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HISTOPATHOLOGICAL RESPONSES IN LIVER, BRAIN AND GILLS OF *Oreochromis niloticus* JUVENILES EXPOSED TO WATER SOLUBLE FRACTIONS OF CRUDE OIL

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ABSTRACT

Aquatic pollution has been shown to induce different changes ranging from biochemical alterations in single cells to changes in whole populations. In this study, qualitative analyses of liver, brain and gills histopathological parameters were evaluated in *Oreochromis niloticus* after exposure to water-soluble fractions of crude oil. After preliminary short-term (96 hours) bioassay, fish were subjected to four sub-lethal concentrations (30, 45, 60, and 75 % of the LC₅₀ corresponding to 28, 41, 55, and 69 mg/l respectively), and a control group using a semi-static renewal method for 90 days. After the exposure period, the liver, brain and gills were harvested, labeled and prepared for photomicrography using standard procedure. Results showed atrophy and necrosis of hepatocytes and degeneration of hepatopancreas in the liver. The histopathological alterations in the brain included mild congestion of cerebral blood vessels, widespread vacuolation of neuropil, necrosis and atrophy of neurons. In the gills, congestion of capillaries, atrophied and necrotic lamellae were detected. Overall, the study revealed that histopathological biomarkers can be relied upon as a suitable tool for accessing long-term impacts of crude oil spillage in the aquatic environment. Therefore, an immediate multi-agency response approach for efficient and effective management of oil spills is recommended.

Keywords: Crude oil, water pollution, fish organs, biomarkers, *Oreochromis niloticus*.

INTRODUCTION

Aquatic pollution is the release of substances from humans into the aquatic environment, which has detrimental effects on living resources, risks to human health, impediments to marine activities like fishing, deterioration of seawater quality,

and reduction of infrastructure (Kumar and Prasannamedha, 2021). An important component of the Sustainable Development Goals (SDG - 14) is the sustainable management and protection of marine and coastal ecosystems from pollution. In Nigeria, aquatic pollution is a serious

problem and it is majorly caused by a variety of sources such as industrial effluent, agricultural runoff, solid waste and oil spills (Akankali and Elenwo, 2015; Eriegha and Sam, 2020; Olufunke, 2022). These pollutants alter the water's properties, thereby affecting the water quality, biodiversity and productivity. Oil spills are endemic to Nigeria's Niger Delta and may occur as a result of pipeline leakage, pipeline rupture, accidental discharges, engineering failure, bunkering (illegal refining of the crude oil) or vandalism (Ayuba, 2012). The exposure of the aquatic environment to oil spills has been shown to pose significant threats to aquatic organisms, particularly fishes, and distortion of ecological balance. The impact of oil spills on the environment has been reported by many investigators (Eriegha and Sam, 2020; Onyena and Sam, 2020). They are acutely lethal in concentrations in a concentration of a few parts per million (ppm) and chronically lethal in sublethal concentrations in parts per billion (ppb) although animals vary widely in their sensitivity (Almeda *et al.*, 2013; Barron *et al.*, 2020). Oil spills like most other sources of pollutants have been shown to cause death of organisms, disrupt biochemical pathways, metabolites, and enzyme systems in their cells and tissues, cause physical damage to their tissues and organs, and changes in chromosomes and genes in gametes, embryonic developments and growth (Eriegha *et al.*, 2017; Eriegha *et al.*, 2019; Eriegha and Sam, 2020; Eriegha, 2023; Nwabueze and Agbogidi, 2010; Olaifa, 2012). The determination of the health status of fish species following exposure to pollutants forms an important aspect of aquatic ecosystem studies (Feist *et*

al., 2015). Histopathological assessment is a common tool for environmental monitoring and assessment. This is because many toxicants are capable of damaging specific cells, tissues, and organs such as the gills, kidneys, brain, intestines, liver, lungs, and gonads of fish (Rohani, 2023; Vali *et al.*, 2022).

The Nile tilapia (*Oreochromis niloticus*) is an economically important freshwater fish (Munguti *et al.*, 2022) and has received the most research. It is popular for its fast growth rate, easy handling, omnivorous nature, hardiness, low dissolved oxygen tolerance, and good taste. Nile tilapia is a common model organism in the field of ecotoxicology and is suitable for monitoring toxin-induced changes in fish health (Eriegha *et al.*, 2017; Hasan *et al.*, 2022a, 2022b). Several studies have addressed the effect of crude oil on the different organs via histopathological assessment in *O. niloticus*. However, in most previous research, very little information is provided on the water quality characteristics after exposure to crude oil, causing these histopathological alterations. Such information is essential in making comparisons of the toxicities of various petroleum products to different aquatic organisms. This study focused on the histopathological responses in the liver, brain, and gill in *O. niloticus* after exposure to sublethal concentrations of water-soluble fractions of crude oil. In addition, crude oil-induced changes in water quality parameters were observed.

