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Full-length Research Article

Bioassay and Histopathological Effects of Water-Soluble Fractions of Crude Oil on *Coptodon guineensis*

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Summary: Acute and subacute effects of crude oil's water-soluble fraction (WSF) and associated histopathological changes in the gills and liver of juvenile *Coptodon guineensis* were investigated. An acute toxicity test (96 hrs) was performed by a static non-renewal method at various concentrations of 0.25, 1, 5, 15, and 30%. Similarly, test organisms were tested in 2.5% sublethal concentration for 28 days. Water quality parameters, heavy metals, and total polycyclic aromatic hydrocarbons (PAHs) were determined using standard methods. After fish exposure to WSF of crude oil, gills and liver were harvested, stained with hematoxylin/eosin, and later prepared for photomicrography. Results revealed the LC50 as 26.73% (26.73 g/L), while total PAHs and heavy metal (Ni, Cd, and Pb) levels varied significantly ($p < 0.05$) in *Coptodon guineensis* throughout exposure. Histopathological alterations were evident in gills and further revealed the presence of deformed secondary lamellae, with corresponding congestion of the passive central vein and cytoplasmic vacuolation in the liver. Apart from toxicity values to unravel the adverse impacts of the bioavailable water-soluble fraction of crude oil on juvenile *Coptodon guineensis*, the histopathological assay revealed underlying sub-lethal responses that can serve as early warning signals.

Keywords: *Bioassay, water-soluble fraction, crude oil, histopathological, Coptodon guineensis.*

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INTRODUCTION

Crude oil is a naturally occurring hydrocarbon with a relatively low viscosity and specific gravity, thus allowing it to float on water. When crude oil spills in a marine environment, there is a tendency to spread over a wide surface area while the slick may remain to drift via wave actions, ultimately extending to open seas, inland waters, and terrestrial habitats (Adofo *et al*., 2022; Centers for Disease Control and Prevention, 2010). As a complex mixture, crude oil consists primarily of aliphatic and aromatic constituent hydrocarbons as well as nitrogen, oxygen, sulfur and trace amounts of cadmium, iron, nickel, vanadium, and lead (Primerano *et al*., 2024; Laws, 2017; Varjani *et al*., 2015 Olaifa, 2012; Edema, 2006). As a raw material and energy source for production of organic compounds, crude oil and its derivatives play important roles in socio-economic development of several nations (Varjani, 2017). In fact, petroleum hydrocarbons constitute one of the most vital organic pollutants in land-dwelling and marine ecosystems, thus have a tendency to contaminate natural waters and ultimately lead to emergence of other fractions including the water-soluble fractions (Ukpaka *et al*., 2020). In actual sense, continuous discharge of crude oil and its derivatives due to tanker accidents or conventional spills may lead to potentially adverse consequences in the ambient environment (George *et al*., 2025; 2014). As crude oil weathers in natural environment, its more readily bioavailable water-soluble fraction will emerge, thus leading to increased contaminant uptake in exposed organisms. Over time, accumulated oil residues in planktonic organisms and sediments may be taken up in higher organisms, resulting in bio-magnification across the aquatic food chain (Ogbeide and Eriyamremu, 2023; Chouksey *et al*., 2004). Thus, the water-soluble fraction of crude oil is assumed to be the freely dissolved component of the petroleum hydrocarbon that constitutes various toxic components, including PAHs, benzene, toluene, ethylbenzene, xylene (BTEX), phenols, heterocyclic compounds and heavy metals. Consequently, these constituents of crude oil can elicit toxic actions in exposed organisms (George, *et al*., 2025; Ambaye *et al*., 2022; Rodrigues *et al*., 2010). Typically, adverse effects due to biota exposure to crude oil residues and their derivatives can range from underlying biochemical responses to visible effects in whole organisms, particularly in early life juvenile stages (Banaee *et al*., 2025; Esteban-Sánchez, *et al*., 2021; Lee *et al*., 2017; Sadani *et al*., 2011). In particular, fish have been reported to more readily absorb dissolved petroleum hydrocarbons from contaminated water (Olaifa, 2012). The

resulting water-soluble fraction of crude oil can potentially induce adverse biological reactions, including irregular movement and ultimately death of exposed organisms. Considering that uptake of crude oil residues adversely impacts gills and other internal organs, leading to substantial damage, histopathological assays can establish contaminants' interactions and sensitivity of relevant organs as well as unravel underlying adverse consequences in exposed organisms (Agbogidi, *et al*., 2024; Pathan *et al*., 2010). Although many previous studies have assessed the potential toxicity of crude oil residues in fish (Esteban-Sánchez, *et al*., 2021; Hagerty and Ramseur, 2010), research information is scarce on the potential health consequences of its water-soluble fraction on juvenile Guinea Tilapia, *Coptodon guineensis*. Therefore, this study assessed the uptake of water-soluble fraction of crude oil, its constituent PAHs and heavy metals load by using histopathological biomarkers of toxicity in juvenile *Coptodon guineensis*.

