WATER SOLUBLE FRACTIONS OF CRUDE OIL DETERIORATES WATER QUALITY PARAMETERS AND ALTERS HISTOPATHOLOGICAL COMPONENTS OF JUVENILE *CLARIAS GARIEPINUS*

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ABSTRACT

In this study, the water quality changes after the introduction of water soluble fractions of crude oil were monitored along-side the accompanying effects on histopathological changes in selected organs of juvenile Clarias gariepinus. After a preliminary short-term (96 hours) static toxicity tests, fish were exposed to four sub-lethal concentrations (30, 45, 60 and 75 % of the LC_{50} corresponding to 67, 101, 135 and 169 mg/l respectively) and a control group containing clean water using a semi-static renewal method for 90 days. Water quality parameters, heavy metals and total petroleum hydrocarbon (TPH) were determined using standard methods. After the exposure period, liver, brain and gill were harvested, labeled and prepared for photomicrography. Chloride, conductivity, salinity, magnesium, biochemical oxygen demand, chemical oxygen demand, total dissolve solids, turbidity and nitrate levels increased significantly ($p<0.05$) with increasing concentration of water soluble fractions (WSF) of the crude oil. Temperature readings and phosphate levels were however not affected ($p>0.05$). Values of TPH increased ($p<0.05$) with increasing concentrations of the crude oil. All analysed heavy metals followed a direct proportional trend as their values increased ($p<0.05$) alongside concentrations. Several histopathological alterations were found in the liver (sinusoidal congestion, atrophy of hepatocytes, hepatocellular degeneration and diffuse coagulation necrosis of hepatocytes), brain (vascular congestion and atrophy of neurons, neuronal necrosis, gliosis and loss of neurons) and gill (moderate loss of the secondary gill lamellae, moderate lamellae atrophy and moderate diffuse lamellae hyperplasia). The intensity of these lesions increased with increase in exposure dose.

Keywords: Toxicity, Petroleum, Heavy metals, Water quality, Organs, *Clarias gariepinus*, LC_{50}

INTRODUCTION

Oil pollution is a growing problem devastating to coastal wildlife especially in areas where activities of crude oil exploration, exploitation and transportation are common. When petroleum mixes with water, it undergoes physical and chemical processes such as evaporation, dissolution, emulsion, photolysis and biodegradation which eventually generate water-soluble fractions (Pérez-Cadahía *et al*., 2004). The Water Soluble Fraction (WSF) of crude oil is that small fraction of oil containing components fully or sparingly soluble in water. Since aquatic organisms directly encounter it, WSF plays an important role in the toxicity of crude oil in aquatic environments (Lari *et al*., 2015). Toxicity tests are bioassays, in which test organisms are exposed, in a laboratory condition, to various concentrations of chemical

toxicants, or dilutions of whole effluents (Odiete, 1999). Organisms exposed to WSF of crude oil take up the dissolved hydrocarbon and react to their effects. The severity of the effects depends on the duration of exposure, the concentration of the components and mode of exposure.

Toxicity tests allow the determination of effects of xenobiotic compounds, providing direct evidence of the biological responses of organisms to contaminants. Due to the fact that organisms from different species vary in their sensitivity towards chemical substances, it is difficult to set standards for protection of species with regard to pollutants in the environment. Extrapolation from one species to another is, therefore, difficult if their relative sensitivities are not known (Hedayati *et al.*, 2010).

Laboratory investigations concerning the toxicity of oil to freshwater fishes are numerous. Crude oil has been known to affect histopathological parameters of fish (Khabakhsh *et al.*, 2014). Although, in most cases, very little information is provided about the actual exposure concentration (total petroleum hydrocarbon and a wide range of water quality variables) used. Such information is essential in making comparisons of the relative toxicities of different petroleum products to aquatic organisms. Considering the reliability of histopathological lesions as biomarkers of stress in fish under toxic conditions (Costa *et al.*, 2009), this present investigation was carried out to determine the variations in water quality and toxicity of water soluble fraction of crude oil to histopathological components of *Clarias gariepinus.*

MATERIALS AND METHODS

Test Animal: Healthy juveniles of *Clarias gariepinus* were procured from Aquatech College of Agriculture Fish Farm, Ibadan, and were transported to the Research Laboratory of the Department Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria. They were maintained in the laboratory for three weeks to allow for acclimation.

