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Toxicological Effects of Crude Oil on the Histopathology of Selected Organs in African Catfish (Clarias Gariepinus Burchell, 1822)

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Abstract: *A twenty-eight (28) day study investigated the toxicological effects of crude oil on the histopathology of selected organs in African catfish (Clarias gariepinus Burchell, 1822) as a bioindicator organism. The research was conducted to evaluate the impact of varying concentrations of crude oil on water quality parameters, swimming and feeding behaviour, mortality rate, and histopathological changes in vital organs of the fish. A laboratory-based experimental design was adopted, where fish were exposed to different concentrations of crude oil (10 ml, 20 ml, 40 ml, 80 ml, and 160 ml) alongside zero (00 ml) control. Physicochemical parameters such as temperature, pH, dissolved oxygen (DO), and water hardness were measured. Results showed a progressive increase in water temperature and hardness with increasing crude oil concentration, while pH and DO levels decreased significantly, indicating deterioration in water quality. Mortality rates increased with both concentration and exposure time, with the highest mortality recorded at 160 ml. The histopathological examination revealed severe tissue damage in exposed fish with increased concentration of crude oil. Observed alterations included lamellae damage, epithelial lifting, and mucus accumulation in the gills; cellular degeneration, vacuolation, and necrosis in the liver; and tubular degeneration and shrinkage of glomeruli in the kidney. These changes demonstrate that crude oil exposure significantly impairs respiratory, metabolic, and excretory functions in fish. The study concludes that crude oil pollution poses serious threats to aquatic organisms and ecosystems by degrading water quality, increasing mortality, and causing structural damage to vital organs. It is therefore recommended that stricter environmental regulations and effective oil spill management strategies be implemented to protect aquatic biodiversity and sustain the community's livelihood.*

Keywords: Crude Oil, Histopathology, Clarias gariepinus, Bioindicator, Toxicity

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INTRODUCTION

Crude oil pollution is a major environmental concern, particularly in oil-producing regions such as the Niger Delta in Nigeria. Hydrocarbon contamination affects aquatic ecosystems, leading to severe toxicological impacts on fish and other aquatic organisms. Petroleum hydrocarbons, in their crude, refined, or spent forms, pose significant risks to human, animal, and plant species (Khan et al., 2017; Smith et al., 2015). The environmental presence of crude and refined oils, often resulting from accidental spills or deliberate sabotage, has devastating consequences (Omoregie et al., 2017). In contrast, the disposal of spent oil is frequently deliberate and indiscriminate, disregarding its polluting effects (Hossain et al., 2019).

Oil pollution in aquatic environments constitutes hazards ranging from impairment to suppressed death (Javed et al., 2020). Toxicological evaluations, including acute/lethal and chronic/sub-lethal observations, are crucial in assessing these hazards (Rahman *et a*l., 2018). The lethal concentration of a substance is determined by its short-term effects on a test population (Kumar et al., 2019). Oil spills have caused widespread destruction of food resources and harmed various aquatic organisms (Adams et al., 2018). Regular assessments are necessary to understand the immediate and potential long-term effects of oil spills on the physiological characteristics of affected species (Omoregie et al., 2017).

Fisheries play a vital role in Nigeria's agricultural sector, with fish accounting for over 40% of animal protein consumption (Federal Department of Fisheries, 2020). Therefore, investigating the pollution effects of crude oil, refinery effluents, and spent oil on aquatic animals, such as catfish, is essential.
The African catfish (*C. gariepinus*) endemic to Africa and it is a hardened fish species, is a widely distributed freshwater species that serves as an important food source and an indicator of aquatic ecosystems' health. Understanding the toxic effects of crude oil on this species is crucial for environmental risk assessment and conservation efforts.

Acute Toxicity: high concentrations of crude oil causes rapid mortality due to respiratory failure (Udo et al., 2020). Chronic Toxicity: sub-lethal exposure leads to growth retardation, oxidative stress, and organ damage (Omoregie & Oyebanji, 2023). Behavioral Changes: crude oil reduces swimming activity and avoidance behaviour of the C. gariepinus (Ezekwe et al., 2021). Oil exploration and extraction activities, including drilling, transportation, and accidental spills, have significant ecological consequences, particularly in aquatic ecosystems. These activities introduce hydrocarbons, heavy metals, and other toxic compounds into water bodies, threatening fish, invertebrates, plankton, and other aquatic organisms. The Niger Delta, the Gulf of Guinea, and other oil-rich regions in Africa have experienced severe biodiversity loss due to prolonged oil pollution. Despite regulatory measures, oil exploration continues to degrade aquatic habitats, leading to: decline in fish populations, loss of sensitive species (e.g., mangroves, mollusks), bioaccumulation of toxins in the food chain, disruption of ecosystem services (fisheries, water purification). Findings align with global studies (Beyer et al., 2022; Adebowale et al., 2021), confirming that oil exploration severely disrupts aquatic ecosystems. Oil exploration leads to habitat degradation, species loss, and long-term ecological damage.

