.6[b]
Platelet	126000.0±0.0[c]	181666.7±11893.0[b]	240666.7±31881.7[a]	218333.3±21926.6[a]

[abc]Means (±Standard error of mean) in the same row having similar superscript are not significantly different at $p < 0.05$

These were not significantly different in the catfish groups exposed to the varying concentrations of petroleum refinery wastewater. On the other hand, levels of white blood cell count and platelets were significantly lowest in the control group than in the catfish groups exposed to the varying concentrations of petroleum refinery wastewater.

White Blood Cell Differentials

The white blood cell differentials of juvenile catfish exposed to petroleum refinery wastewater is presented in Table 4. Levels of LP and EP were significantly higher in the control group. However, levels of LP and EP recorded in the catfish groups exposed to the varying concentrations of petroleum refinery wastewater were not significantly different. On the other hand, level of HP was significantly lowest in the control group than those exposed to the varying concentrations of petroleum refinery wastewater. HP level was also not significantly different in the catfish groups exposed to the varying concentrations of petroleum refinery wastewater.

Levels of MCV, MCHC and MCH were not significantly different between the control group and those exposed to the varying concentrations of petroleum refinery wastewater. Basophils were not detected in the catfish groups' exposed to 100 ml and 300 ml of the petroleum refinery wastewater. Basophil levels were also not significantly different in the control group and those exposed to 200 ml of the petroleum refinery waste water.

Histopathological Examination

Plate 1 – 9 present the histopathology of *C. gariepinus* gills, heart and liver exposed to different concentrations (0 ml, 100 ml, 200 ml and 300 ml) of petroleum refinery wastewater for 7, 14 and 21 days respectively.

Table 4: White blood cell differentials of juvenile catfish exposed to petroleum refinery wastewater

	Control	100 ml	200 ml	300 ml
LP	70.00±1.15[a]	51.67±10.84[b]	56.67±7.26[b]	48.33±9.77[b]
HP	21.00±0.58[b]	45.00±14.43[a]	34.67±5.90[a]	42.00±8.19[a]
MC	3.00±0.29[a]	3.33±0.33[a]	3.00±0.58[a]	3.67±0.33[a]
EP	5.00±0.58[a]	3.67±0.33[b]	2.67±0.67[b]	2.67±0.33[b]
Basophils	1.00±0.12[a]	0.00±0.00[b]	0.67±0.33[a]	0.00±0.00[b]
MCV	135.00±2.31[a]	193.00±35.16[a]	179.67±40.17[a]	143.67±13.48[a]
MCHC	31.80±0.46[a]	31.10±0.29[a]	29.47±1.23[a]	29.63±1.82[a]
MCH	43.20±0.12[a]	60.43±11.51[a]	52.50±10.76[a]	42.33±1.77[a]

[abc]Means (±Standard error of mean) in the same row having similar superscript are not significantly different at $p < 0.05$

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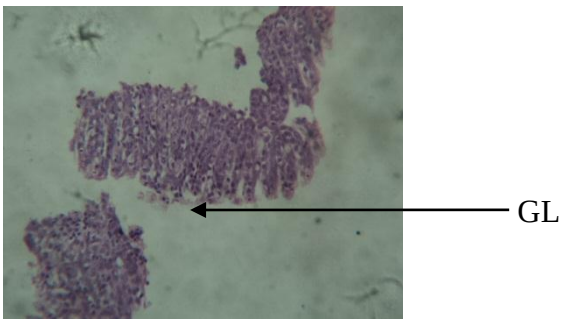
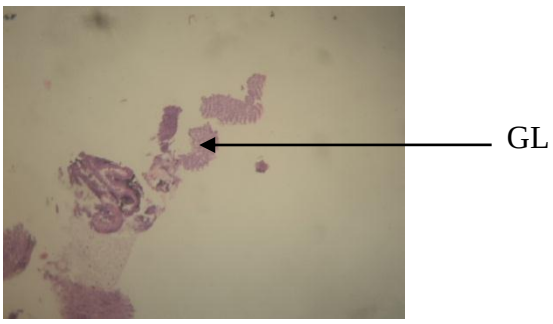
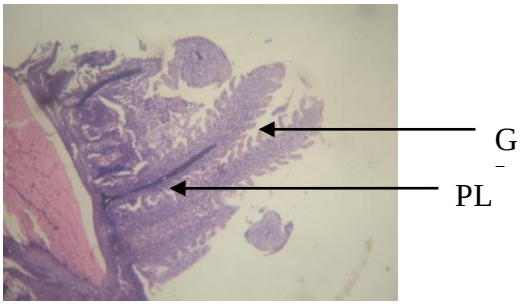


