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Histopathological Changes on the Gills of the African Catfish Fingerlings Exposed to Light and Heavy Crude Oils

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Abstract
Histopathological changes on the gills of the catfish, C. gariepinus (mean total length, 15.40±0.57 cm, mean weight, 22.08±2.51 g) exposed to light and heavy crude oils were studied in static toxicity bioassay over a 96-hour period after acclimation of test organisms in the laboratory. There was initial range finding tests to determine the concentrations of the toxicants to be administered on the test organisms in the definitive tests. The test organisms were exposed to five concentrations (1.0, 1.5, 2.0, 2.5 and 3.0%) of both toxicants and a control (0.0%) in static renewal bioassay. Histopathological changes on the gills of untreated fish showed normal parallel arranged gill filaments while lamellae of gill of exposed fish suffered different levels of damage including distension and fusion, and were also hypertrophic and necrotic. The control group had greater pH, temperature, and dissolved oxygen levels than the treated group. The greater concentration of accumulated polycyclic aromatic hydrocarbons (PAHs) in heavy crude oil may have contributed to the more degenerative alterations observed on the gills in comparison to light crude oil. Hence, a baseline toxicological study on the constituent and components of light and heavy crude oils is recommended so as to ascertain the level of PAHs in both oils.

Key Words: Bioassay, histopathological, light crude oil, heavy crude oil, polycyclic aromatic hydrocarbons (PAHs).

Introduction: Crude oil spillage which could occur as accidental spill during the shipping and leakage from the underground pipes are becoming a common phenomenon on a global scale and they have over the years led to the pollution of the world aquatic ecosystem (Omoregie and Ufodike, 2000, Vinod, Nitin, Temin, Jogendra and Pankaj, 2020). On the other hand, one of the causes of deliberate discharge of crude oil to the environment is sabotage (Numbere, 2018); Nyong,E. E. &Nweze,N.J (2012). Crude oil can be classified based on its chemical composition with regard to its relative density either as light or heavy crude oil and this is based on the American Petroleum Institute (API) gravity which reflects how light or heavy a crude oil is compared to water. Light crude oil is liquid petroleum that has low density, flows freely at room temperature, has low viscosity, low specific gravity and high API gravity (more than 20°) due to the presence of a high proportion of light hydrocarbon fractions (Hu, 2017, Sojinu and Ejeromedoghene, 2019 and Chrysalidis and Kyzas, 2020). Heavy crude oil on the other hand is any liquid petroleum with an API gravity less than 20° (Dusseault, 2001).

Histopathological changes in animal tissues are powerful indicators of prior exposure to environmental stressors and are the net result of adverse biochemical and physiological changes in an organism (Palanisamy, Sasikala, Malikaraj, Bhuvaneshwari and Natarajan, 2011). Fish gill is a multipurpose organ that, in addition to providing for aquatic gaseous exchange, plays a dominant role in osmotic and ionic regulation, acid-base regulation and excretion of nitrogenous wastes (Evans, Piemarini and Choe, 2005). According to Saravana-Bhavan and Geraldine (2000), toxic substances can injure gills, thus reducing the oxygen consumption and disrupting the osmo-regulatory function of aquatic organisms.

In Nigeria, the African catfish (*Clarias gariepinus*) is a common tropical and commercially important fish species to a wide range of consumers. The flesh of this species is oily and tasty and is highly priced in the market (Akande and Ajayi, 2002). In additon, the younger fish species like the fingerlings are adversely affected in most pollution cases due to their vulnerability; hence the choice for fingerlings in this study. Previous studies on the effects of crude oil on this species include those of Nwadukwe and Ayinla (2004), Nwadukwe, Okoro, Iwalewa and Ayoabu-Cookey (2004) and Nwadukwe, Ayaobu-Cookey; Nyong E. E, *et al* (2023) and Matanmi (2006). According to Fernandes and Mazon (2003), the fish gills are the prime target organs of all pollutants due to their extensive surface in contact with the external and internal medium. They also noted that changes in the gill morphology and morphometric are important biomarkers providing a rapid method for detection of the effects of pollutants. Akaishi, De Assis, Jaakobi, Eiras-Stofella, St. Jean, Couteany, Lima, Wagener, Scofield, and Ribeiro (2004); Nyong,E. E**.** &Nweze,N.J (2012) observed some histopathological changes in various organs of fish (ovaries, gill and liver) exposed to hydrocarbons. Despite the large number of crude oil spills in Nigeria, very little is known about the histopathological changes on the gills of *C. gariepinus* fingerlings exposed to light and heavy crude oils. The objective of this study was to determine the histopathological changes on the gills of *C. gariepinus* fingerlings exposed to light and heavy crude oil.