Aim and Objectives of the Study

Aim of the Study

The aim of this research work was to examine the Toxicological Effects of Crude Oil on the Histopathology of Selected Organs in African Catfish (*C. gariepinus* Burchell, 1822).

Objectives of the Study

The specific objectives of the study were to:

- Evaluate the sub-lethal toxicity of crude oil on *C. gariepinus*.
- Examine histological changes in gills, liver, and kidney tissues of exposed fish.
- Assess the impact of crude oil and survival rate of the *C. gariepinus.*

MATERIALS AND METHODS

Study Design

Experimental Fish: Juvenile *C. gariepinus* (mean weight: 50g).
Crude Oil Source: Bonny Light Crude Oil obtained from NNPC.

Sub-lethal Exposure (28 Days)

Fish exposed to 0%, 10%, 20%, and 40%
Swimming rate, feeding behaviour, and mortality were recorded.

Histopathological Analysis

Gills, liver, and kidney tissues were preserved in 10% formalin, processed, and stained with H&E.

Experimental Design

Sub-lethal toxicity: 28-day exposure to 10%, 25%, and 50% of derived 96-h + control; 3 replicates (10 fish species per replicate). Endpoints were measured at day 0, 7, 14, and 28.

Data Analysis

Data tested for normality (Shapiro-Wilk) and homogeneity (Levene's). One-way or two-way ANOVA + Dunnett/Turkey post hoc for parametric data. Kruskal-Wallis + Dunn's for histopathology scores. Chi-square of physicochemical parameters with fish mortality (P-value). Pearson's correlation and PCA for biomarker relationships. Significance at ($P < 0.05$). Software: SPSS Model 2024.

RESULTS AND DISCUSSION

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Effect of Crude Oil Treatments on Feeding Habit of *Clarias gariepinus*

The one-way analysis of variance revealed that crude oil treatment exerted a highly significant effect on the feeding habit (tables 1 & 2) of *C. gariepinus*, as evidenced by **F(3, 59) = 26.21, p < 0.001**. This result indicates that the mean feeding scores varied markedly across the four treatment groups, with feeding activity progressively declining as crude oil concentration increased. Since higher feeding scores represent poorer feeding performance, the trend observed across treatments confirms that exposure to crude oil impaired the normal feeding response of the fish. Post hoc comparisons using Turkey HSD showed that the control group (T_1) differed significantly from T_2, T_3, and T_4, while T_2 did not differ significantly from T_3 but differed from T_4. Furthermore, no significant difference was observed between T_3 and T_4, indicating that severe feeding impairment had already set in at T_3 and persisted through T_4. The Dunnett test, which compares all treatments with the control, further emphasized that T_4 exhibited a significantly poorer feeding response relative to the control and lower concentrations. This pattern demonstrates that increasing crude oil concentration severely disrupted the feeding behavior of the fish, with the highest level of impairment occurring at the highest treatment.

Table1: Analysis of Variance for Juveniles' Feeding Habit

Source	SS	df	MS	F	p
Between Groups	51.218	3	17.073	26.21	< .001
Within Groups	38.433	59	0.651		
Total	89.651	62			

Table2 : Turkey HSD Post Hoc Test for Juveniles' Feeding Habit

Comparison	Mean Difference	SE	p	95% CI Lower	95% CI Upper
T1 vs T2	-1.11	0.29	0.002	-1.88	-0.34
T1 vs T3	-1.86	0.29	< .001	-2.63	-1.09
T1 vs T4	-2.13	0.26	< .001	-2.82	-1.45
T2 vs T3	-0.75	0.33	0.115	-1.62	0.12
T2 vs T4	-1.03	0.3	0.006	-1.82	-0.23
T3 vs T4	-0.28	0.3	0.792	-1.07	0.52