MATERIALS AND METHODS

Experimental fish

Healthy Nile tilapia juveniles with a good physical appearance were purchased

from a reputable fish farm and transported to the laboratory in oxygen bags. The average body weight of the tilapia was 8.6 g. The experimental fish were first kept in the laboratory for 2 weeks in natural photoregime conditions in 30 L experimental tanks containing 20 fish each. The experimental tanks were filled with 20 L of the test solution and covered with a lid made of fine polyethylene gauze screen of 1mm mesh size to prevent the fish from jumping out of the containers. They were fed with commercial feed, Coppens® *ad libitum* two times a day during the acclimation period.

Exposure Assessment

The juveniles of Nile tilapia were treated with four different sub-lethal doses (30, 45, 60 and 75% of the LC₅₀ corresponding to 28, 41, 55, and 69 mg/l respectively) of WSF of crude oil and a control group containing clean water for 90 days. The selected concentrations were based on previous research on tilapia (Eriegha *et al.*, 2017). The test was performed using a semi-static renewal method in which the exposure medium was exchanged every 3 days to maintain the strength of the toxicant and minimize the level of ammonia. The experimental fish were fed 2 times a day. After 90 days of treatment, fish were sacrificed to collect liver, brain, and gill from each aquarium. Soon after the collection, organs were fixed in Bouin's fluid for 24 h. The tissues were preserved in 70% alcohol for histopathological procedures.

Histopathology

Histopathology was carried out following the method of Ibrahim *et al.* (2014) with little modification. The

preserved liver, brain, and gills were dehydrated in ethanol and cleared using pure chloroform. After that, the tissues were embedded in paraffin wax with a melting point of 60–70 °C, and blocks were prepared for sectioning. The blocks were cut maintaining about 5 µm width and were fixed on slides. The samples were then left for air drying the whole night. Finally, the samples were stained with Eosin and Hematoxylin and mounted in DPX for extending their durability. The stained slides were studied under a digital light microscope at 400 × magnifications, and photographs were taken by a digital microscopic camera.

Water Quality Analysis

Water quality parameters and heavy metals concentrations of the experimental setup such as pH, alkalinity, chloride, conductivity, salinity, total hardness, calcium, magnesium, total dissolved solids, total solids, biochemical oxygen demand, chemical oxygen demand, turbidity, nitrate, sulphate and phosphate were monitored periodically using standard methods (APHA, 2017).

Data Analysis

Information on the water quality properties of the culture conditions of the experimental fish was expressed as mean ± SD (standard deviation). Mean values were compared using one-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test using the software, SPSS Version 22.

RESULTS

The results of the physico-chemical characteristics of water samples from the

various concentrations of crude oil used for the bioassay are presented in Table 1. Chloride, conductivity, salinity, magnesium, biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity, and Nitrate levels increased with an increase in the volume of crude oil introduced in culturing systems. A sharp increase in COD levels was observed upon the introduction of

crude oil as the control had 110.50 ± 3.54 mg/l whereas the minimum concentration had 209.00 ± 8.49 mg/l. Also, BOD levels in the various test solutions were significantly ($P < 0.05$) higher than the BOD levels of the control experiment. pH, total solids, and sulphate levels decreased with an increase in the concentration of crude oil.

Table 1: Values of Physico-chemical parameters during exposure of juvenile *O. niloticus* to water soluble fractions of Crude oil (Mean $\pm$ Standard deviation).