Materials and Methods: Collection and Acclimation of Experimental Fish: The experimental fish comprising four hundred and thirty-two (432) apparently healthy fingerlings of *C. gariepinus* (9 weeks old, mean total length, 15.40±0.57 cm, SEM; mean weight, 22.08±2.51 g, SEM) were sourced from Jossy Farms, Ugbiyokho Qtrs, Ekenwan, Benin City. The fish which were held in open top glass aquaria tanks (50x25x26cm^3) containing 20 L of borehole water were allowed to acclimate to laboratory conditions for one week, during which period the fingerlings were fed to satiation with 2mm coppens fish feed. The water was renewed every 24 hours to prevent the accumulation of waste metabolites and food particles before using them for the bioassay study.

Experimental Setup and Design: Acute toxicity tests were conducted to compare the toxicities of light and heavy crude oils on *C. gariepinus* fingerlings over a 96-hour period (Hoffman, Rattner, Burton and Cairns, 2003). The bioassay study was carried out in the laboratory of the Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. The bioassay method employed was the one outlined by APHA (1998) and Scott, Zubot, MacKinnon, Smith and Fedorak (2008). The experiment was designed as a factorial experiment laid out in Completely Randomized Design (CRD) with two crude oil types (light and heavy crude oils) using six treatments for each crude oil containing a control (0.0%) and concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume. Each of the treatments was replicated thrice to give a total of 36 experimental units containing 432 fingerlings of *C. gariepinus* (12 fingerlings in each test tank). The control (0.0%) which also has 12 fingerlings of *C. gariepinus* was not contaminated with the test toxicants.

Preparation of Test Solutions and Application of Test Toxicants

Experiment 1 (Range – Finding Test): The crude oils were collected from the Nigerian Petroleum Development Company (NPDC), Oredo Field, Edo State. A preliminary (range – finding) test as described by Cheruiyot, Lee, Mwangi, Wang, Lin, N., Lin, Y., Cao, Zhang and Chang-Chien (2015), Frigaard, Paso and De Souza Mendes (2017), Nyong,E. E**.** &Nweze,N.J (2012) Kamal, Adewunmi, Sultan, Al-Hamad and Mehmood (2017), Fritt-Rasmussen, Wegeberg, Gustavson, Sørheim, Daling, Jørgensen, Tonteri and Holst-Andersen (2018), Gounder (2019), Yesilyurt and Cesur (2020) and Joel, Amajuoyi and Dede (2009) was conducted to determine the main experimental concentrations for the light and heavy crude oils. Based on the results of the range – finding test, the following concentrations of 1.0, 1.5, 2.0, 2.5

and 3.0% by volume of the toxicants and a control (0.0%) were prepared for the definitive test.

Experiment 2 (Definitive Test): The second stage of the study gave details of the actual experimental concentrations of the toxicants as described by Aqeel, Jamil and Yusoff (2014), Silva (2023) and Abowei, J., Alfred-Ockiya, Allison and Abowei, M. (2005). For the light crude oil against *C. gariepinus*, a sample of it was diluted with borehole water to obtain concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume while for the heavy crude oil against *C. gariepinus*, a sample of it was also diluted with borehole water to obtain concentrations of 1.0, 1.5, 2.0, 2.5 and 3.0% by volume.

Exposure Procedure: A sample of twelve (12) fingerlings was randomly placed in each of the experimental tanks and appropriate test solutions were added. Monofilament nettings were used to cover the tanks to prevent the test organism (fish) from jumping out of the tanks. During the study, fish mortality and swimming behaviour were monitored. Gill samples were obtained from exposed fish and control, preserved in 10% formal saline for further gill examination.