Test-chemicals: Crude oil was obtained from the Afiesere Oil Field, near Ughelli in Delta State of Nigeria and was transported to the Department of Chemistry, Faculty of Science, University of Ibadan where water soluble fraction (WSF) of the oil was prepared using the method of Anderson *et al.* (1974). The properties of the WSF used for the experiment is provided in Table 1. Different concentrations of the WSF were prepared by diluting the stock WSF with freshwater.

Bioassay: A preliminary short-term (96 hours) static toxicity tests were performed to evaluate the toxicity of WSF to *C. gariepinus* using the methods of Reish and Oshida (1987) and Odiete (2003). Nominal concentrations for the short term assay were 56, 100, 180, 320, 576 mg/l and a control without addition of crude oil. Mortalities recorded were expressed as percentages of the test populations and the median lethal concentration (LC_{50}) values were calculated by using regression equation method of Probit analysis. The 96 hours LC_{50} determined through Probit analysis for juveniles of *C. gariepinus* after exposure to WSF was 224.74 mg/l. To evaluate the effects of the crude oil on histopathological components, fish (average weight 5.1 ± 0.3 g) were exposed to four sub-lethal concentrations (30, 45, 60 and 75 % of the LC_{50} corresponding to 67, 101, 135 and 169 mg/l respectively) and a control group containing clean water in 30 L experimental tanks containing 20 fish each. All treatments and controls were conducted in triplicate. 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. Experimental fish were fed *ad libitum* twice daily with a commercial feed containing 42 % crude protein. Natural photoperiod was maintained throughout the experiment. 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 assay was carried out for 90 days.

Water Quality Analysis: Water quality parameters such as temperature, dissolved oxygen and pH of the experimental set up was monitored on a periodic basis using standard methods (APHA, 1998). Heavy metals and total hydrocarbon contents were determined using the methods of FAO/SIDA (1983) and Olaifa (2012) respectively.

Histopathological Analysis: Liver, brain and gills were harvested from the fish and labeled. Tissues were fixed in Bouin's fluid for 24 hours, washed with 70 % ethanol and dehydrated through a graded series of ethanol. They were embedded in paraffin, sectioned at 5 µm thickness, stained with haematoxylin and eosin, examined microscope and photomicrography.

Statistical Analysis: Information on the physical and chemical properties of the culture conditions of the fish were expressed as mean ± SD (standard deviation). Mean values were compared using one-way analysis of variance (ANOVA) followed by the Tukey's test. The significance level adopted was 95 % ($p<0.05$). Statistical analyses were performed using the software SPSS Version 20.

RESULTS

Property of the Water Soluble Fraction of the Crude Oil: The results of the physical and chemical properties of the WSF of the crude oil used for the bioassay are presented in Table 1. The pH value at 25.5 ^{o}C was 6.94. Values of alkalinity chloride and total hardness of the WSF of the oil were 173 mg$CaCO_3$/L, 16.3 mg/L and 169 mg$CaCO_3$/L respectively. Total petroleum hydrocarbon recorded in the sample was 1820 mg/L. Lead, copper, cadmium, chromium, nickel and iron were found in the following proportion respectively: 0.92, 0.50, 0.53, 0.44, 0.36 and 1.02 mg/L.

Water Quality Characteristics during Bioassay: The physicochemical characteristics of water samples from the various concentrations of crude oil used for the bioassay are summarized in Table 2. pH values showed slight reduction with increase in concentration from 6.81 ± 0.28 in control to 6.57 ± 0.01 in highest concentration.