MATERIALS AND METHODS

Test organism (*Coptodon guineensis*): Guinean Tilapia (*Coptodon guineensis*) (Gunther, 1862) is a brackish water euryhaline fish species found along the west coast of Africa. The Juvenile stage of the brackish fish was sourced from the Department of Aquaculture, Nigerian Institute for Oceanography and Marine Research (NIOMR), Lagos, Nigeria. The fish were collected as fries on day 14 after hatchery and allowed to acclimatize prior to utilization for an acute toxicity test that lasted for 21 days. The body weights of the fish fries were 0.07 - 0.10 g, while the length ranged from 1.4 to 1.6 cm. The fish were relocated to the toxicity test laboratory in a 56 x 41 x 35 cm plastic tank at a density of 50 fries per litre. Subsequently, fish were transferred to similar acclimation glass tanks at the same density.

Extraction of water-soluble fraction: Water-soluble fraction of crude oil was prepared according to procedures described by Bamidele and Eshagberi (2015) and Faksness *et al*. (2020), but with slight modifications. One part of oil was added to nine parts of filtered seawater at 10 ppt (1:9, v/v) in a 1L glass volumetric flask. The flask was capped with a stopper and covered with aluminum foil to minimize the evaporation of volatile components of the crude oil. Subsequently, the mixture was placed on a magnetic stirrer and stirred continuously for 24 hr at 250 rpm. The solution was later transferred into a separating funnel and allowed to stand for 8 hrs.. Afterwards, the aqueous phase was drained out and designated as 100% water-soluble fractions of crude oil. The stock solution was stored in a refrigerator for 24 hr and thereafter used for further bioassay experiments.

PAHs and heavy metal analysis: PAHs were analyzed using a previously developed method (Maskaoui and Hu, 2009). A 5g of the harvested fish was weighed into a mortar and homogenized in anhydrous sodium sulphate (previously baked at 160 oC for 24 hr). The homogenized sample was transferred to an amber bottle for a cold extraction in 50 mL dichloromethane for 30 mins in a sonication bath (USEPA, method 3550c; Sun *et al*., 1998; Nwaichi and Ntorgbo, 2016; Sogbanmu *et al*., 2019). The resulting extract was dried to 1 mL, prior to application to a silica gel column (4 mm i.d. × 90mm) as a further clean-up procedure. Extracts for PAH analysis were further evaporated under a gentle stream of nitrogen until 100 µL. A clean extract was reconstituted in 2 mL 2, 2, 4-trimethylpentane and later transferred into glass vials for gas chromatography analysis. The samples were assessed for all 16 US EPA priority PAHs (naphthalene, acenaphthylene, acenapthene, fluorene, phenanthrene, anthracene, fluoranthenes, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranathene, benzo[k]fluoranathene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a.h] anthracene, and benzo[g,h, i]perylene) using a GC-FID Agilent 7890 device. Prior to analysis, PAH analytical standards were used to calibrate the gas chromatograph.

Heavy metal analysis in fish tissues: The APHA-AWWA-WEF (2012) protocols according to Bello *et al*. (2019), with slight modifications were used for heavy metal analysis. A 5 g of fish muscle was weighed into a 250 mL conical flask. A further 25 mL of digestion reagent (HNO3:H2O2) in a 1:1 v/v ratio was added and allowed to stand for 30 mins in order to cool the initial reaction with the samples. Later, samples in flasks were heated for 1hr on the hot plate in a fume cupboard until 110 oC, till the volume of the contents was 5 mL. Afterwards, the sample was removed and allowed to cool for 30 mins prior to a filtration step using Whatman paper. Filtrates were collected in a standard flask and filled with deionized water to a volume of 50 mL prior to storage in a plastic dispensing bottle. Metal levels in all sample extracts were determined in an Atomic Absorption Spectrophotometer (AAS) (air-acetylene flame) (model PG AA500). A deuterium background correction was used for the metals, while blank corrections were applied for each set of the analyses. Accuracy was assessed by analyzing three replicates of samples.