Indices	Concentration of WSF of Crude Oil				
	0% (Control)	30%	45%	60%	75%
pH	6.89 ± 0.02^b	6.88 ± 0.01^{ab}	6.74 ± 0.15^{ab}	6.70 ± 0.01^{ab}	6.57 ± 0.10^a
Alk	131.00 ± 1.41^a	130.00 ± 1.44^a	126.50 ± 6.36^a	127.50 ± 3.54^a	127.00 ± 2.12^a
Cl	7.88 ± 0.09^a	8.20 ± 0.08^{ab}	8.47 ± 0.11^{bc}	8.69 ± 0.07^{cd}	8.79 ± 0.06^d
Cond	596.50 ± 0.71^a	606.50 ± 3.54^{ab}	620.50 ± 10.61^{bc}	631.00 ± 1.41^c	652.50 ± 2.12^d
Sali	0.21 ± 0.01^a	0.26 ± 0.01^b	0.26 ± 0.00^b	0.28 ± 0.01^b	0.31 ± 0.01^c
TH	107.50 ± 3.54^a	114.50 ± 0.71^a	124.00 ± 1.41^b	129.00 ± 1.41^{bc}	134.50 ± 0.71^c
Ca	40.65 ± 0.50^{ab}	42.00 ± 0.71^b	42.55 ± 0.64^b	41.25 ± 0.35^{ab}	39.75 ± 0.35^a
Mg	2.81 ± 0.07^a	2.99 ± 0.07^a	3.38 ± 0.04^b	3.61 ± 0.09^{bc}	3.91 ± 0.08^c
TDS	323.00 ± 5.66^c	316.00 ± 2.83^c	293.00 ± 4.24^b	280.50 ± 6.36^{ab}	265.50 ± 4.95^a
TS	507.50 ± 3.54^a	546.00 ± 5.66^b	779.00 ± 15.56^e	710.00 ± 1.41^d	620.50 ± 6.36^c
BOD	7.99 ± 0.15^a	9.60 ± 0.14^b	10.65 ± 0.21^c	11.40 ± 0.14^d	11.85 ± 0.07^d
COD	110.50 ± 3.54^a	209.00 ± 8.49^b	216.00 ± 8.49^b	230.00 ± 1.41^b	255.00 ± 4.24^c
Turb.	3.88 ± 0.04^a	6.05 ± 0.35^b	7.32 ± 0.45^{bc}	8.25 ± 0.35^c	9.69 ± 0.27^d
NO ₃ -N	2.46 ± 0.42^a	4.75 ± 0.34^b	6.02 ± 0.40^b	7.60 ± 0.28^c	8.74 ± 0.27^c
SO ₂ ⁻⁴	9.30 ± 0.13^c	9.49 ± 0.02^c	8.43 ± 0.11^b	7.85 ± 0.35^{ab}	7.05 ± 0.21^a
PO ₄	1.23 ± 0.02^a	1.31 ± 0.01^a	1.25 ± 0.06^a	1.26 ± 0.01^a	1.20 ± 0.03^a

*Means with different superscripts in the same row are significantly different ($p < 0.05$). Where: Alk= Alkalinity (mgCaCO₃/L), CL= Chloride (mg/L), Cond.= Conductivity (μ homs/cm), Sal= Salinity (g/kg), TH= Total Hardness (mgCaCO₃/L), Ca= Calcium (mg/L), Mg= Magnesium (mg/L), TDS= Total Dissolved Solids (mg/L), TS= Total Solids (mg/L), BOD= Biochemical Oxygen Demand (mg/L), COD= Chemical Oxygen Demand (mg/L), Turb= Turbidity (FTU), NO₃-N= Nitrate (mg/L), SO₂⁻⁴= Sulphate (mg/L) and PO₄ = Phosphate (mg/L).

The results of heavy metal concentrations and total petroleum hydrocarbon content of the water used for the bioassay are presented in Table 2. Total petroleum hydrocarbon was not detected in the control water sample but its value increased from 161.5 mg/l in the minimal concentration of oil to 268 mg/l in the highest concentration. A corresponding increase in values of heavy metals detected with increasing concentrations of WSFs of

crude oil was also observed. However, zinc was barely detected in water samples from the two highest concentrations of the oil. Increasing demand for petrochemicals has led to increased concentrations of petroleum hydrocarbons in the environment. Values obtained for Total Petroleum Hydrocarbon (TPH) increased significantly ($P < 0.05$) as the concentration of the WSF of the crude oil increased.

Table 2. Total Petroleum Hydrocarbon and Heavy metals content (mg/L) of water treated with water- soluble fraction of crude oil (Mean $\pm$ Standard deviation).