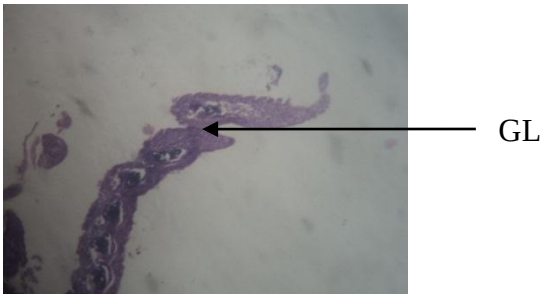
A - Gills exposed to 100ml



B - Gills exposed to 200ml



C – Gills exposed to 300ml



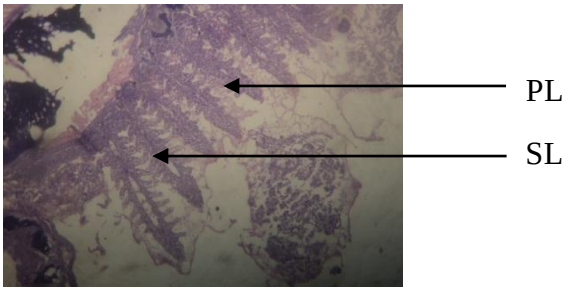
D - Gills exposed to 0ml (Control fish)

Plate 1A – D: Gills exposed to 100 ml, 200 ml, 300 ml and 0 ml of petroleum refinery wastewater for 7 days

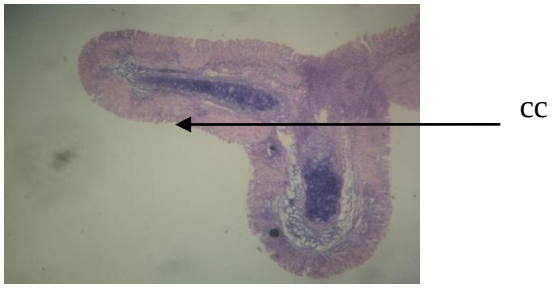
Note

GL – Gill Lamellae

PL – Primary Lamellae



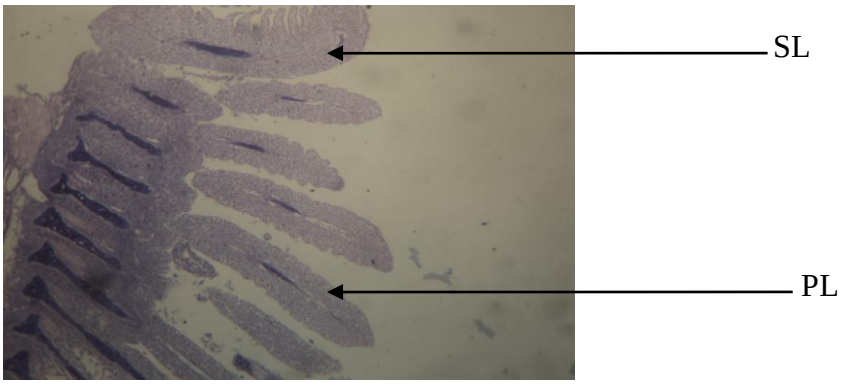
A: Gills exposed to 100ml



B – Gill expopsed to 200ml

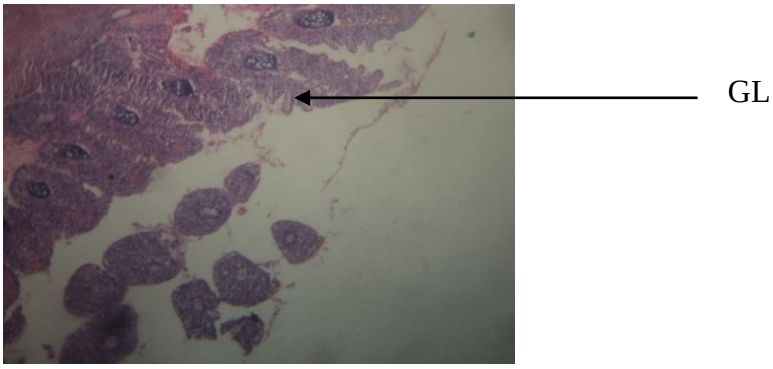
Moderate proliferative thickening of the epithelium of primary (PL) and secondary (SL) gill lamellae appear normal.