Acute toxicity study: Acute test to assess the potential effects of a water-soluble fraction of crude oil on *Coptodon guineensis* was conducted according to the OECD guidelines (1993), in a static non-renewal regime. Exposures were performed in triplicates in 2L glass aquarium tanks (28cm x 13cm x 15cm). Various exposure levels tested include 0.25, 1, 5, 15, and 30% of the WSF of crude oil after each was constituted in the same habitat water as the source of fish.

Sub-lethal toxicity study: Twenty juvenile fish were exposed to 30L of the test solution in a glass aquarium tank (56 x 41 x 35cm). The weights of the fish were in the range of 9.5 - 12 g, while the standard and total lengths were 7 - 7.5 cm and 8.8 - 9.5 cm, respectively. Exposed organisms were depurated for 48 hr in clean water prior to commencement of the bioassay, in order to remove residual contaminants attached to exposed organisms. In order to assess bioaccumulation in exposed fish, individual organisms were subjected to a sub-lethal concentration of 2.5% WSF of crude oil.

Fish maintenance, exposure duration, and water quality analysis: Fish were exposed to 2.5% of WSF of crude oil for 28 days and fed compounded tilapia feed daily at 5% body weight. Exposure water was renewed every 4 days while individual fish was sacrificed every 7 days to harvest

the gills, liver and muscles for histopathological analysis, PAH, and heavy metal levels.

Water quality parameters including temperature, pH, conductivity, dissolved oxygen (DO), total dissolved solids (TDS), salinity, and turbidity of the experimental setup were monitored periodically using standard methods (APHA-AWWA-WEF, 2012).

Bioaccumulation factor (BAF): The bioaccumulation factor was determined after fish exposure to WSF of crude oil according to Kalfakakour and Akrida-Demertzi (2000) and Rashed (2001) as follows:

$$BAF = \frac{Ca}{Cw}$$

Where Ca = concentration accumulated in tissue of the juvenile fish; Cw = concentration in exposure water

Histopathological assay of *Coptodon guineensis* gills and liver tissues: At the end of 28-day exposure, fish gills and liver harvested from control as well as exposed individuals were preserved in 10% buffered normal saline. Fixed tissues were dehydrated at various levels in graded ethanol at 70 - 95%, cleared in xylene and subsequently embedded in paraffin blocks. Later, the samples were cut using a rotary microtome at a thickness of 4 - 5 µm. The resulting tissue sections were stained with hematoxylin and eosin, tested by light microscopy and photographed (Avwioro, 2014; Suvarna *et al*., 2013).

Data analysis: At the end of fish exposure in WSF of crude oil, lethal concentration (LC50) values were determined by Probit according to Finney (1971) and Akçay (2013) using the IBM SPSS Statistics Package (version 26). A one-way ANOVA was used to analyse concentration and time of exposure independently. Tukey HSD multiple posthoc tests were used to assess the significance of the differences across mean values, while a Bray-Curtis cluster analysis was applied to physico-chemical parameters of the water-soluble fraction of crude oil in relation to the control test.

RESULTS

Physico-chemical parameter values for WSF of crude oil: Mean values and Standard deviation for physico-chemical parameters determined throughout the duration of fish exposure in WSF of crude oil are shown in Table 1. Application of the Bray-Curtis index analysis (Figure 1) shows similarities in test water properties at category I (temperature, DO, conductivity, and pH), category II (TDS), and category III (turbidity and salinity).

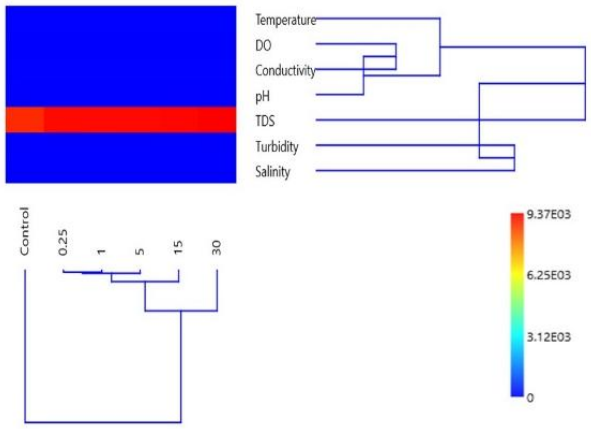

Figure 1:
Hierarchical clustering (Bray-Curtis similarities index) of physico-chemical parameters of WSF of crude oil.