Histopathological (Tissue Processing) Procedure: Gill samples preserved in 10% formal saline were processed for histological examination. The tissues (gill samples) were processed in 70% and 90% alcohol for 1 hour each for dehydration. They were further processed for dehydration in 96% alcohol in two changes for 1 hour each and also in absolute (100%) alcohol in two changes for 1 hour each, after which the tissues were transferred to xylene in two changes for 1 hour each.The tissues were then transferred to two changes of wax for impregnation for 2 hours each (that is, a total of 4 hours in the molten paraffin wax) to give the tissue a support. After impregnation, the tissues were embedded in a metal mold and molten paraffin wax was poured into it and allowed to solidify. After embedding, tissues were sectioned into 3 – 5 microns using the microtome and placed on the clean slide.The sections and slide preparations were made for histological investigation under light microscope using the method described by Krause (2001). Sections were stained in Haematoxylin and Eosin to demonstrate the tissue general structure (Macia, Tan, Vieira, Leach, Stanley, Luong, Maruya, McKenzie, Hijikata, Wong, Binge, Thorburn, Chevalier, Ang, Marino, Robert, Offermanns, Teixeira, Moore, and Mackay, 2015 and Dey, 2023). Sections were then mounted in DPX (Diphthal Xylene) mountant and examined under low power (x10) microscope. The photomicrographs were taken using a photomicrographic microscope.

Statistical Methods: Data generated were subjected to analysis of variance (ANOVA) test at 5% level of significance, using a GENSTAT Computer Software (version 12.00 for windows). Significant means were separated using the Least Significant Difference (LSD) (Ajayi, Adeoye and Shittu, 2017, Adelani, Ogunsanwo and Awobona, 2020, Kumar, Carter, Mir, Sehgal, Agarwal and Paterson, 2022 and Wani, Jiang, Hossain, Burritt, Rouached and Liu, 2023).

Results and Discussion: Physico-Chemical Parameters: The physico-chemical parameters measured during the 96-hour exposure are presented in table 1. The Dissolved Oxygen (D.O.) levels, pH and temperature values in the control were higher than those in the treatment of both toxicants. The findings demonstrated that while temperature values increased with increasing concentrations of both toxicants, dissolved oxygen and pH values in the contaminated tanks of both toxicants declined. Tanks contaminated with light crude oil (concentrations of 0.0, 1.0, and 1.5% by volume) did not exhibit significantly different dissolved oxygen levels from tanks contaminated with heavy crude oil ($P>0.05$); nevertheless, there was a significant difference between the tanks with 2.0, 2.5, and 3.0% by volume. Additionally, there was no significant difference ($P>0.05$) between the temperature values of light and heavy crude oil-contaminated tanks, but there was a significant difference (pH values) between the light and heavy crude oil-contaminated tanks.

Table 1: Water Quality Parameters in Control and Test Tanks containing *C. gariepinus* fingerlings.

	D. O. (mg/l)		pH		Temperature (^{O}C)	
Concentration (%)	**LCO**	**HCO**	**LCO**	**HCO**	**LCO**	**HCO**
0.0	6.20^{a}	6.24^{a}	6.67^{a}	6.76^{b}	27.57^{a}	27.72^{a}

1.0	4.65^{b}	4.76^{b}	6.31^{c}	6.62^{d}	26.61^{b}	26.96^{b}
1.5	4.45^{c}	4.45^{c}	6.27^{e}	6.62^{f}	26.62^{c}	27.12^{c}
2.0	4.00^{d}	4.13^{e}	6.19^{g}	6.59^{h}	26.66^{d}	27.31^{d}
2.5	3.62^{f}	3.49^{g}	5.95^{i}	6.56^{j}	26.83^{e}	27.32^{e}
3.0	2.95^{h}	2.55^{i}	5.94^{k}	6.50^{l}	26.86^{f}	27.33^{f}
	LSD(0.123)		**LSD(0.029)**		**LSD(0.901)**	
	SED(0.062)		**SED(0.015)**		**SED(0.451)**	

NOTE: Means with identical superscript between the crude oil types do not differ significantly while means with non-identical superscript differ significantly (P = 0.05).
LCO = Light Crude Oil, HCO = Heavy Crude Oil, LSD = Least Significant Difference, SED= Standard Error of the Difference between two means

Histopathological Changes on the Gills Exposed to both Crude Oils: Results of histopathological investigation of the gill sections from the control showed normal parallel arranged gill filaments (Plate 1: Controls 1 and 2). However, lamellae of gills of exposed fish suffered different levels of damage including distension and fusion, and were also hypertrophic and necrotic. Severity of damage on the gills was concentration-dependent (Plates 1 and 2). The photomicrographs showed that the heavy crude oil caused more degenerative changes on the gills than the light crude oil.