Effect of Crude Oil Treatments on Juveniles' Swimming Habit of *C. gariepinus*

Similarly, the one-way analysis of variance indicated a significant effect of crude oil treatment on the swimming habit (tables 3 & 4) of the fish, with **F(3, 59) = 21.85, p < 0.001**. This finding shows that swimming behavior differed significantly among the treatment groups, and the deterioration in swimming activity corresponded with increasing crude oil concentration. Fish in the control treatment maintained active swimming behavior, whereas those in higher concentrations gradually shifted to middle and bottom dwelling, eventually becoming compacted as exposure increased. The Turkey post hoc analysis revealed that the control group differed significantly from T_2, T_3, and T_4, while T_2 and T_3 were not significantly different from each other. In addition, T_3 and T_4 showed no statistical difference, suggesting that abnormal swimming behavior had become fully established by the third treatment level and remained severe at the highest concentration. These results confirm that crude oil exposure significantly alters locomotor activity and normal swimming patterns of *C. gariepinus*, reflecting increasing physiological stress with rising concentration.

Table 3: Analysis of Variance for Juveniles' Swimming Habit

Source	SS	df	MS	F	p
Between Groups	44.171	3	14.724	21.85	< .001
Within Groups	39.766	59	0.674		
Total	83.937	62			

Table 4: Turkey HSD Post Hoc Test for Swimming Habit

Comparison	Mean Difference	SE	p	95% CI Lower	95% CI Upper
T1 vs T2	-1.11	0.3	0.002	-1.89	-0.32
T1 vs T3	-1.86	0.3	< .001	-2.64	-1.07
T1 vs T4	-1.91	0.26	< .001	-2.61	-1.22
T2 vs T3	-0.75	0.34	0.125	-1.64	0.14
T2 vs T4	-0.81	0.31	0.051	-1.61	0
T3 vs T4	-0.06	0.31	0.998	-0.86	0.75

Effect of Crude Oil Treatments on the Mortality of *Clarias gariepinus*

In contrast to the behavioral responses (table 5), the one-way analysis of variance showed that crude oil treatment did not produce a statistically significant effect on mortality during the period of observation, as indicated by **F(3, 59) = 1.38, p = 0.257**. Although some mortality was recorded in the higher treatment groups, particularly in T_4, the variation across treatments was not large enough to reach statistical significance within the acute exposure duration. Both Turkey and Dunnett post hoc comparisons confirmed the absence of significant differences (table 6) in mortality between the control and the treatment groups. This outcome suggests that mortality was not an immediate response to crude oil exposure within the timeframe considered, whereas behavioral alterations occurred much earlier and were more sensitive indicators of toxic stress. The result therefore highlights that feeding and swimming behaviors serve as early warning signs of crude oil toxicity before significant mortality becomes evident.

Table 5: Analysis of Variance for Mortality *Clarias Gariepinus*

Source	SS	Df	MS	F	p
Between Groups	4.313	3	1.438	1.38	0.257
Within Groups	61.337	59	1.04		
Total	65.651	62			

Table 6: Turkey HSD Post Hoc Test for Mortality *C. Gariepinus*

Comparison	Mean Difference	SE	p	95% CI Lower	95% CI Upper
T1 vs T2	0.07	0.37	0.997	-0.9	1.05
T1 vs T3	-0.51	0.37	0.512	-1.49	0.46
T1 vs T4	-0.48	0.33	0.457	-1.35	0.38
T2 vs T3	-0.58	0.42	0.504	-1.68	0.52
T2 vs T4	-0.56	0.38	0.467	-1.56	0.45
T3 vs T4	0.03	0.38	1	-0.98	1.03

Chi-Square Analysis of Water Quality Parameters

The Chi-square test revealed that there is a statistically significant association between treatment and dissolved oxygen levels (table 7), as indicated by the Pearson Chi-square value ($\chi^2 = 36.000$, df = 9, $p < 0.001$). Since the p-value is less than 0.05, the null hypothesis of no association is rejected. This implies that the distribution of dissolved oxygen categories varies significantly across the treatment groups.

The likelihood ratio ($\chi^2 = 33.271$, $p < 0.001$) further supports this result, confirming the robustness of the association. In addition, the linear-by-linear association ($\chi^2 = 10.623$, $p = 0.001$) indicates a significant trend, suggesting that dissolved oxygen levels change progressively with increasing treatment levels.

However, it is important to note that 100% of the cells have expected counts less than 5, which violates one of the key assumptions of the Chi-square test. This suggests that while the result is significant, it should be interpreted with caution.