Table 1: **Properties of water soluble fraction used for the bioassay**

Parameter	Value
Apparent Colour (K_2PtCl_6)	5
Appearance	Clear
Temperature (^{o}C)	25.5
pH (at stated temperature above)	6.94
Alkalinity (mg$CaCO_3$/L)	173
Chloride (mg/L)	16.3
Total Hardness (mg$CaCO_3$/L)	169
Calcium (mg/L)	60
Magnesium (mg/L)	9.73
Total Dissolved Solids (mg/L)	321
Total Solids (mg/L)	377
Biological Oxygen Demand (mg/L)	6.97
Chemical Oxygen Demand (mg/L)	764
Turbidity (FTU)	38.5
Nitrate (mg/L)	2.18
Sulphate (mg/L)	27.4
Phosphate (mg/L)	3.18
Total Petroleum Hydrocarbon (mg/L)	1820
Lead (mg/L)	0.92
Copper (mg/L)	0.50
Cadmium (mg/L)	0.53
Chromium (mg/L)	0.44
Nickel (mg/L)	0.36
Iron (mg/L)	1.02

FTU - Furan Transform Unit

Total dissolve solids also increased significantly ($p<0.05$) with increase in concentration from 358.5 ± 3.54 mg/l to 265.50 ± 4.95 mg/l. Temperature readings and phosphate levels were not significantly ($p>0.05$) affected with increase in concentration of WSF of crude oil. No definite trend was observed in values of alkalinity, total hardness, calcium, total solids and sulphate. Chloride, conductivity, salinity, magnesium, biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity and nitrate levels increased significantly ($p<0.05$) with increasing concentration of water soluble fractions of crude oil.

Heavy Metals: Data on heavy metal and total petroleum hydrocarbon of the analyzed water used for bioassay in *C. gariepinus* is shown in Table 3.

Table 2: Values of physico-chemical parameters during exposure of juvenile *C. gariepinus* to water soluble fractions of crude oil

Parameter	% Concentration of LC_{50}				
	0%	30%	45%	60%	75%
pH	6.81 ± 0.28^{c}	6.77 ± 0.04^{bc}	6.72 ± 0.00^{bc}	6.65 ± 0.07^{ab}	6.57 ± 0.01^{a}
Alkalinity ($mgCaCO_3/L$)	130.00 ± 1.41^{ab}	123.00 ± 2.83^{a}	138.00 ± 4.95^{b}	138.00 ± 2.83^{b}	137.00 ± 1.41^{b}
Chloride (mg/L)	5.88 ± 0.15^{a}	6.74 ± 0.21^{b}	7.60 ± 0.07^{c}	8.61 ± 0.08^{d}	8.83 ± 0.15^{d}
Conductivity (µhoms/cm)	518.50 ± 12.02^{a}	570.50 ± 7.79^{b}	612.00 ± 4.24^{c}	631.00 ± 15.56^{c}	717.5 ± 2.12^{d}
Salinity (g/kg)	0.26 ± 0.01^{a}	0.29 ± 0.01^{ab}	0.31 ± 0.01^{bc}	0.34 ± 0.01^{c}	0.34 ± 0.01^{c}
Total Hardness ($mgCaCO_3/L$)	107.5 ± 3.53^{bc}	95.45 ± 2.05^{ab}	88.70 ± 1.69^{a}	112.00 ± 4.24^{c}	119.00 ± 2.83^{c}
Calcium (mg/L)	39.70 ± 0.42^{bc}	36.25 ± 0.35^{ab}	34.95 ± 1.06^{a}	38.70 ± 0.99^{abc}	42.00 ± 1.41^{c}
Magnesium (mg/L)	2.02 ± 0.05^{a}	3.49 ± 0.05^{b}	3.86 ± 0.01^{bc}	4.11 ± 0.15^{c}	4.62 ± 0.16^{d}
Total Dissolved Solids (mg/L)	358.5 ± 3.54^{d}	334.50 ± 13.44^{cd}	315.00 ± 5.66^{bc}	304.00 ± 1.41^{b}	265.50 ± 4.95^{a}
Total Solids (mg/L)	779.00 ± 1.14^{b}	680.50 ± 13.44^{b}	735.5 ± 6.36^{b}	716.00 ± 8.49^{b}	501.00 ± 69.30^{a}
Biochemical Oxygen Demand (mg/L)	9.31 ± 0.28^{a}	14.85 ± 0.92^{b}	17.25 ± 0.35^{c}	20.60 ± 0.57^{d}	21.90 ± 0.30^{d}
Chemical Oxygen Demand (mg/L)	106.50 ± 4.95^{a}	214.50 ± 4.95^{b}	238.50 ± 3.53^{c}	257.50 ± 10.61^{c}	282.50 ± 2.12^{d}
Turbidity (FTU)	4.31 ± 0.28^{a}	7.15 ± 0.35^{b}	9.27 ± 0.52^{c}	9.70 ± 0.04^{cd}	10.60 ± 0.28^{d}
Nitrate (mg/L)	4.52 ± 0.54^{a}	5.92 ± 0.11^{b}	7.35 ± 0.21^{c}	9.95 ± 0.08^{d}	11.55 ± 0.21^{e}
Sulphate (mg/L)	10.05 ± 0.21^{b}	8.67 ± 0.37^{a}	9.26 ± 0.23^{ab}	8.42 ± 0.12^{a}	8.35 ± 0.35^{a}
Phosphate (mg/L)	1.31 ± 0.02^{a}	1.31 ± 0.27^{a}	1.26 ± 0.08^{a}	1.20 ± 0.02^{a}	1.20 ± 0.01^{a}