Moderate proliferative thickening of the covering epithelium; intact cartilageneous core. (cc)



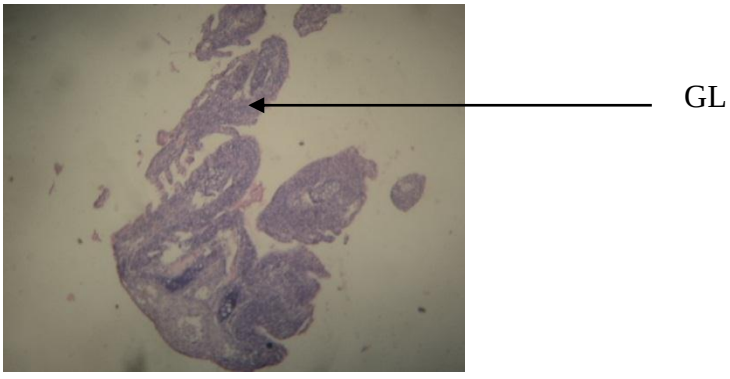
C – Gills exposed to 300ml
Marked proliferative thickening of epithelium
of both primary (PL) and secondary (SL) gill lamellae

Plate 2A - C: Gill exposed to 100 ml, 200 ml and 300 ml of petroleum refinery wastewater for 14 days
Note
SL – Secondary Gill Lamellae
PL – Primary Lamellae



A – Gills exposed to 100ml

There are numerous gill lamellae (GL) in transverse section, but no visible lesion observed



B: Gills exposed to 100ml

There are numerous gill lamellae (GL) in transverse section, but no visible lesion observed

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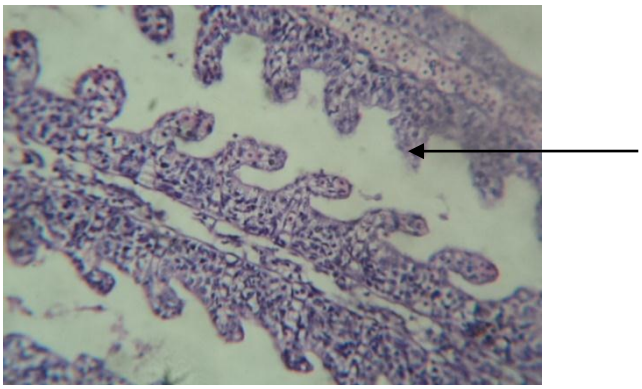
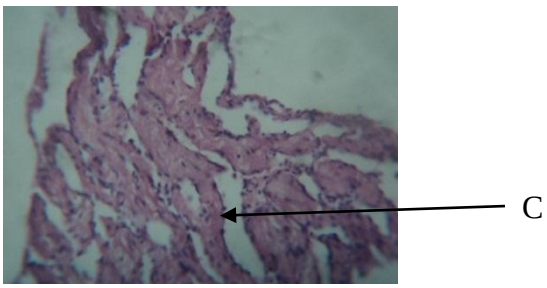


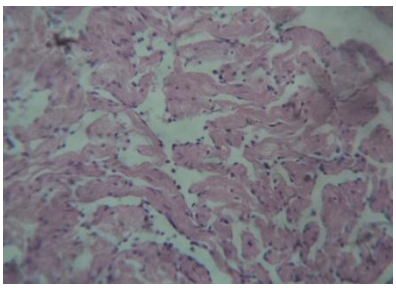
C – Gills exposed to 300ml

There are numerous gill lamellae (GL) in transverse section, but no visible lesion observed.
Plate 3A - C: Gill exposed to 100ml, 200ml and 300ml of petroleum refinery wastewater for 21 days