Table 7: Chi-Square Test for Dissolved Oxygen

	χ^2	df	P
Pearson Chi-Square	36	9	< .001
Likelihood Ratio	33.27	9	< .001
Linear-by-Linear Association	10.62	1	0.001
N	12		

Similarly, the Chi-square analysis for pH (table 8) shows a significant relationship between treatment and pH levels ($\chi^2 = 36.000$, $df = 9$, $p < 0.001$). The null hypothesis is therefore rejected, indicating that pH distribution differs significantly among the treatment groups.

The likelihood ratio ($\chi^2 = 33.271$, $p < 0.001$) confirms the strength of this association, while the linear-by-linear association ($\chi^2 = 10.195$, $p = 0.001$) suggests a consistent trend in pH variation across treatments.

As with dissolved oxygen, all cells (100%) have expected counts below 5, indicating a violation of Chi-square assumptions. Therefore, the significance of this result should be interpreted cautiously.

Table 8: Chi-Square Test for pH

Test	χ^2	Df	p
Pearson Chi-Square	36	9	< .001
Likelihood Ratio	33.27	9	< .001
Linear-by-Linear Association	10.2	1	0.001
N	12		

The Chi-square test for alkalinity (table 9) also indicates a significant association between treatment and alkalinity levels ($\chi^2 = 12.000$, df = 3, p = 0.007). Since the p-value is less than 0.05, the null hypothesis is rejected, implying that alkalinity levels vary significantly across treatments.

The likelihood ratio ($\chi^2 = 13.496$, p = 0.004) further supports this finding, while the linear-by-linear association ($\chi^2 = 6.600$, p = 0.010) indicates a significant trend across treatment levels.

However, similar to the previous variables, all cells have expected counts less than 5, which violates the assumptions of the Chi-square test. This limitation suggests that the results, although significant, should be interpreted with caution.

Table 9: Chi-Square Test for Alkalinity

Test	χ^2	df	p
Pearson Chi-Square	12	3	0.007
Likelihood Ratio	13.5	3	0.004
Linear-by-Linear Association	6.6	1	0.01
N	12		