Different letters indicate significant difference mean values among treatments (P < 0.05).

Table 3: Total petroleum hydrocarbon and heavy metals content of water treated with water-soluble fraction of crude oil

Parameter	% Concentration of LC_{50}				
	0%	30%	45%	60%	75%
Total Petroleum Hydrocarbon (mg/L)	ND	217.5 ± 10.61^{a}	275.50 ± 30.41^{ab}	344.50 ± 28.99^{bc}	377.00 ± 5.65^{c}
Lead (mg/L)	ND	0.06 ± 0.00^{a}	0.14 ± 0.01^{b}	0.19 ± 0.01^{c}	0.24 ± 0.01^{d}
Copper (mg/L)	ND	0.06 ± 0.01^{a}	0.09 ± 0.00^{b}	0.10 ± 0.01^{b}	0.14 ± 0.01^{c}
Cadmium (mg/L)	ND	0.05 ± 0.01^{a}	0.09 ± 0.00^{b}	0.11 ± 0.01^{b}	0.16 ± 0.00^{c}
Chromium (mg/L)	ND	0.03 ± 0.00^{a}	0.05 ± 0.01^{b}	0.07 ± 0.00^{c}	0.14 ± 0.00^{d}
Nickel (mg/L)	ND	0.02 ± 0.01^{a}	0.05 ± 0.01^{b}	0.08 ± 0.00^{c}	0.13 ± 0.01^{d}
Iron (mg/L)	0.23 ± 0.00^{a}	0.52 ± 0.01^{b}	0.63 ± 0.02^{c}	0.67 ± 0.03^{c}	0.77 ± 0.02^{d}
Zinc (mg/L)	ND	ND	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01

Different letters indicate significant difference mean values among treatments (P < 0.05). ND = Not detected

Total petroleum hydrocarbon values increased significantly ($p<0.05$) as concentrations of water soluble fractions of crude oil increases. Although, TPH was not detected in the control group without crude oil. Apart from zinc, all heavy metals analysed and detected in these water samples followed a direct proportional trend as their values increased significantly ($p<0.05$) alongside concentrations. Analysed metals were not detected in control groups with the exception of iron containing 0.23 mg/l. Zinc was not detected in both control and minimal concentration (67 mg/l) but maintained a steady value even with increased levels of crude oil.