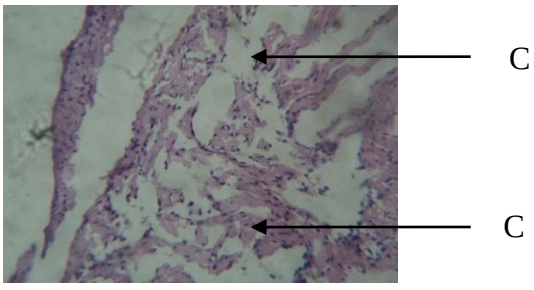
A – Heart exposed to 100 ml

No visible lesion



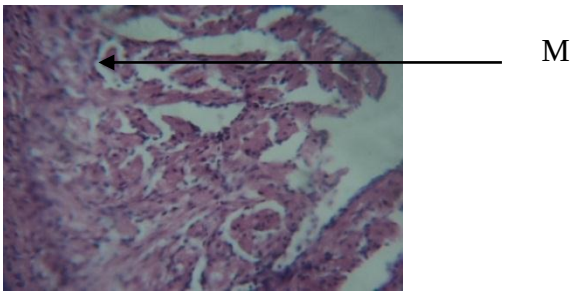
B – Heart exposed to 200 ml

No visible lesion



C – Heart exposed to 300 ml

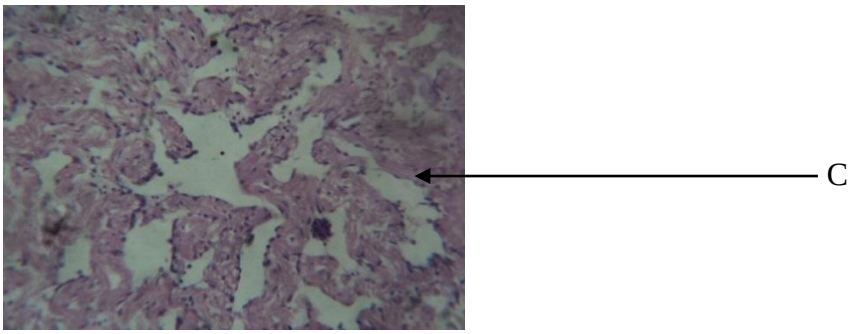
Myocardium (M) appears thinned out with moderate depletion of cardiomyocytes (C)



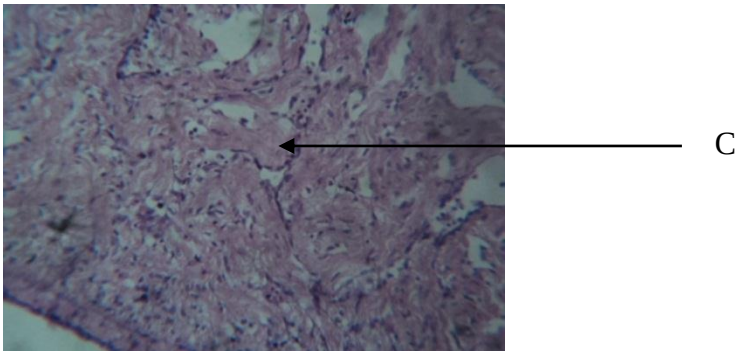
D - Heart exposed to 0 ml (Control)

No visible lesion with normal

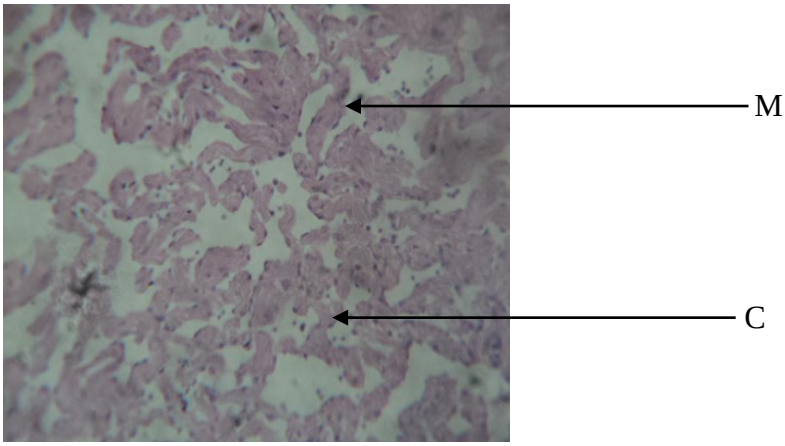
Plate 4A - D: Heart exposed to 100ml, 200ml, 300ml and 0ml of petroleum refinery wastewater for 7 days



A – Heart exposed to 100 ml



B – Heart exposed to 200 ml



C – Heart exposed to 300 ml
Moderate reduction in size of the myocardium (M) with depleted numbers of cardiomyocytes (C)

Plate 5A - C: Heart exposed to 100 ml, 200 ml and 300 ml of petroleum refinery wastewater for 14 days