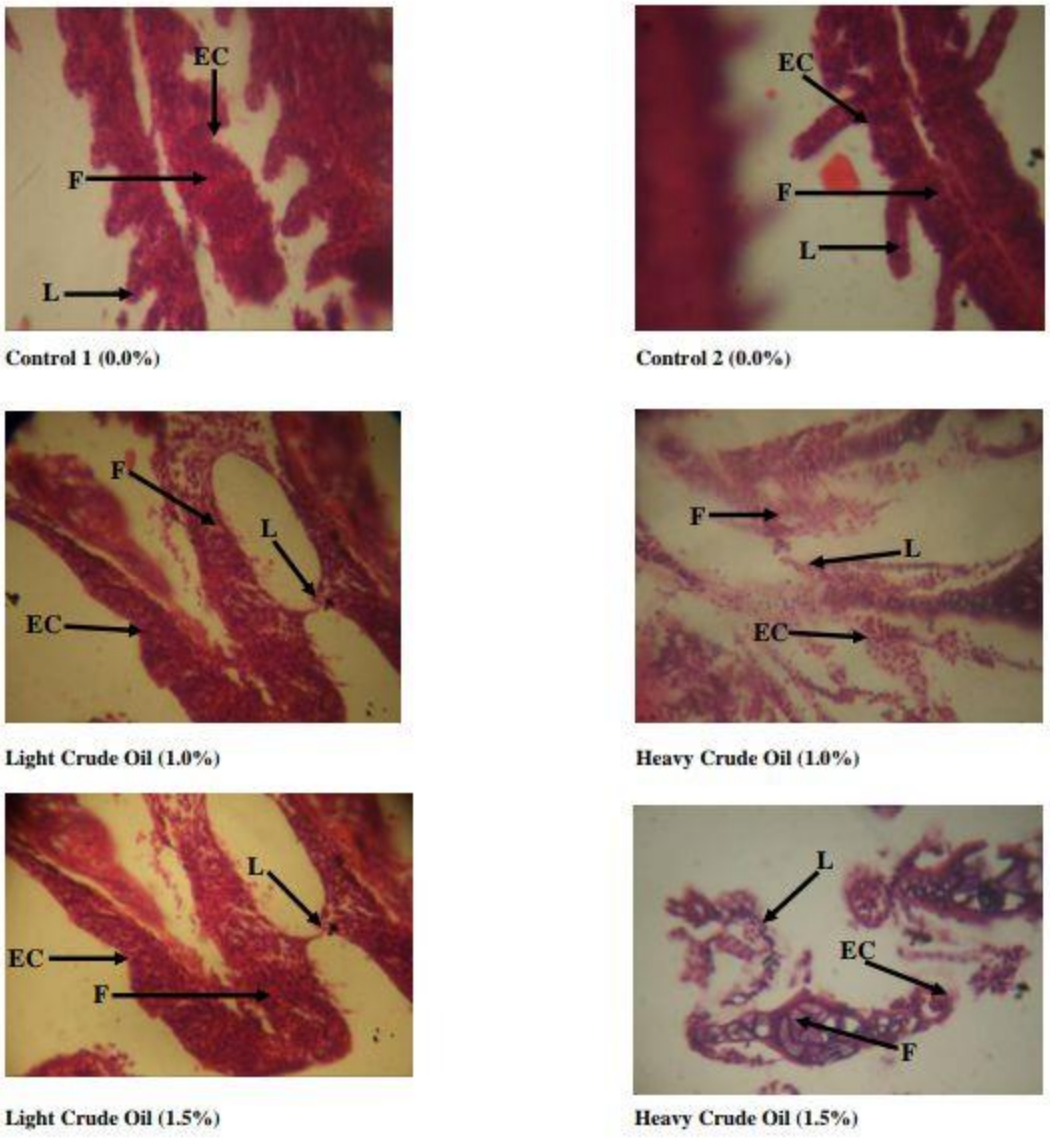

Plate 1: Photomicrograph of the Gills of *Clarias gariepinus*:

Control 1 (0.0%) and Control 2 (0.0%) = Normal aspect of the gills showing the Filament (F), Lamellar (L) and Epithelia Cells (EC). Heavy crude oil at 1.0% and 1.5% showed more degenerative changes on the gills than the light crude oil of corresponding concentrations.