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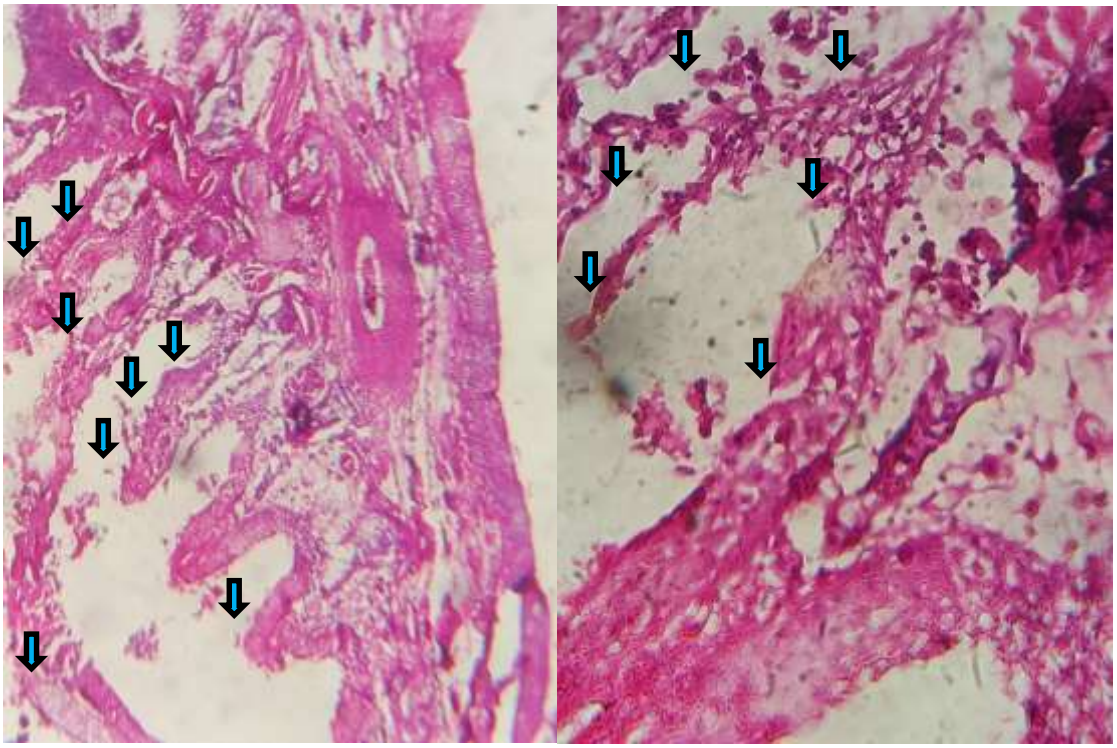
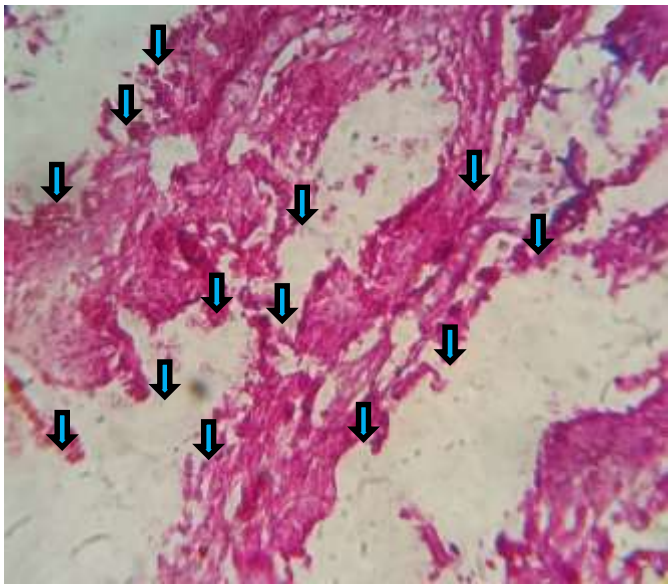
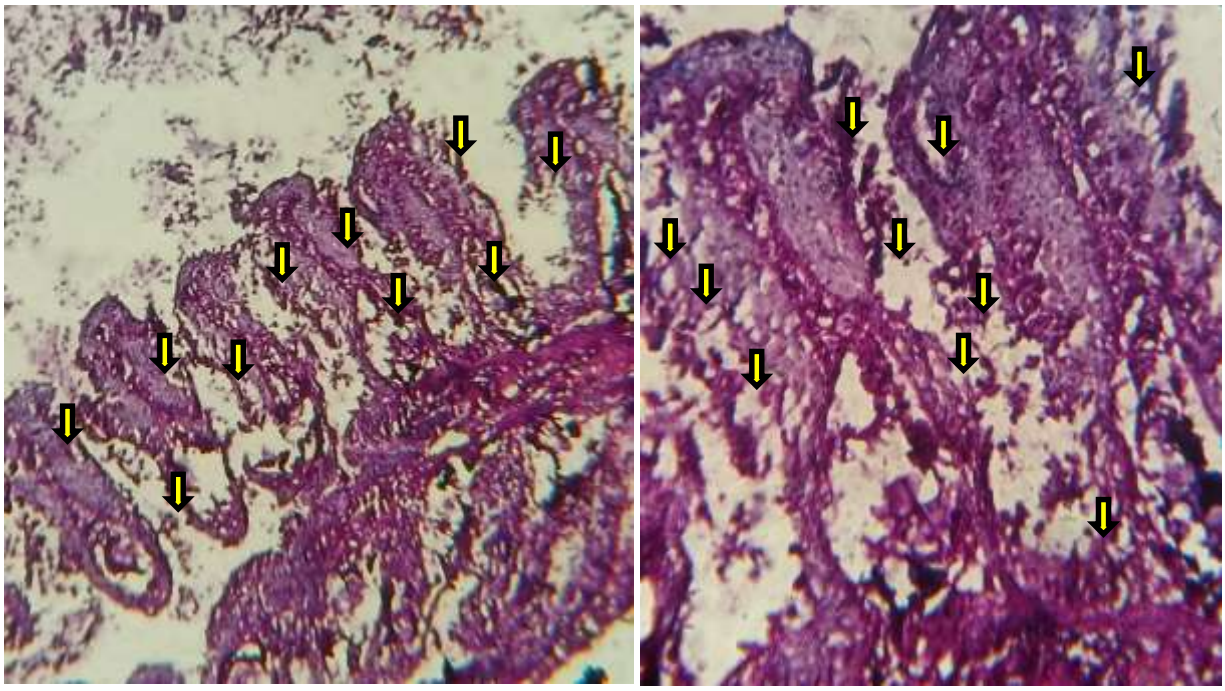
HISTOLOGY PHOTOMICROGRAPHS OF CRUDE OIL TREATMENTS ON CLARIAS GARIEPINUS