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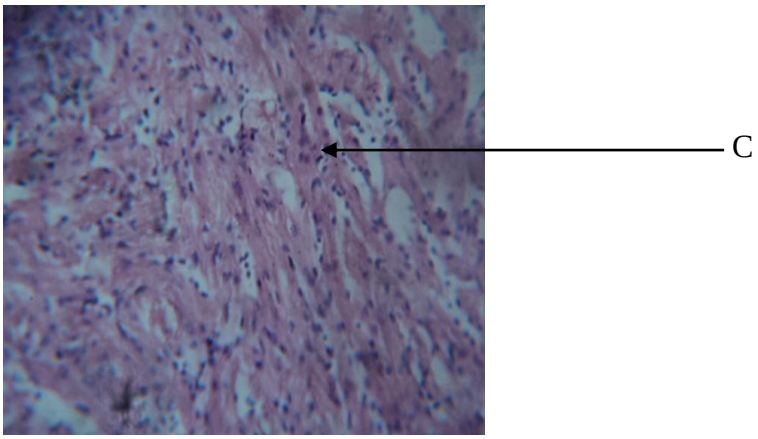
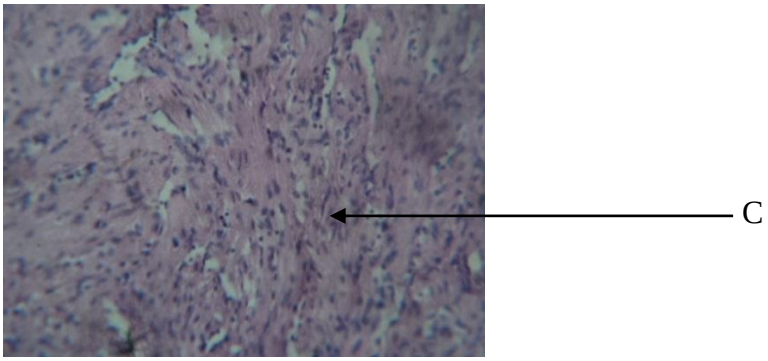


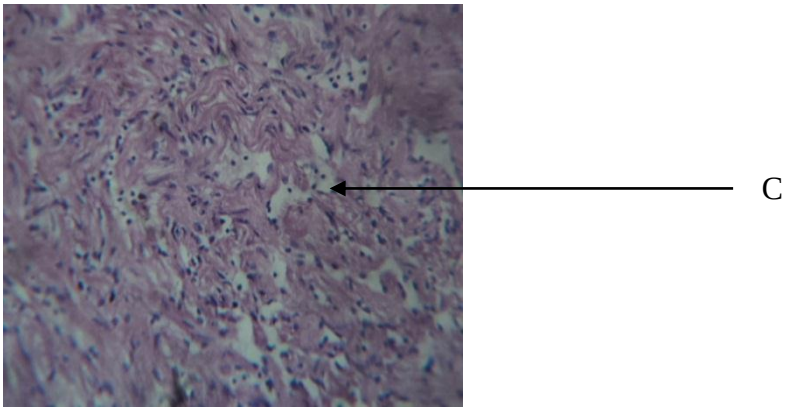
A - Heart exposed to 100 ml

No visible lesion



B – Heart exposed to 200 ml

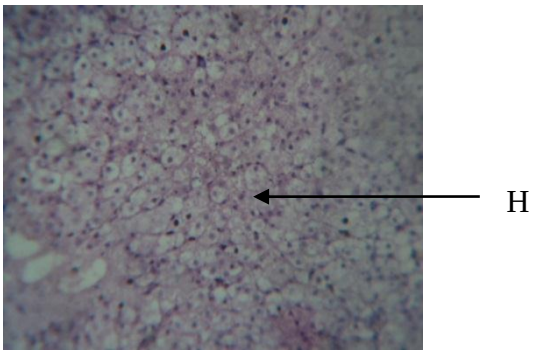
No visible lesion



C – Heart exposed to 300 ml

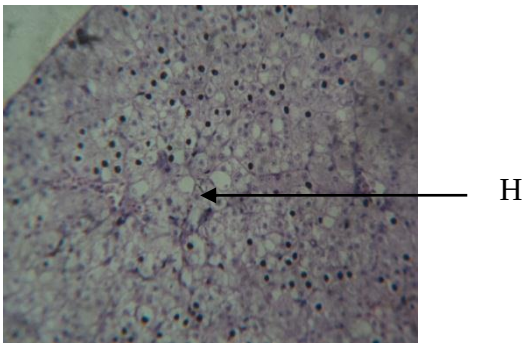
No visible lesion

Plate 6A - C: Heart exposed to 100ml, 200ml and 300ml of petroleum refinery wastewater for 21 days