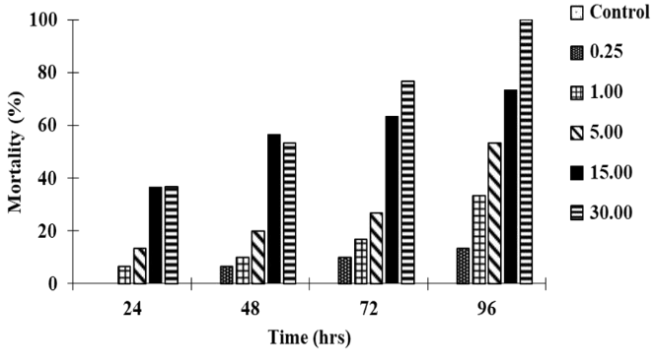

Figure 2:
Mortality of *C. guineensis* after 96hr exposure to various concentrations of WSF of crude oil

Acute toxicity: Lethal concentration (LC50) values obtained from *Coptodon guineensis* exposure to various concentrations of water-soluble fraction of crude oil until 96 hr are shown in Table 2. Mortality increased with the duration of exposure in fish treated in WSF of crude oil (Figure 2). At the end of 96 hr, a cumulative mortality of 13.3% was recorded at the lowest exposure concentration. Meanwhile, 100% mortality of fish was recorded at the highest exposure concentration.

Table 1:
Physico-chemical parameter values of WSF of crude oil in a 96 hr fish test

Conc.	Temperature (ºC)	pH	Conductivity (mS/cm)	DO (mg/L)	TDS (mg/L)	Salinity (‰)	Turbidity (mg/L)
0.0	26.6±0.1	7.6±0.0	14.4±0.1	8.8±0.1	8857.3±6.4*	8.7±0.0	0.0±0.0
0.25	27.0±0.0	7.6±0.0	14.9±0.1	8.2±0.2	9250.0±2.0*	8.7±0.0	0.0±0.0
1.0	27.1±0.2	7.7±0.0	15.0±0.1	8.3±0.1	9254.7±4.2*	8.7±0.0	0.1±0.0
5.0	27.1±0.0	7.8±0.0	15.1±0.1	8.0±0.0	9259.3±1.2*	8.6±0.0	0.8±0.0
15.0	27.0±0.0	7.8±0.0	15.6±0.1	7.9±0.0	9285.3±5.0*	8.7±0.0	1.2±0.0
30.0	27.1±0.0	7.8±0.0	15.2±0.1	7.7±0.1	9374.0±4.0*	8.8±0.0	5.9±0.0

DO: Dissolved oxygen, TDS: Total dissolved solids, * indicates significant level at $p<0.05$

Table 2:
Lethal concentration (LC_{50}) values for WSF of crude oil in a fish test

Time (hr)	LC_{50} (g/L)	lower / upper limits (95% conf.) (g/L)	
24	499.9	238.2	2,671.3
48	200.4	108.0	552.4
72	85.5	51.2	160.7
96	26.7	16.4	42.0

Accumulation of PAHs in C. guineensis exposed to WSF of crude oil: Fish body burden and the corresponding bioaccumulation factors (BAFs) increased with exposure duration until 28 days (Figure 3). Meanwhile, the cumulative uptake of PAHs peaked at 50.0 mg/kg with a BAF value of 276.7 at the end of exposure (Table 3).

Accumulation of heavy metals in C. guineensis exposed to WSF of crude oil: The selected heavy metals (Nickel, Cadmium and Lead), were determined in C. guineensis exposed to WSF of crude oil and the corresponding BAF values are shown in tables 3 and 4.

Histopathology of the gills in C. guineensis exposed to WSF of crude oil: Figure 4 (A, B, C, D and E) shows the outcome of histopathological examination of gills from *Coptodon guineensis*, which suggests there was no noticeable effects due to treatment in WSFs of crude oil upon 7day exposure. At day 14, however, there were observable toxicity effects due to the WSFs of crude oil, which was evident in the gills of *Coptodon guineensis* and showed deformed secondary lamellae but appeared mild at day 21. On day 28 (the final day of exposure), the secondary lamellae were severely deformed and damaged.