Gills T_1: Necrosis of the gill filaments (blue arrows). Hematoxylin and eosin stain. Magnification x100 and x400 respectively



Gills T_1: Necrosis of the gill filaments (blue arrows). Hematoxylin and eosin stain. Magnification x400



Gills T_2:Necrosis of the gill filaments (yellow arrows). Hematoxylin and eosin stain. Magnification x100 and x400 respectively

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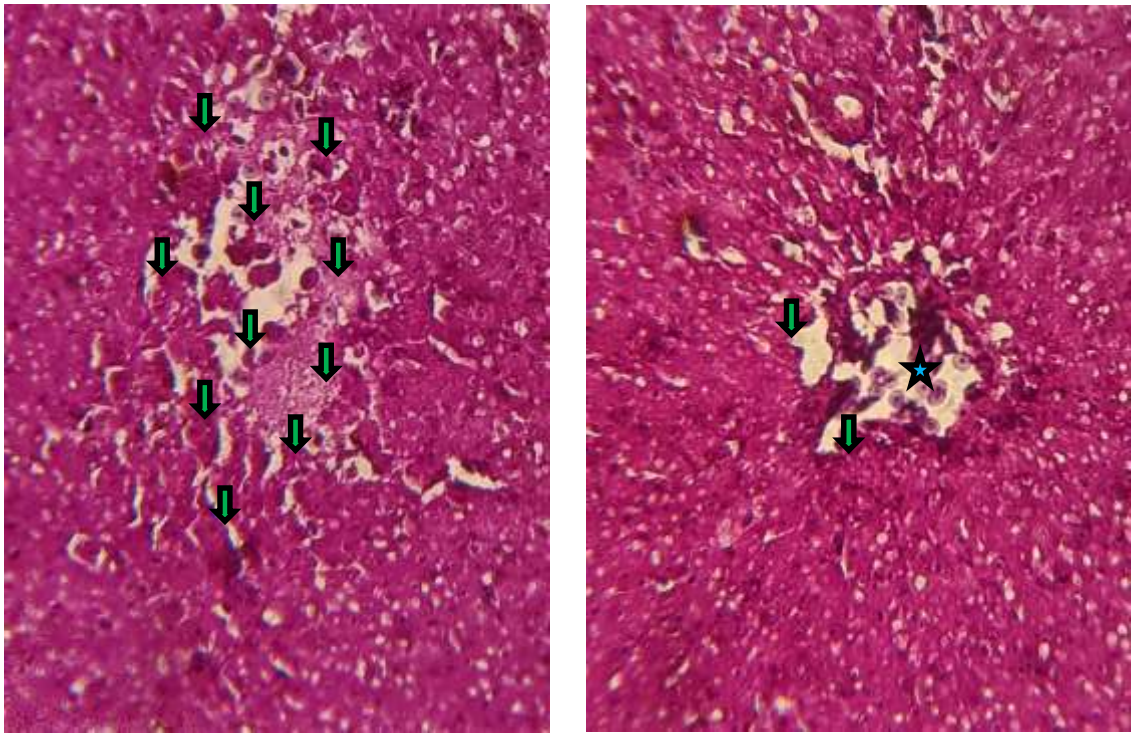
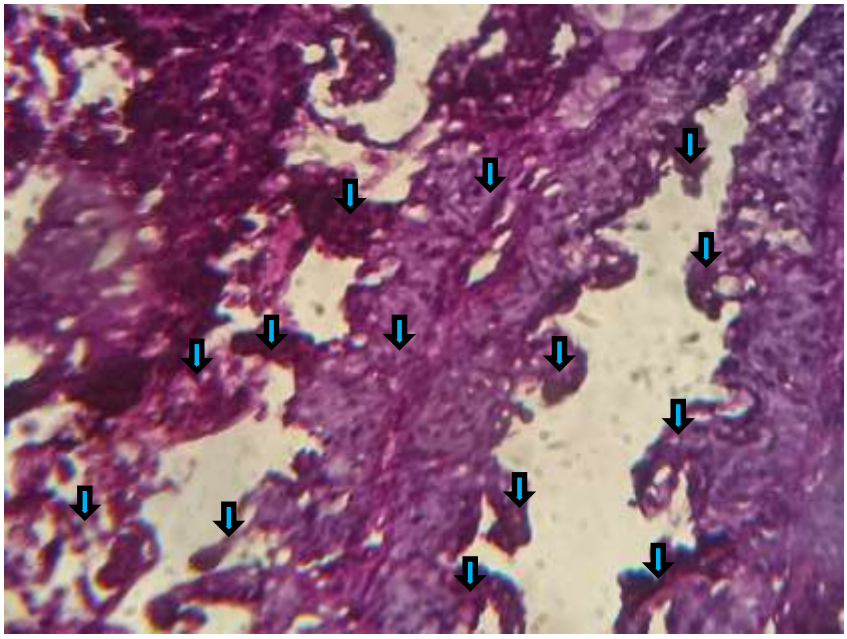
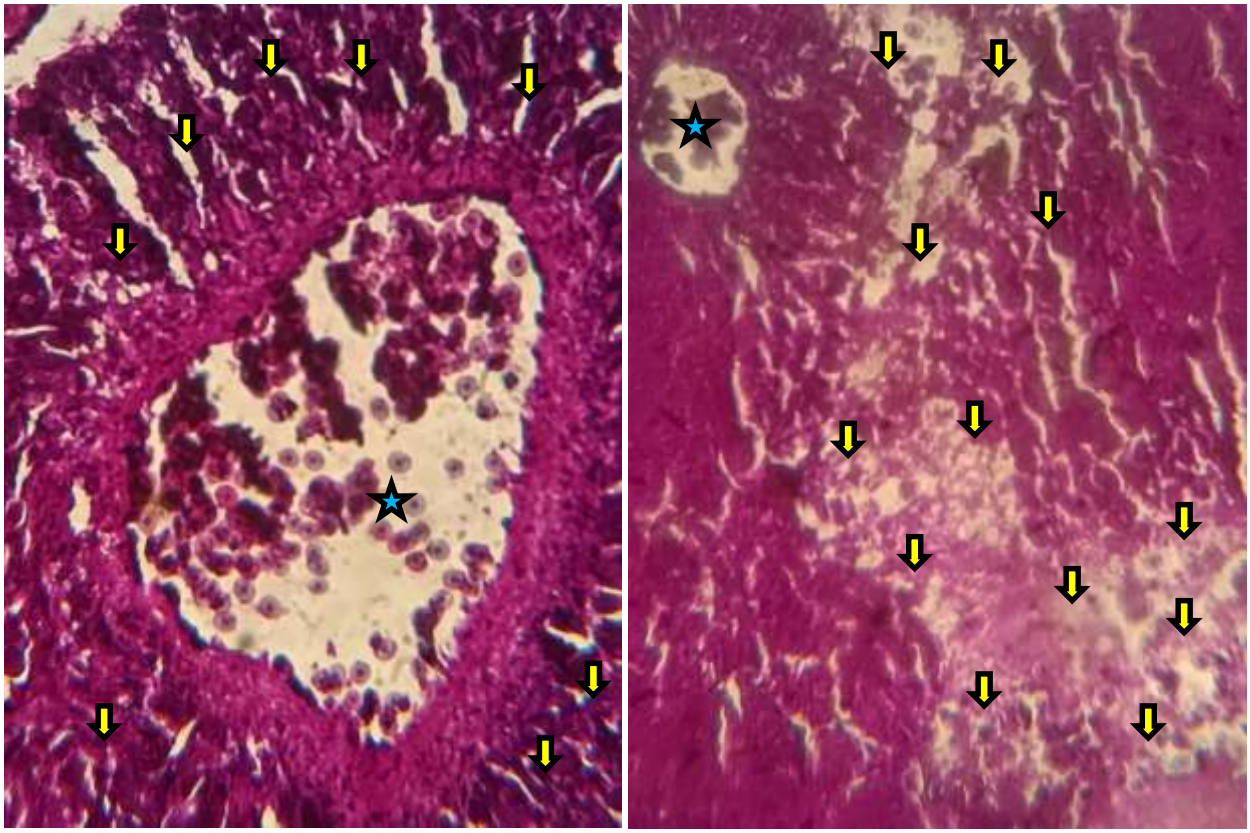