Histopathology: Photomicrographs of livers of juvenile *C. gariepinus* after exposure to WSF of crude oil is presented in Figure 1.

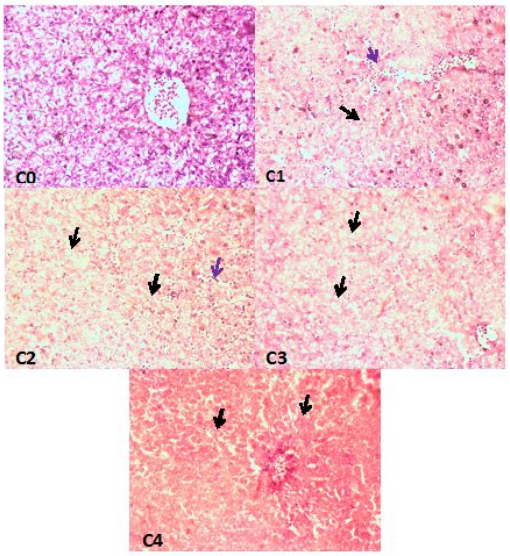

Figure 1: Photomicrographs of the liver of *Clarias gariepinus* exposed to WSF of crude oil. ***Key:*** *C0 - No observable lesion; C1 - Sinusoidal congestion (purple arrow) and atrophy of hepatocytes (black arrows); C2 - Sinusoidal congestion (purple arrow) and atrophy of hepatocytes (black arrows) and hepatocellular degeneration; C3 - Diffuse coagulation necrosis of hepatocytes, C4 - diffuse severe hepatocellular necrosis and atrophy. H&E x400*

Unexposed *C. gariepinus* showed a normal vacuolation of hepatocytes due to accumulation or storage of fat/glycogen with absence of lesson, necrosis, pigments, malignancy, inflammation and inclusion bodies. The liver of all exposed group had lost their typical architecture and clearly exhibited observable alterations. Main alterations observed were sinusoidal congestion, atrophy of hepatocytes, hepatocellular degeneration, diffuse coagulation necrosis of hepatocytes, diffuse severe hepatocellular necrosis and atrophy. The intensity of observable lesions increased with increase in exposure dose. Higher frequencies of the most severe alterations were observed in the highest concentration (C4) containing 75 % of LC_{50} of WSF of crude oil.

Photomicrographs of Brains of juvenile *C. gariepinus* after exposure to WSF of crude oil are presented in Figure 2.

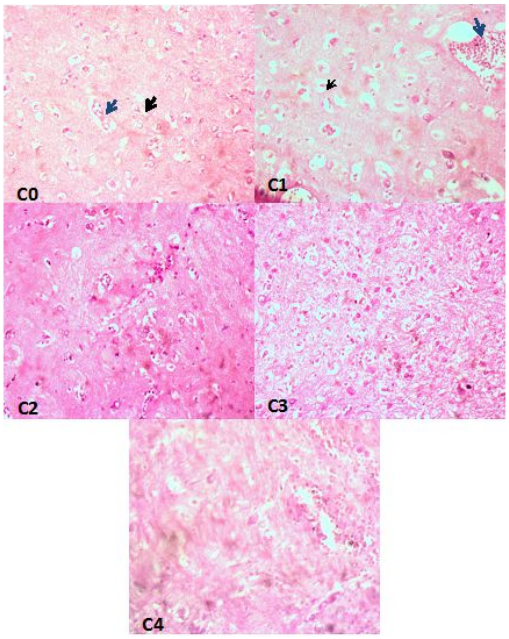

Figure 2: Photomicrographs of the brain of *Clarias gariepinus* exposed to WSF of crude oil. ***Key:*** *C0 - There is no observable lesion [normal neurons (black arrow) and vessels (blue arrow)]; C1 - vascular congestion and atrophy of neurons; C2 - moderate gliosis; C3 - neuronal necrosis and gliosis; C4 - loss of neurons and gliosis. H&E x400*

Histopathological observations of the brain of unexposed fish showed a typical structural organization with normal neurons and vessels without lesions. Juvenile *C. gariepinus* exposed to WSF of crude oil revealed vascular congestion and atrophy of neurons, moderate gliosis,

neuronal necrosis and gliosis and loss of neurons and gliosis.