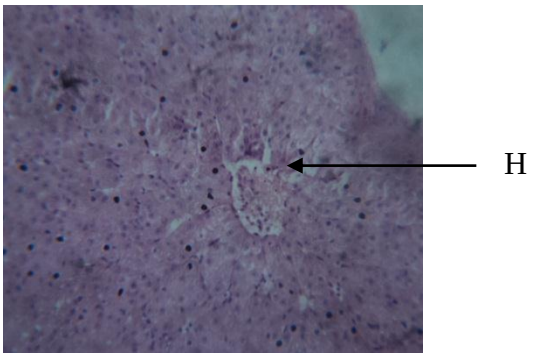
A – Liver exposed to 100 ml

Widespread, variably-sized, moderate cytoplasmic vacuoles in hepatocytes (H)



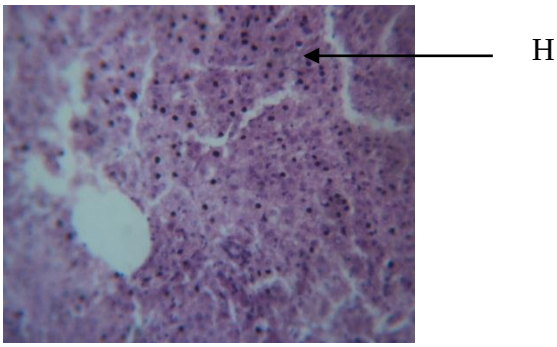
B – Liver exposed to 300 ml

Marked, widespread vacuolar change of hepatocytes (H)



C – Liver exposed to 200 ml

Closely apposed hepatic plates; hepatocytes (H) are devoid of cytoplasmic vacuoles



D - Liver exposed to 0 ml (Control)

There are closely apposed hepatic plates; a few hepatocytes (H) contained

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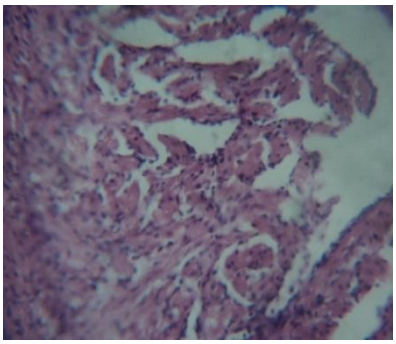
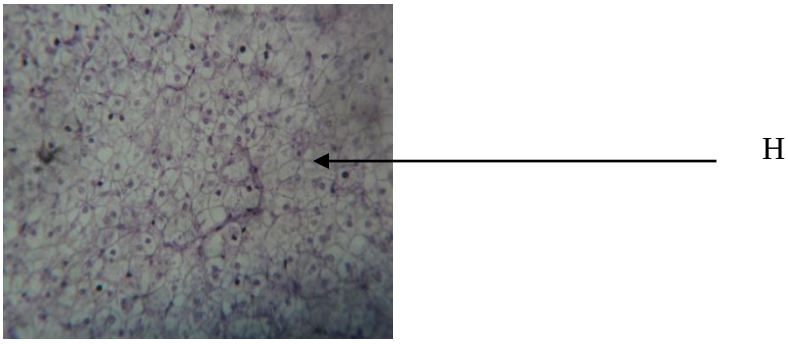


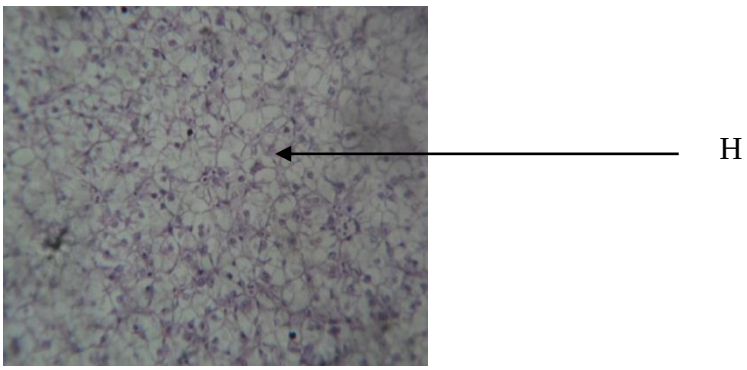
A – Liver exposed to 100 ml

Widespread marked vacuolar change of hepatocytes (H) (hepatocytes contain large clear cytoplasmic vacuoles)