Liver T_2: Liver T4: severe necrosis of the hepatocytes (green arrows); portal vein (blue star). Hematoxylin and eosin stain. Magnification x400



Gills T_4: Necrosis of the gill filaments (blue arrows). Hematoxylin and eosin stain. Magnification x400



Liver T_4: severe necrosis of the hepatocytes (yellow arrows); portal veins (blue stars). Hematoxylin and eosin stain. Magnification x400

DISCUSSION

The present study demonstrated that crude oil exposure had a significant negative effect on the feeding habit of *Clarias gariepinus*, with feeding activity progressively declining as concentration increased. Fish in the control group maintained active feeding behavior, whereas those exposed to higher concentrations exhibited semi-active to poor feeding responses. This pattern indicates that crude oil contamination interferes with appetite, feeding drive, and possibly the metabolic efficiency of the fish. The observed impairment in feeding behavior suggests physiological stress and reduced energy acquisition, which may ultimately affect growth and survival if exposure persists.

This finding is consistent with earlier reports discussed in Chapter Two. For instance, Omoregie et al. (2017) reported that exposure to crude oil significantly altered behavioral patterns in *Clarias gariepinus*, including reduced feeding activity. Similarly, Rahman et al. (2018) noted that fish exposed to petroleum hydrocarbons exhibited noticeable behavioral stress responses, particularly loss of appetite and sluggish feeding. The reduction in feeding activity observed in this study therefore aligns with the documented behavioral disruptions associated with hydrocarbon toxicity. Furthermore, Nwamba et al. (2022) explained that hydrocarbon contamination often leads to hypoxic conditions due to reduced dissolved oxygen levels, which can suppress metabolic processes and reduce feeding efficiency. This mechanism provides physiological support for the present findings, as stressed fish typically prioritize survival over feeding.

The results also agree with the concept of chronic and sub-lethal toxicity described in the literature review. According to Omoregie and Oyebanji (2023), sub-lethal crude oil exposure can lead to growth retardation and organ dysfunction, both of which are closely linked to reduced feed intake. Feeding inhibition may occur as a protective response to stress, or as a consequence of impaired gill function and reduced oxygen uptake, which limits the energy available for normal activities. Additionally, crude oil contains polycyclic aromatic hydrocarbons (PAHs) and other toxic components (Adebayo et al., 2021) that may disrupt endocrine and neurological regulation of appetite, further contributing to reduced feeding.

The progressive worsening of feeding behavior across treatments in this study suggests a concentration-dependent toxic effect, which is in agreement with Kumar et al. (2019), who reported that the severity of toxic responses in fish increases with pollutant concentration. The absence of significant difference between the highest treatment levels in the post hoc test suggests that feeding impairment may reach a threshold beyond which additional concentration increases produce minimal additional behavioral change. This plateau effect has also been observed in toxicological studies where severe stress responses stabilize once maximum physiological disruption is reached.

Importantly, while mortality did not differ significantly across treatments during the observation period, feeding behavior showed a strong and significant alteration. This supports the literature assertion that behavioral indices are more sensitive early indicators of toxicity than mortality endpoints (Rahman et al., 2018). Therefore, the findings of this study strongly align with previous research presented in Chapter Two, confirming that crude oil exposure significantly disrupts feeding behavior in *Clarias gariepinus* and that behavioral responses serve as reliable early markers of aquatic toxic stress.

CONCLUSION

This study clearly demonstrates that crude oil exposure has a significant adverse effect on the behavioural responses of *C. gariepinus*, particularly in terms of feeding and swimming habits. The findings revealed a consistent, concentration-dependent decline in feeding activity and a marked alteration in swimming behaviour as crude oil concentration increased. These behavioural disruptions indicate that crude oil contamination induces physiological stress, impairs normal biological functions, and interferes with the overall well-being of the fish.

Furthermore, the study established that behavioural parameters such as feeding and swimming habits are more sensitive and reliable early indicators of toxic stress than mortality. Although some mortalities were observed at higher concentrations, the differences were not statistically significant within the exposure period, suggesting that lethal effects may require longer exposure durations to manifest. Therefore, sub-lethal effects occur earlier and can serve as critical warning signals of environmental pollution.

Recommendations

Based on the findings of this research work, the following recommendations are made:

- Regulatory bodies should enforce stricter controls on crude oil exploration, transportation, and discharge to prevent contamination of aquatic ecosystems.
- Continuous monitoring of water quality in oil-producing and oil-impacted areas should be carried out to detect early signs of pollution using behavioural indicators in fish.
- Feeding and swimming behaviours should be adopted as standard for early-warning biomarker on environmental impact assessments and toxicological studies.
- Oil companies and relevant stakeholders should implement effective spill prevention strategies and rapid response mechanisms to minimize environmental damage.
- Communities in oil-producing regions should be educated on the dangers of crude oil pollution and the importance of protecting aquatic ecosystems.
- Future studies should focus on long-term (chronic) exposure effects, including growth performance, reproductive capacity, and physiological changes in *C. gariepinus* and other aquatic species.

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