Histopathological alterations on the gills of juvenile *C. gariepinus* after exposure to WSF of crude oil are presented in Figure 3. General structure of the gills in control fishes was typical for teleost. Each of the four gill arches supported two hemibranches composed on numerous filaments bearing lamellae separated with large interlamellar spaces.

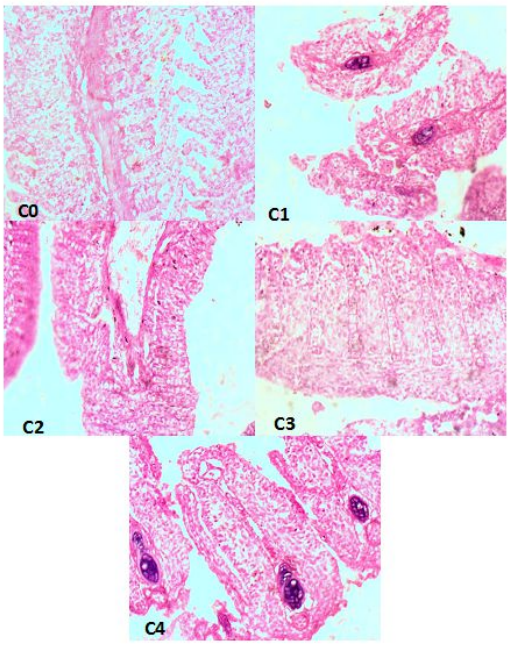

Figure 3: Photomicrographs of the gills of *Clarias gariepinus* exposed to WSF of crude oil. ***Key:*** *C0 - no observable lesion; C1 - There is moderate loss of the secondary gill lamellae; C2 - moderate lamellae atrophy; C3 - moderate diffuse lamellae hyperplasia; C4 - diffuse lamellae hyperplasia. H&E x400*

There is moderate loss of the secondary gill lamellae. Clear evidence of tissue damage was observed in all exposed group except control group which showed no observable lesion. Observable alterations include moderate loss of the secondary gill lamellae, moderate lamellae atrophy, moderate diffuse lamellae hyperplasia.

DISCUSSION

Property of the Water Soluble Fraction of the Crude Oil: The value of total petroleum hydrocarbon (1820 mg/l) recorded in water soluble fractions of the crude oil used for the assay was considerably higher than the report of Ogbeibu (2011) that documented values of 35.79 and 827.3 mg/l for water and sediment analysis respectively in Ethiope-Benin River after a spill. Presence of some metals has also been linked with pollution. Values of metals in WSF of crude oil utilized for the assay were, however, slightly lower in comparison to the values obtained (Ogbeibu, 2011) in the Ethiope-Benin River. Differences in these values could be attributed to weathering which include spreading, evaporation, dissolution, dispersion into the water column, water-in-oil emulsification, photochemical oxidation, microbial degradation, adsorption to suspended particulate matter and stranding on the shore or sedimentation to bottom sediments of oil after spillage (Wang and Stout, 2007). Toxicological results with WSF of petroleum products are often difficult to compare, because different types of petroleum and different ways of preparing its WSFs (Ziolli and Jardim, 2002).

Water Quality Characteristics: A large number of studies have shown that WSF of crude oil can reduce water quality condition and thereby the overall well-being of aquatic organisms (Santos *et al*., 2016). The values of the water quality parameters measured indicated that WSFs of crude oil adversely affected the water quality variables in the culture systems. As the concentration of the xenobiotic increased with time the values of the physicochemical parameters were observed to fluctuate when compared with the control.