Parameters	Concentration of WSF of crude oil				
	0%	30%	45%	60%	75%
TPL	ND	161.5 $\pm$ 4.95 ^a	197.00 $\pm$ 1.41 ^b	200.00 $\pm$ 5.67 ^c	268.00 $\pm$ 4.24 ^d
Pb	ND	0.05 $\pm$ 0.00 ^a	0.09 $\pm$ 0.00 ^b	0.14 $\pm$ 0.01 ^c	0.18 $\pm$ 0.00 ^d
Cu	ND	0.04 $\pm$ 0.01 ^a	0.06 $\pm$ 0.00 ^b	0.08 $\pm$ 0.01 ^{bc}	0.09 $\pm$ 0.01 ^c
Cd	ND	0.04 $\pm$ 0.00 ^a	0.07 $\pm$ 0.01 ^b	0.09 $\pm$ 0.00 ^c	0.11 $\pm$ 0.01 ^c
Cr	ND	0.02 $\pm$ 0.00 ^a	0.04 $\pm$ 0.01 ^a	0.08 $\pm$ 0.01 ^b	0.09 $\pm$ 0.00 ^b
Ni	ND	0.02 $\pm$ 0.00 ^a	0.05 $\pm$ 0.01 ^b	0.06 $\pm$ 0.01 ^b	0.08 $\pm$ 0.00 ^c
Fe	0.23 $\pm$ 0.00 ^a	0.42 $\pm$ 0.01 ^b	0.48 $\pm$ 0.01 ^c	0.53 $\pm$ 0.02 ^{cd}	0.57 $\pm$ 0.01 ^d
Zn	ND	ND	ND	ND	ND

* Means with different superscripts in the same row are significantly different ($p < 0.05$). where: TPL=total petroleum hydrocarbon, Pb=lead, Cu=copper, Cd=cadmium, Cr=chromium, Ni=nickel, Fe=iron, Zn=zinc

The photomicrographs of liver of *O. niloticus* after exposure to WSF of crude oil are presented in Figure 1. Alterations observed in the liver of *O. niloticus* were coagulation necrosis of zone 1 (periportal) hepatocytes and melano-macrophages with lipofuscin pigments, severe centrilobular necrosis and atrophy of hepatocytes, diffuse

degeneration and necrosis of hepatocytes and degeneration of hepatopancreas. These alterations were more severe with increasing crude oil concentrations. Unexposed *O. niloticus* revealed that the hepatic plates are closely packed and the hepatocytes generally have finely reticulated cytoplasmic appearance.