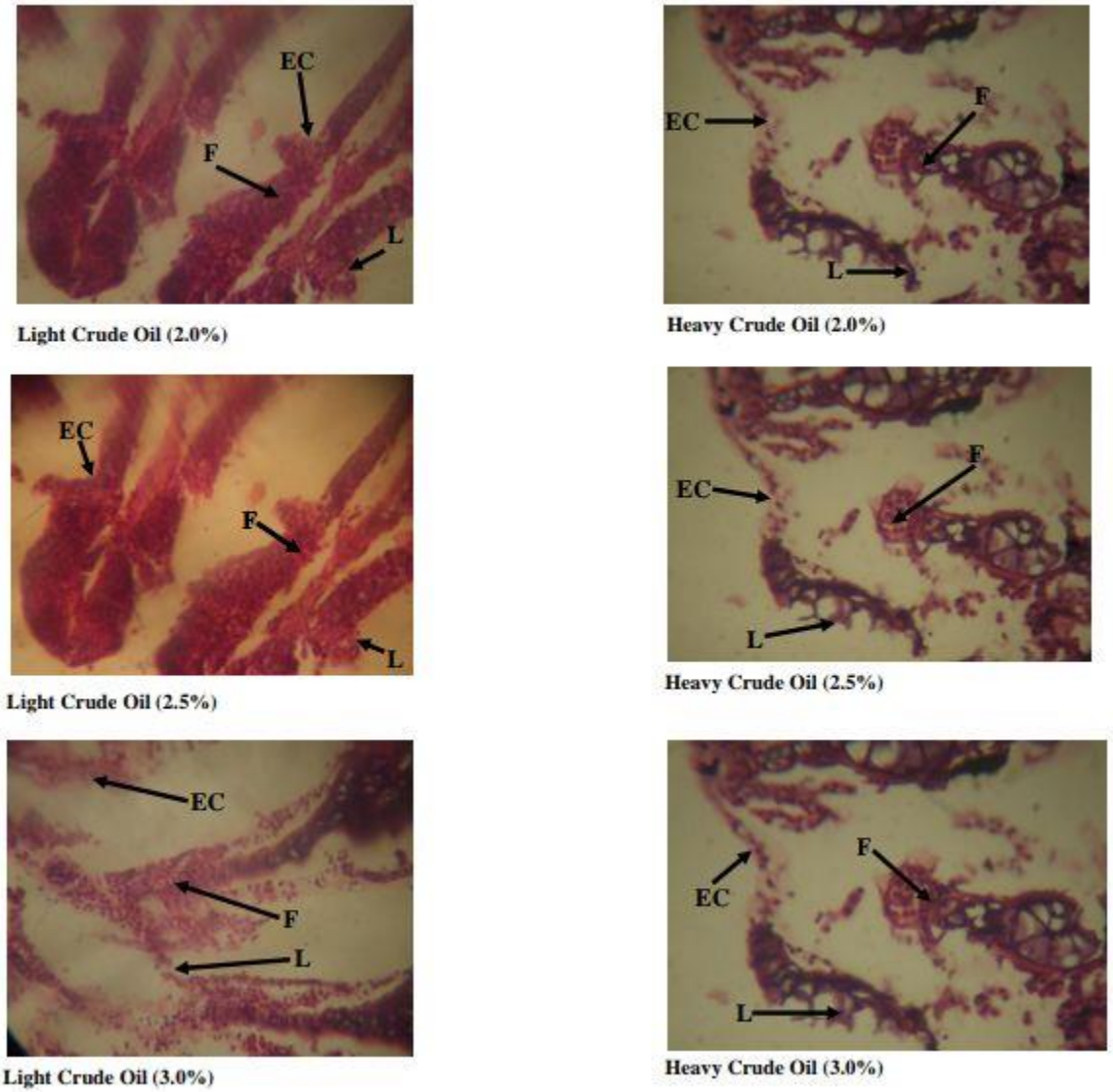

Plate 2: Photomicrograph of the Gills of *Clarias gariepinus*:

Heavy crude oil at 2.0%, 2.5% and 3.0% showed more degenerative changes than the light crude oil of corresponding concentrations as a result of the high accumulation of PAHs of heavy crude in the gills compared to the light crude. The highest concentration (3.0%) of both oils showed the greatest damages.

The percentage mortality increased with increase in the concentration of both toxicants over time of exposure. This was evident from test results which showed 16.67% and 100% mortalities after 24 hrs for 1.0 and 3.0% concentrations by volume respectively for the light crude oil and 8.33% and 44.44% mortalities after 24 hrs for 1.0 and 3.0% concentrations respectively for the heavy crude oil. The histopathological studies showed that the gills of the untreated fish revealed normal parallel arranged gill filaments while the lamellae of gills of exposed fish suffered different levels of distension and fusion, and were also hypertrophic and necrotic. Severity of damage on the gills was concentration-dependent. This result may be because toxicants had been reported to cause structural damage to the respiratory lamellae of the gills. Several histopathological abnormalities in *Clarias gariepinus* due to the exposure to diesel oil (Jabed, Syed, Shams, Hossain, Munzuru, and Shahjahan, 2022), supported this findings. It has been also reported histopathological gill damage due to the exposure of crude oil in yellow perch (*Perca flavescens*), goldfish (*Carassius auratus*) and *Prochilodus lineatus* (Nero, Farwell, Lister, Van Der Kraak, Lee, Van Meer, MacKinnon and Dixon, 2006 and Simonato, Guedes and Martinez, 2008).