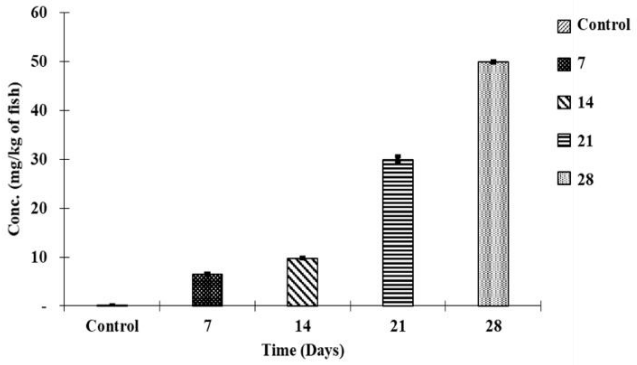

Figure 3:
Accumulation of PAHs in *C. guineensis* exposed to WSF of crude oil. Error bars represent the standard deviation of mean values

Table 3:
Heavy metals in crude oil water-soluble fractions on *Coptodon guineensis*. The data represents the mean of the triplicate samples and the standard deviation

Days	Cadmium (Cd)	Nickel (Ni)	Lead (Pb)
Control	BDL	0.35±0.03	BDL
7	BDL	3.56±0.12	1.88±0.13
14	0.07±0.01	3.25±0.15	1.50±0.10
21	0.02±0.00	3.25±0.18	1.70±0.02
28	0.02±0.00	3.56±0.13	1.88±0.11

BDL= Bellow Detection Limit

Table 4:
Bioaccumulation factors (BAF) of PAHs and selected heavy metals in *C. guineensis* exposed to water-soluble fractions of crude oil

Days	PAHs	Cadmium (Cd)	Nickel (Ni)	Lead (Pb)
7	36.3	BDL	334.0	360.9
14	54.2	25.3	305.4	288.7
21	165.9	7.3	305.4	327.2
28	276.7	7.3	333.9	360.3

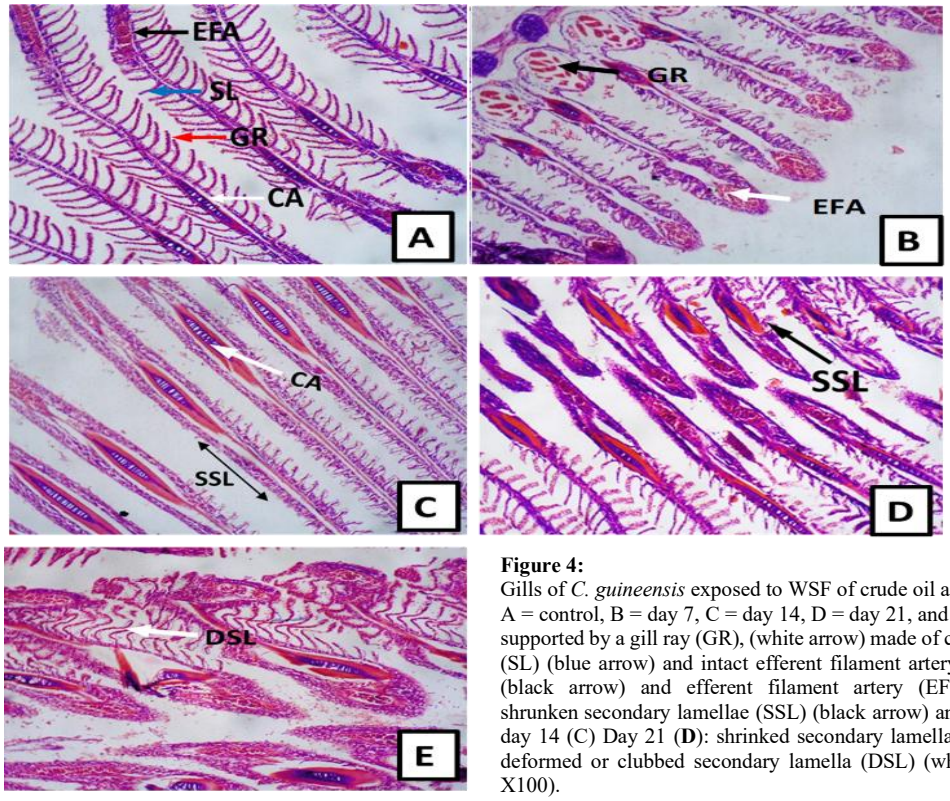

Figure 4:
Gills of *C. guineensis* exposed to WSF of crude oil after histopathological examinations. A = control, B = day 7, C = day 14, D = day 21, and E = day 28. (**A**) Gill filament (red arrow) supported by a gill ray (GR), (white arrow) made of central cartilage (CA). Secondary lamellae (SL) (blue arrow) and intact efferent filament artery (EFA) (black arrow), the gill ray (GR) (black arrow) and efferent filament artery (EFA) (white arrow) at day 7 (**B**) and shrunken secondary lamellae (SSL) (black arrow) and central cartilage (CA) (white arrow) at day 14 (C) Day 21 (**D**): shrinked secondary lamellae (SSL) (black arrow). On day 28 (E), a deformed or clubbed secondary lamella (DSL) (white arrow) was observed (H & E. mag. X100).