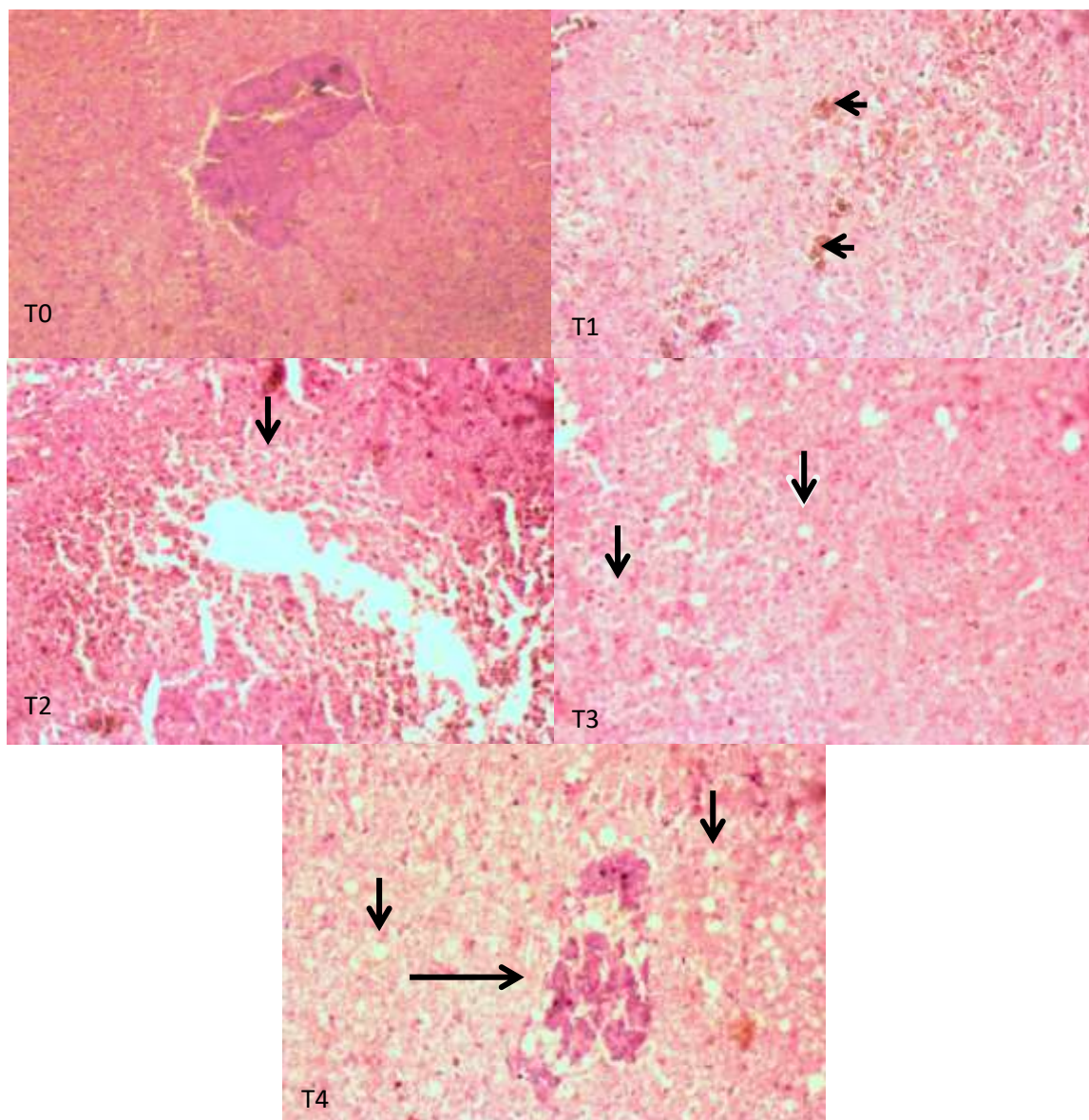


Figure 1. Photomicrographs of the Liver of *O. niloticus* exposed to water-soluble fraction of crude oil of Crude oil. Photomicrographs of the Liver of *O. niloticus* exposed to water-soluble fraction of crude oil of Crude oil. Where: T0 - Hepatic plates closely-packed with hepatocytes generally finely reticulated cytoplasmic appearance. There is marked congestion of hepatic sinusoids. T1 - coagulation necrosis of zone 1 (periportal) hepatocytes and melano-macrophages (short arrows) with lipofuscin pigments; T2 - There is severe centrilobular (zone 3) necrosis and atrophy of hepatocytes (short arrows); T3 - There is diffuse degeneration and necrosis of hepatocytes (medium-sized arrows); T4 - There is diffuse degeneration and necrosis of hepatocytes (medium-sized arrows), and degeneration of hepatopancreas (long arrow) HE x400.

Photomicrographs of the brain of juvenile *O. niloticus* after exposure to WSF of crude oil are presented in Figure 2. Observable lesions were multiple focal of neuronal necrosis, mild congestion of cerebral blood vessels, moderate diffuse

gliosis, widespread vacuolation of neuropil, Angulation, necrosis, and atrophy of neurons with a few gliosis. The intensity of lesions increased with increasing levels of WSF of crude oil.