The morphological changes in the gills recorded in this study have also been reported in *Astyanax* sp. after 96-hr brief exposure to water soluble fraction of crude oil (Akaishi *et al*., 2004) and *Clarias gariepinus* under brief or prolonged exposure to plant extracts (Onusiriuka and Ufodike, 2000, Fafioye, Adebisi, and Fagade, 2004). Furthermore, fusion of secondary lamellae, gill hyperplasia, and oedema had been reported in fish exposed to petroleum hydrocarbons (Dede and Kaglo, 2001). In some other studies, brief exposure to toxicants for about 96 hours have produced irreversible changes in the gills (Ceiqueira and Fernandes, 2001, Fernandes and Mazon, 2003). Lastly, Gabriel, Amakiriand, and Ezeri (2007) in their work on Haematology and gill pathology of *Clarias gariepinus* exposed to refined petroleum oil (kerosene) under laboratory conditions reported that the changes observed in the gills were adaptations by the fish to cope with challenge of the toxicants.

The heavy crude oil showed more severe damage on the gills than light crude. This is as a result of the higher PAHs concentration of the heavy crude oil in the exposed fish compared to the PAHs concentration of the light crude oil. This finding agrees with the work of Martin (2011) who tested the comparative toxicity and bioavailability of heavy fuel oils to fish using different exposure scenarios. He stated that the result of his research indicated that the amount and nature of hydrocarbons partitioning from oil will vary with the type of oil tested and exposure scenario, and assumed that risks to fish will be greatest for those scenarios that release the highest concentrations of alkyl PAH.

The dissolved oxygen levels and pH values of the control were observed to be higher than those of the treatments of both toxicants. This observation corroborated the reports of Gabriel *et al*. (2007) who studied the haematology and gill pathology of *Clarias gariepinus* exposed to refined petroleum oil (kerosene) under laboratory conditions. The dissolved oxygen levels in the tanks of both toxicants decreased with increasing concentration of the toxicants. This finding agrees with the reports of Awoyinka, Atulomah, E. and Atulomah, N. (2011) and Nyong E. E, *et al* (2023) who reported that a significant decrease in oxygen was observed in their comparative study on the effects of crude oil on juveniles of *C. gariepinus* and *C. anguillaris* to cause anaerobic decomposition of organic matter in water, resulting in the formation of noxious and toxic substances such as hydrogen sulphide and methane which ultimately have deleterious effect on aquatic life.Also, the pH values in the tanks of both toxicants decreased with increasing concentration. This finding agrees with the works of Awoyinka *et al.* (2011) who reported decrease in pH which posed lethal effects on the juveniles of *C. gariepinus* and *C. anguillaris*. Sunmonu and Oloyede (2006) observed a similar trend and suggested that this was probably due to deposition of carbonic acid or its metabolites into the medium and mucus secretion from the fish into the water environment in their bid to survive.

However, values for temperature increased with increased concentration of both toxicants. The increased temperature with increasing concentration of the toxicants could be responsible for the increased mortality relative to the concentration. Beckford (2018), Da Silva, Camargo and Treichel (2023) and Huq, Wu, Guan, Ling, De and Roy (2023) in their work on water quality assessments reported that increase in temperature increased the rate of chemical reactions and decreased the solubility of gasses (especially oxygen) in water. This consequently increased the respiratory rates of aquatic organisms and led to increased oxygen consumption and decomposition of organic matter.

Conclusion/Recommendation: Findings from this study showed that heavy crude oil caused more damage on the gills of the fish than light crude oil. The accumulated PAHs from the light crude oil were less than that of the heavy crude oil which could be as a result of its high volatility owing to the fact that some quantities must have been lost due to evaporation. The study also showed that the African catfish gills can serve as a biomarker of crude oil toxicity due to its ability to make use of limited oxygen supply in contaminated waters.

However, a baseline toxicological study on the constituent and components of light and heavy crude oils is therefore recommended so as to ascertain the level of PAHs in both oils. Also, the study showed how highly toxic crude oil is and can adversely affect the aquatic environment in the case of a spill; thus, its pollution should be prevented.

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