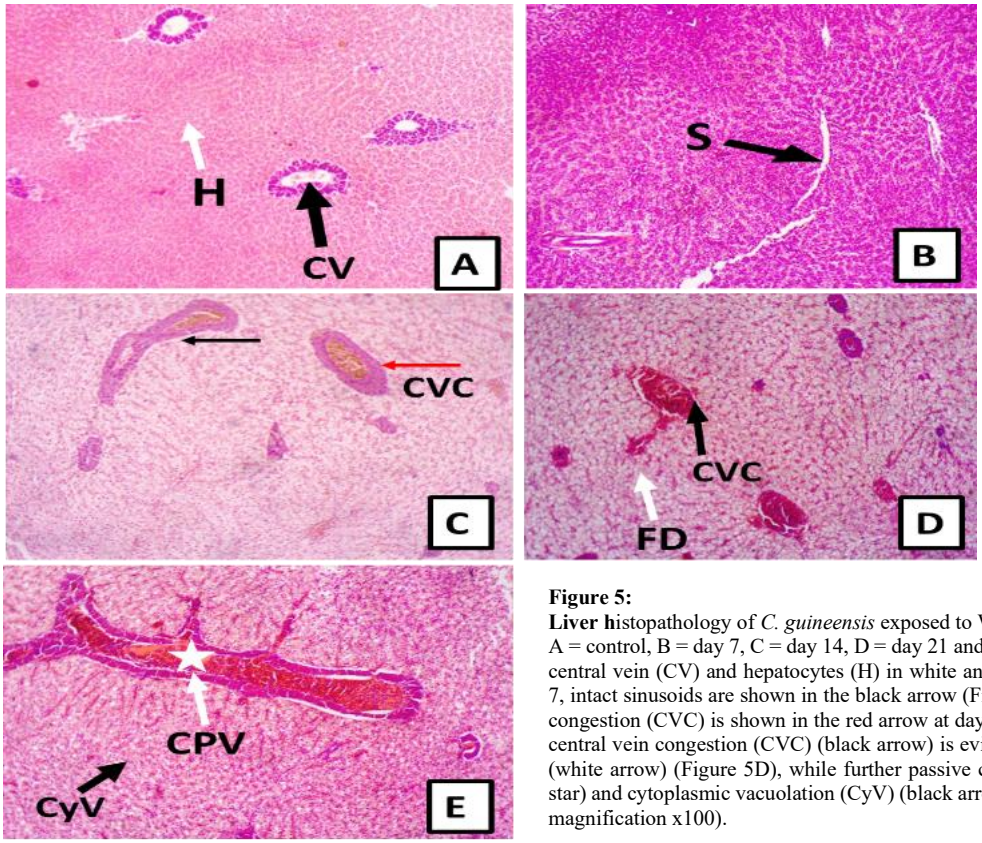

Figure 5:
Liver histopathology of *C. guineensis* exposed to WSF of crude oil.
A = control, B = day 7, C = day 14, D = day 21 and E = day 28**. Figure 5A shows** intact central vein (CV) and hepatocytes (H) in white and black arrows, respectively. At day 7, intact sinusoids are shown in the black arrow (Figure 5B), while passive central vein congestion (CVC) is shown in the red arrow at day 14 (**Figure 5C). At** day 21, passive central vein congestion (CVC) (black arrow) is evident, coupled with fat droplets (FD) (white arrow) (Figure 5D), while further passive congestion portal vein (CPV) (white star) and cytoplasmic vacuolation (CyV) (black arrow) are shown in Figure 5E. (H & E. magnification x100).

Histopathology of liver in C. guineensis exposed to WSF of crude oil: The outcome of histopathological analysis of liver from the control population showed a normal structure of the liver with undamaged central vein and hepatocytes (Figure 5 (A, B, C, D and E)). The first seven days of exposure revealed that there were no noticeable effects of wate soluble fractions of crude oil on liver tissue of *Coptodon guineensis*. However, at day 14, there were noticeable toxicity of the WSFs of crude oil, which was evident in congestion of the passive central vein of the liver. Also, damage to the central vein, congestion, dilated sinusoids, and the fat droplet at day 21 were observed. On day 28, the final day of exposure, cytoplasmic vacuolation was noticed.