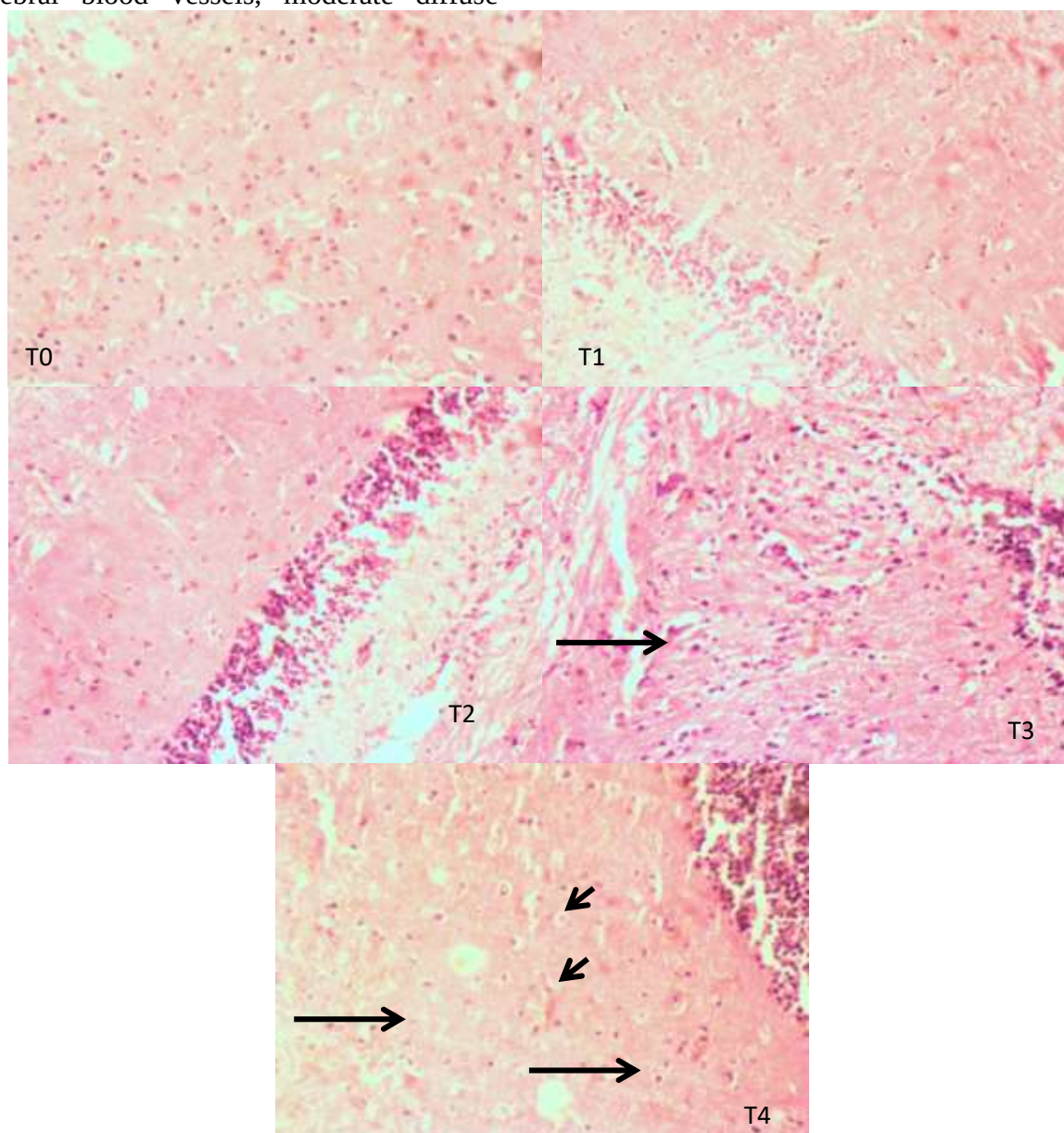


Figure 2: Photomicrographs of the Brain of *O. niloticus* exposed to water-soluble fraction of crude oil. Where: T0 - Neurons and neuropil appear normal; T1 - There are multiple focal of neuronal necrosis. There is mild congestion of cerebral blood vessels; T2 - There are multiple focal of neuronal necrosis. There is mild congestion of cerebral blood

vessels; T3 - Moderate diffuse gliosis (long arrows), are multiple focal of neuronal necrosis. There is widespread vacuolation of neuropil. There is mild congestion of cerebral blood vessels; T4 - Angulation, necrosis and atrophy of neurones (medium-sized arrows) with a few gliosis (long arrows) H&E X400.

Histopathological alterations on the gills of juvenile *O. niloticus* after exposure to WSF of crude oil are presented in Figure 3. Observable alterations after exposure to crude oil were

moderate hyperplasia of secondary lamellae, congestion of capillaries, focal atrophy of secondary lamellae, moderate diffuse lamellae hyperplasia, and atrophied and necrotic lamellae.