B – Liver exposed to 200 ml

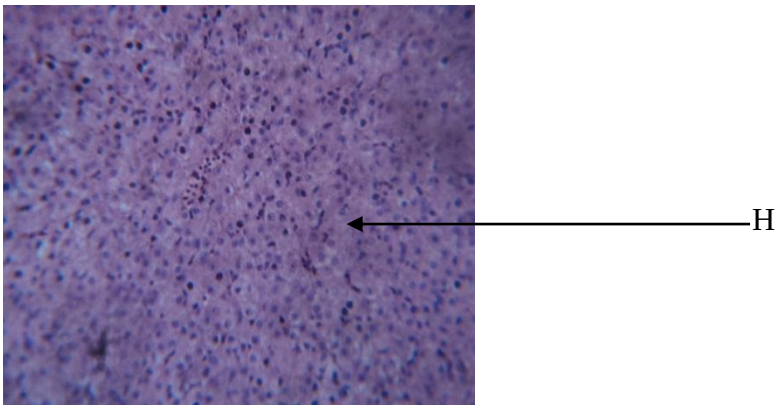
Hepatocytes (H) contain large, clear cytoplasmic vacuoles with each nucleus at the centre of the cell



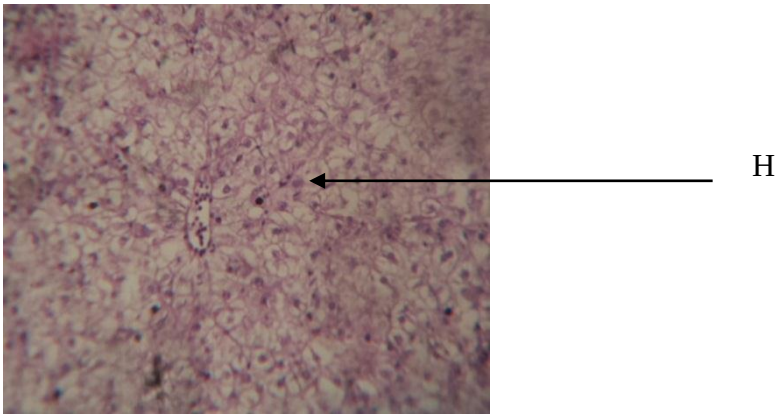
C – Liver exposed to 300 ml

Widespread, multiple clear cytoplasmic vacuoles in hepatocytes; the nuclei in some hepatocytes (H) are pushed to the periphery (eccentrically placed)

Plate 8A - C: Liver exposed to 100ml, 200ml and 300ml of petroleum refinery wastewater for 14 days

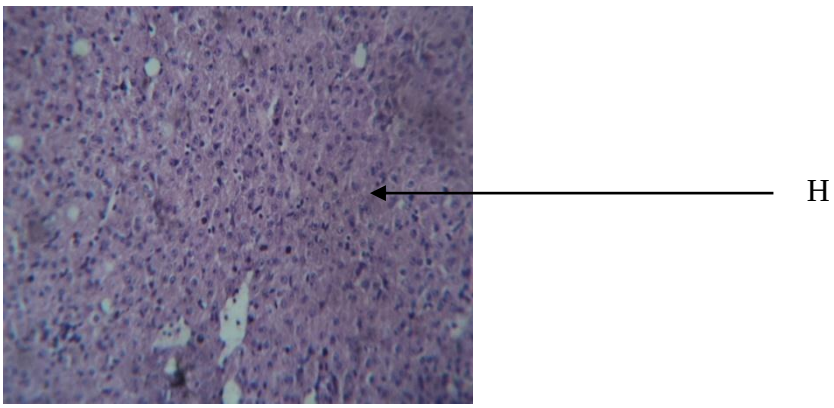


A – Liver exposed to 100 ml
Hepatocytes (H) appear normal; however there is mild congestion of the central veins



B – Liver exposed to 200 ml

Multiple foci of moderate vacuolar change of hepatocytes (H) appear



C – Liver exposed to 300 ml

There are a few foci of mild vacuolar change of hepatocytes (H)

Plate 8A - C: Liver exposed to 100ml, 200ml and 300ml of petroleum refinery wastewater for 21 days

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DISCUSSION

The exposure of living systems to crude oil may manifest in various forms including changes in physical performance and/or biochemical changes. In the present study, evidences are presented to address the survival of fish in a crude oil polluted environment. Ships can pollute waterways and oceans in many ways. Oil spills can have devastating effects. While being toxic to marine life, polycyclic aromatic hydrocarbons (PAHs), found in crude oil, are very difficult to clean up, and last for years in the sediment and marine environment (Panetta, 2003).