DISCUSSION

The water quality characteristics were relatively steady at various tested concentrations of 0.1, 0.25 and 0.5%. The observed consistency in values is in agreement with Anwar *et al*. (2022), who reported that DO and pH did not vary significantly (P≥0.05) in the exposure test with WSF of crude oil when compared with the control vessels. The overall health of aquatic species can be negatively impacted by WSF from crude oil, according to Santos *et al*. (2016). Consistency in values of water quality parameters indicates that the observed changes were due to fish exposure to WSF of crude oil, not as a result of variations in water quality characteristics. For most water quality characteristics, values were within acceptable ranges for the sustenance of aquatic life according to the FEPA/FMEnv (1991) and not likely to contribute to the toxicity of the water-soluble fraction.

A cumulative mortality of 13.3% recorded at the lowest exposure concentration and 100% mortality of fish at the highest exposure concentration, suggest that crude oil residues may have accumulated over the period of the fish test to exert adverse consequences in exposed individuals. According to Vroumsia *et al*. (2014), toxicants can exert adverse impacts on fish and other aquatic organisms by impairment of transmission of nerve impulses, impacting vital body organs including the liver and gills, or altering haematological parameters (Friday *et al*., 1996; Chindah *et al*., 2001 and 2004). When one considers the variation in LC50 values from this test compared to previous studies with Tilapia (Akaishi *et al*., 2004; Dighiesh *et al*., 2019), it becomes pertinent to establish standard protocols for preparation of WSF of crude oil for bioassay studies. More so, the discrepancy in toxicity values compared to previous research can be attributed to the difference in source of the crude oil used for the test.

The BAF values varied greatly over the period of exposure, this implies that PAHs were taken up and accumulated in exposed fish, considering that uptake may have been greater than contaminant metabolism and subsequent elimination. Other previous research suggests that soluble hydrocarbons tend to partition more actively into animal tissues compared to other components of the environment; thus, this may explain the observed greater accumulation in fish tissues (Anyakora *et al*., 2006; Gravato and Santos, 2002). Therefore, fish is a good bio-indicator to assess contaminant uptake in biota.

Body burden for selected heavy metals, Nickel, Cadmium and Lead While Cadmium showed substantial

accumulation at day 14, further extension of exposure duration did not reveal any significant toxicant accumulation in fish tissues. The fact that these metals tend to dissolve sparingly in water-soluble fractions of crude oil, may suggest that uptake and accumulation of bioavailable fractions were slow within the duration of exposure. Also, According to a prior study by Sobhanardakani *et al*. (2011), heavy metals are rapidly absorbed by living things and are highly soluble in aquatic environments. Also, in contaminated aquatic ecosystems, Barakat (2011) stated that heavy metals have been discovered in the gills, livers, and muscles of many fish species, which is in agreement with the results of the current study as the investigated metals were found to accumulate in the muscles of the fish species used as the day of exposure progressed. Farombi *et al*. (2007) attributed the uptake, concentration, and accumulation of heavy metals in the tissues of animals as recorded in this study to the possession of metal-binding proteins in animal tissues. Due to their extreme toxicity to both humans and aquatic life, heavy metals have been utilized as markers of pollution (Omoigberale and Ogbeibu, 2007). According to Edema (2012), the WSF of crude oil can alter the physical, ionic, and heavy metal composition of the aquatic environment.