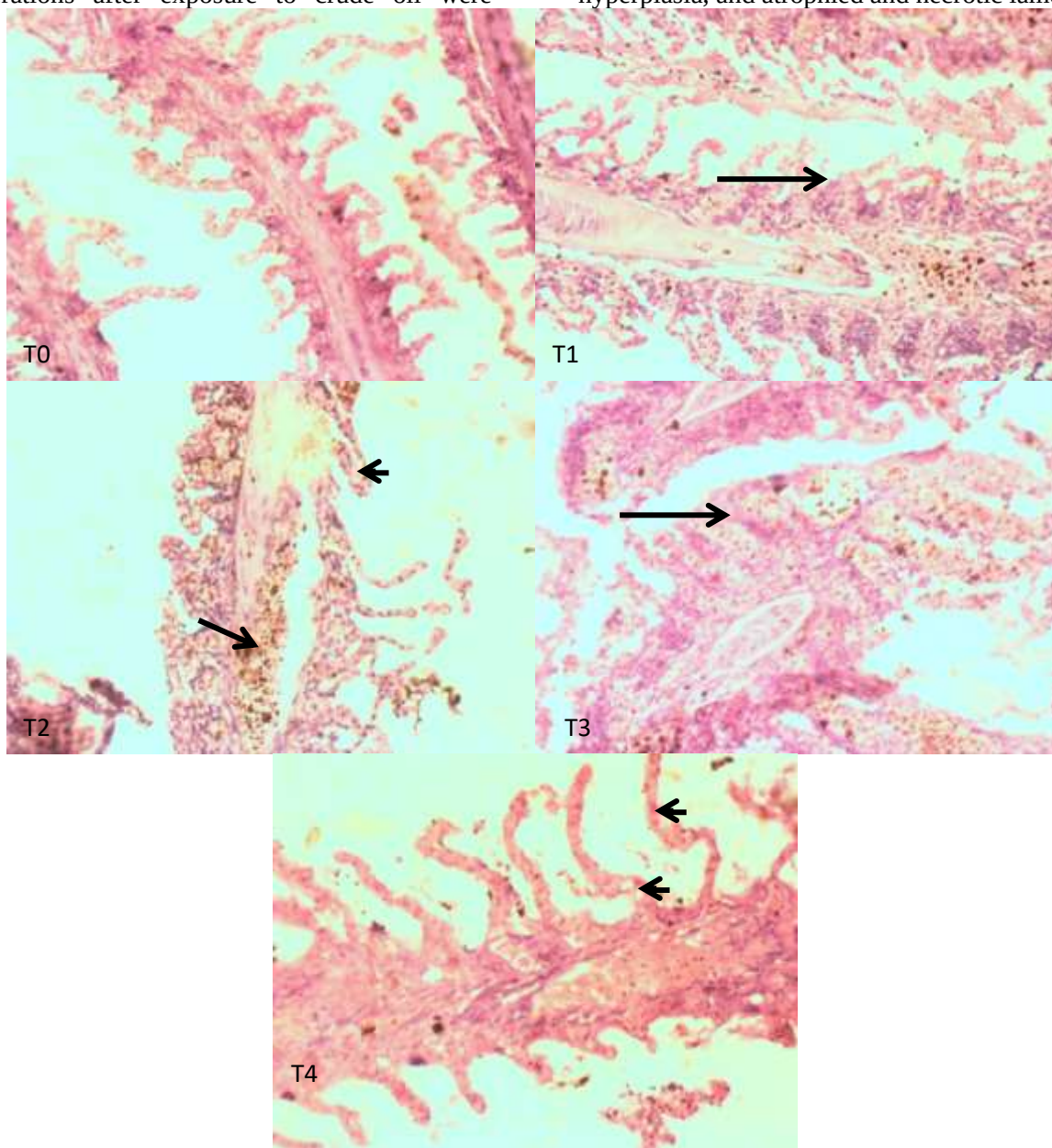


Figure 3: Photomicrographs of the Gill of *O. niloticus* exposed to water-soluble fraction of crude oil. Where: T0- No observable lesion; T1- Moderate hyperplasia of secondary lamellae (long arrows); T2- There is congestion of capillaries (medium-sized arrows) and focal atrophy of secondary lamellae (short arrow); T3- There is capillary congestion and moderate diffuse lamellae hyperplasia (long arrow); T4- The lamellae are atrophied and necrotic (short arrows) H&E x400.

DISCUSSION

A large number of studies have shown that the WSF of crude oil can reduce water quality conditions and thereby the overall well-being of aquatic organisms (Eriegha *et al.*, 2019; Santos *et al.*, 2016). Dissolved oxygen is considered an important water quality parameter in the assessment of the degree of pollution in natural water (Mahmoud *et al.*, 2008). Therefore, the reduced DO levels with increasing concentration of WSF of the crude oil could be attributed to biological processes, respiration, and oxidation of the organic matter in water and sediments (Tayel *et al.*, 2008). Many factors such as temperature, salinity, amount of mixing between air and water, pH, photosynthesis activity of phytoplankton, submerged plant and aeration by living organisms, decomposition of organic matter, and pollution affect the amount of oxygen in natural water (Das and Acharya, 2003). The obvious inverse relationship that existed between BOD and DO levels is explainable, given the likely biological activity that could accompany DO usage (Obasohan *et al.*, 2011). This observation supported earlier reports by Eriegha *et al.* (2019) and Nwabueze and Agbogidi (2010). Temperature is a significant water quality variable in aquatic systems and it is a key parameter that influences the physical, chemical, and biological transformations in the aquatic environment (Tayel *et al.*, 2008). The temperatures recorded during this study