Fish naturally are found in water with pH tending towards neutrality (Cote, 2006). The reduction in pH values of crude oil polluted water as observed in the present study may be due to deposition into the medium of carbonic acid arising from the reaction between carbon IV oxide (from crude oil) and water. The acidic nature makes the medium inhabitable for the experimental catfish, as they required a neutral medium for survival. In an attempt to address this, the final pH values tend towards neutral in all the treatment groups. This may be attributable in part to some secretions arising from the slimy nature of the catfish into the water environment in their bid to survive. It could also be as a result of the water vapour from the atmosphere having a diluting effect on the acidic nature of the water and consequently causing it to be relatively neutral. These propositions may however require further clarification.

The non-significant effect observed in the initial and final weights of catfish cultured in well water (control) may be due to apparent conducive environment to which the catfish were subjected (i.e. crude oil free condition). However, the observation with respect to the reduction in the weight of catfish exposed to crude oil polluted water may be due to the water-soluble fractions of crude oil (such as organic and inorganic compounds as well as short chain polycyclic aromatic hydrocarbons) which may pose physical threat to fishes (Hodson *et al.*, 2007).

Significant reduction in the weight of the experimental catfish could be due to the coverage of water surface by crude oil. Crude oil hinders the dissolution of oxygen, and also oxygen content in blood is reduced (Val and Almeid-Val, 2009). This implies that the experimental catfish were starved of oxygen required for the breakdown of the food consumed and as such affecting their growth. It is also possible that the crude oil affected the taste and smell of the feed resulting in lower food intake by the catfish. This is in accordance with the report of Hill *et al.* (2000) which reported that an effective weight loss could occur on modification of diet and physical activities by virtue of decline in energy production.

The behavioural response that was exhibited by *C. gariepinus* on exposure to petroleum refinery wastewater could be due to respiratory impairment and nervous system failure caused by the toxicant. Similar observations was made by Fakayode (2005) who reported that hyperactivity of fish due to introduction to an unfavorable environment is the primary and principal sign of nervous system failure which is due to chemical poisoning that affect physiological and biochemical activities.

Pal and Konar (2001) observed that disruption of the functioning of the nervous system of fish might be the cause of slow and lethargic swimming, erratic movement and loss of equilibrium. Similarly, Oshode *et al.* (2008) observed that exposure of *C. gariepinus* to acute concentrations of leachate from municipal solid waste landfill causes behavioural abnormalities, which include; agitated swimming, loss of equilibrium, air gulping and death.

Thus, the catfish switch to anaerobic respiration for energy production to take care of metabolism. It may therefore be inferred from this study that one of the mechanisms by which catfish could survive in a crude oil polluted environment is the use of anaerobic approach. This could be the reason for the thickening lesions in the gill lamellae on the second week and also the presence of numerous lamellae by the third week. Result on histopathology performed on gills show no visible lesion except from the thickening of the lamellae which was in line with Adeyemo (2005) findings. This is because catfish can always switch to anaerobic respiration, making use of its air chamber outgrowths on its head. Ramesh (2018) concluded that catfish can survive anaerobically for hours during which the water surface may be clearing from oil droplets covering it.

Analysis of the histological sections revealed gross structural disintegration in the glomerulus and hepatic cells when compared with the control. Gross breakage in the glomerular cells which is more pronounced in catfish exposed to the highest concentrations of wastewater (300 ml). This observation indicated that tissue disintegration increases as the concentrations of wastewater in the water increases. Similarly in the liver, gross tissue disintegration was observed in catfish exposed to 200 ml wastewater as evidenced by the presence of large open spaces. Loekle *et al.* (2003) described these open spaces as areas of tissue disintegration. The study confirmed that exposure of petroleum refinery wastewater to *C. gariepinus* fingerlings has insignificant haematological and histopathological changes in the gills, liver and heart of the fish in contrast to the report of Olujimi *et al.* (2016) on leacheates from Olusosun and Igando landfill sites.

CONCLUSION

The study indicated that survival of the *C. gariepinus* in petroleum wastewater depend on the use of anaerobic approach with extensive structural damage in gills, heart and liver of the catfish. The effects on the hepatocytes of the liver may bring about reduced haematological activities. Further research can be done on lethal effects of this petroleum refinery wastewater and the histopathological changes on brain, skin, kidney and other fish organs should be examined.

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