Gills of C. guineensis are essential respiratory features that allow frequent water flow over them to facilitate the absorption of dissolved oxygen from surrounding water. Considering the volume of water flowing over the gills, there is a tendency for both the dissolved and suspended contaminants to partition over the relatively large surface area of the respiratory organ. Thus, gills of fish exposed over a long period in a contaminated water system can bioaccumulate contaminants, which ultimately can elicit negative physiological responses in whole organisms. The challenge, however, is that contaminants in trace levels over short exposure durations may not substantially elicit visible deleterious effects in a whole fish if assessed by conventional toxicity endpoints. It is therefore necessary to assess underlying subtle toxicity outcomes via histopathological examination of fish tissues. This approach can serve as early warning signs to visualize potential structural changes in tissues prior to actual manifestation of severe life-threatening toxic outcome in whole organisms. For the control tests in this study, histopathological examination of gills did not result in any visible structural defects in the first 7 days (Figures 4A, 4B). At day 14, however, gills from C. guineensis treated in WSF of crude oil showed structural alterations that include deformation of the secondary lamellae (Figure 4C). A further extension of exposure duration to 21 - 28 days revealed more severe deformity in the secondary lamellae and consequently damage to the structure of the gills (Figure 4D, 4E). Overall, structural deformities were apparent in the gills of C. guineensis, which can constitute enormous health threat to exposed individuals and other fish species. These observations are consistent with the findings by Dighiesh *et al*. (2019), which reported localized hyperplasia and adhesion of secondary gill lamellae, coupled with congestion of blood vessels, in red tilapia exposed to WSF of crude oil over a 96 hr period. Similarly, the results from this study are in agreement with the outcome of research by Brand *et al*. (2001), who reported hyperplasia of secondary lamellae in the pink salamander, Oncorhynchus gorbusha exposed to crude oil. Also, Khan (2003) in a previous study of three marine fish species exposed to petroleum hydrocarbons reported substantial pathological changes, including hyperplasia of the epithelium lining the lamellae, mild necrosis and distortion, which ultimately will hamper the performance of gill filaments in diffusion of oxygen across the gill lamellae. A consequence of the physiological alterations includes reduced surface area for gaseous exchange, which will lead to development of hypoxic condition in exposed fish (Elahee and Bhagwant, 2007).

Apart from its role in maintenance of homeostasis, glucose and lipid metabolism, liver is an essential organ in contaminant detoxification (Mohamed, 2009). Liver histopathology in control population of C. guineensis showed normal structures of healthy central vein, which is consistent with the observations at day 7 of fish exposure in WSF of crude oil (Figure 5A and 5B). At day 14, however, congestions of the passive central vein were apparent in liver upon fish exposure to WSF of crude oil (Figure 5C). Further extension of exposure duration to day 21 revealed more severe damage of the central vein, congested and dilated sinusoids, coupled with the presence of fat droplets (Figure 5D). Subsequently, cytoplasmic vacuolation was observed in exposed population at day 28 (Figure 5E). Apart from operational accidents at oil rigs, crude oil contamination of marine ecosystems can also result from sabotage and activities of pirates at sea, thus leading to weathering of crude oil residues. Ecological implications of various existing routes of fish exposure to water soluble fractions from contaminating crude oil is huge, considering that oil spills can reoccur in natural marine environment. Therefore, histopathological evidence from this study suggests that WSF of crude oil can elicit various sub-lethal toxic responses in C. guineensis in a manner that increased with the duration of exposure. The results presented in this study are consistent with a previous research, which reported liver as one of the most adversely affected organs in fish exposed to WSF of hydrocarbons. It further suggests increased tendency and role of the liver to detoxify and eventually eliminate contaminants, which may have led to the observed necrosis in liver. The observed effect is expected to be more pronounced given that necrotic fish liver may not regenerate new cells. These findings are in agreement with the reports by Akaishi *et al*. (2004) and Kakkar *et al*. (2011), who observed corresponding changes in liver tissues of Astyanax sp. and Channa punctatus after exposure to WSF of crude oil, respectively. Also, the results reported by Brand *et al*. (2001) and Khan (2003) after histopathological examination of liver in freshwater and marine water fish species exposed to petroleum hydrocarbons, revealed necrosis and cellular inflammation. If one considers the frequency of threats due to petroleum hydrocarbons, it is possible that complex constituents of the WSF of crude oil can elicit toxicity in exposed fish and possibly in other marine organisms, even in trace levels. The challenge however is that toxicity of several contaminating substances are mainly assessed by using visible toxicity endpoints, which in some cases are not able to detect subtle underlying responses in exposed population. Thus, histopathological evidence can provide clues on ecological health and serve as early warning signs in event of pollution.

Various bioassay-based indices to assess water quality rely on visible parameters that often require a substantial

level of contamination in order for toxicity signatures to manifest in exposed organisms. Research evidence suggests that petroleum hydrocarbon contamination of marine water, even in low concentrations, can elicit deleterious consequences in a chronic fish test. Alternatively, histopathological examination of fish tissues as shown in this study, unravelled toxicity evidence at realistic treatment levels and short exposure time, which can serve as an early warning signal in events of crude oil contamination of marine water bodies. The results further suggest that apart from other complex constituents of crude oil, heavy metals and PAHs were detected in test water. Also, the WSF of crude oil elicited histopathological changes in gills and liver tissues even at environmentally realistic treatment levels. Utility of the WSF in this study provides a basis to assess the uptake and toxicity potential of the bioavailable portion of crude oil in the exposed population.

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