were within range for tropical fish (Ovie and Ovie, 2014). The significant reduction in values of Total Dissolved Solids (TDS) observed in this study suggested that it can be used as a surrogate indicator of general water quality. The water becomes progressively unacceptable as TDS rises (Moran, 2018). The increase in conductivity levels may be attributed to the high content of dissolved ions and cations occasioned by the introduction of the WSF of the oil. High heavy metal concentrations in waters of Nigeria's Niger Delta are often products of pollutants arising from crude oil explorations, anthropogenic wastes, and other industrial and domestic sewages that find their way through various channels (Brades *et al.*, 2004). The absence of zinc in this investigation was consistent with the findings of Nwajei *et al.* (2014) in samples obtained from a Niger Delta creek. Olaifa (2012), however reported zinc as the most abundant heavy metal in water treated with WSF of crude oil. In general, values of heavy metals obtained in this study were lower than the composition and proportion reported by Okojie *et al.* (2012) for sediments collected from Ijana River. This variation may be attributed to the adsorption and accumulation of metals in bottom sediments as the latter being repositories of heavy metals.

The occurrence of necrosis in the liver of fish after exposure to crude oil was a direct reflection of the amount of xenobiotics present during the detoxification

process. Necrosis in some areas of liver tissue may be due to the excessive work required by the fish to remove toxins during the detoxification process by the liver (Ayoola, 2008; Olufayo and Alade, 2012). The vacuolation of the hepatocytes might indicate an imbalance between the rates of synthesis of substances (Topić Popović *et al.*, 2023). Oxygen deficiency as a result of gill degeneration is the most common cause of cellular degeneration in the liver (Mohamed, 2001). The studies of Thophon *et al.*, 2003 and Hassan *et al.* (2022b) also reported that the liver plays a major role in detoxification and biochemical transformation. Therefore, an aberration in the liver cells may be a strong indication of the presence of pollutants in the water. The liver is also one of the most susceptible organs affected by the water-soluble fraction of crude oil. Due to its high sensitivity to pollutants, the liver can be used as an indicator for environmental monitoring. Thus, changes in its structure can be used in the assessment of the well-being of fish. Histopathological changes observed in the brain agreed with those of Eriegha *et al.* (2019) that reported severe lesions in the brain after exposure to toxicants. Different regions in the fish brain are concerned with various kinds of important roles. The hindrance of tissue of a region in the brain by these obsessive changes may lead to the decrease of the specific function in fish. This alters the physiological and behavioral elements of the fish (Ahmad *et al.*, 2021).

Histopathological alterations such as inflammation, oedema of cells, and severe cytomias of cells and oedema have also been reported by Nwakanma and Hart (2013) in the brain of *P. barbarus* after exposure to

oil-based mud. Ayoola (2008) documented spongiosis, severe congestion, and mononuclear infiltration in *O. niloticus* exposed to glyphosate herbicide. Other studies also reported diverse histopathological alterations in the brain tissue of fish after exposure to xenobiotics. Mild vacuolar changes in the cerebrum have also been reported in *Labeo rohita* after exposure to hexachloro cyclohexane (Basanta and Subhas, 2000). Ayoola and Ajani (2008) also documented mononuclear invasion, neuronal degeneration, and spongiosis in the brain of *C. gariepinus* after exposure to cypermethrin. The histopathological alterations in the gills were very pronounced and produced convincing evidence that the WSF of crude oil could pose a serious health challenge to fish. This may be because the gills remain in direct contact with pollutants in the water and are the main route for absorption of toxins contained in the water (Mishra and Mohanty, 2008; Hassan *et al.*, 2022b). Crude oil hydrocarbons have also been shown to stimulate structural abnormalities in the branchial respiratory epithelium (Rosety-Rodríguez *et al.*, 2002). Histopathological alterations observed in this study could lead to a general reduction in the efficiency of gill filaments to aid in the diffusion of oxygen across the gill lamellae resulting in the development of a hypoxic condition within the fish (Elahee and Bhagwant, 2007; Eriegha *et al.*, 2017). Apart from the impairment of respiratory functions, observable lesions in the gills also tend to impair osmoregulatory functions (Tang and Au, 2004).

CONCLUSION

The present study has demonstrated that crude oil pollution causes histopathological alterations in the liver, gills, and brain of *Oreochromis niloticus*. Histopathological findings can thus be used to predict contaminant exposure, particularly for sub-lethal and chronic effects. The findings also demonstrated that the concentration of toxicant determined the degree of tissue change and alterations. However, because changes in gene expression associated with toxicity are frequently more sensitive and characteristic of the toxic response than pathology endpoints, more research on genotoxicity testing should be conducted. Stringent measures to prevent new oil spills and effective remedial methods to cushion the effects of such spills are strongly advised.

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