Dissolved oxygen levels in the present study reduced with increasing concentration of WSFs of the crude oil. Edema and Asagba (2007) reported similar reduction in DO after exposure to the different levels of WSF of crude oil. In the present study, biological oxygen demand (BOD) increased from 9.31 ± 0.28 mg/l in control to 21.90 ± 0.30 mg/l in highest concentration of WSF of crude oil. Chattopadhyay *et al.* (1988) indicated 10 – 20 mg/1 as the optimum BOD range for fish culture in effluent or polluted waters. This observation supports earlier reports of Baden (1982) that a corresponding increase in biological oxygen

demand and a decrease in dissolved oxygen content are typical of water bodies contaminated with crude oil.

In this study, the hydrogen ion concentration (pH) levels of the water was within the FEPA acceptable range of 6.0 to 9.0 for the sustenance of aquatic life and may unlikely contribute to the toxicity of the water soluble fraction. The decreased in pH value as concentration increases observed in this study is in agreement with the findings of Giari *et al.* (2012) that reported decrease in pH level after a spill. There was a significant increase ($p<0.05$) in conductivity values as concentration of WSF of crude oil increases. 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 (APHA, 1998). The increased levels nitrate with increasing concentration observed in this study may be attributed to the suppression of nitrosomonas (ammonia-oxidizing proteobacteria) and nitrobacters (nitrite-oxidizing proteobacteria) by the WSF of the crude oil (Ahmed, 2007). There was an increase in salinity levels with corresponding increase in the concentration of the oil. Increasing salinity observed in this study might have influenced the activities of these bacteria. A similar observation has been documented by Edema (2009).

Total Petroleum Hydrocarbon and Heavy metals: Highest value of TPH obtained during this bioassay (377.00 mg/l in 75 % LC_{50}) was however different from the values recorded in crude oil polluted water. Olufemi *et al.* (2011) have reported TPH values of 73.5 in water surface from Ubeji River in Delta State of Nigeria. Similarly, Osuji and Nwoye (2007) noted a range of 3400 – 6800 mg/kg in soils of polluted section of Owaza in the Niger Delta. They attributed this higher value to the persistent oil spillage occurring in the region. Such high levels hydrocarbon can affect both aquatic flora and fauna which are essential for biogeochemical cycle and ultimately nutrient availability (Osuji *et al.,* 2004).

Heavy metals have been used as indicators of pollution as a result of their high toxicity to human and aquatic life (Omoigberale and Ogbeibu, 2007). The introduction of WSF of crude oil into the aquatic system can increase the ionic, heavy metals and physical characteristics of aquatic environment (Edema, 2012). Nwajei *et al*. (2014) have reported higher values than the result obtained from this study in surface water and sediments from Crayford Creek in Warri. High heavy metal content in waters of the Niger Delta region of Nigeria are often products of pollutants arising from crude oil explorations, anthropogenic wastes and other industrial and domestic sewages that find their ways through various channels (Braide *et al*., 2004). Cadmium values obtained from this study was higher than the values reported by Ubiogoro and Adeyemo (2017) from some rivers and creeks in Delta State of Nigeria. The highest level of lead (0.24 ± 0.01 mg/kg) was recorded in the concentrations containing 75 % of LC_{50}. This value was lower than those obtained by Puyate *et al*. (2007) for Orogodo River (4.39 mg/kg).

Histopathology

Liver: Histopathological examinations in the liver of exposed juveniles of *C. gariepinus* clearly indicate alterations such as centrilobular degeneration, atrophy (reduction in number and volume of cells and/or a decreasing amount of intercellular substances) of hepatocytes and multiple foci of hepatocellular coagulation necrosis. The severity and frequency of organ lesions were found to be more pronounced in fish treated with higher concentrations of the WSF of crude oil. Several studies have demonstrated links between exposures to crude oil and hepatic lesions in different fish species. Flounder (*Pleuronectes americanus*) collected near an oil refinery showed various histological lesions in the liver which were believed to be indicative of the impact of the oil on the health of the fish (Khan, 1998). Amadi *et al*. (2015) have also observed vascular and cellular degeneration, hyperplastic hepatocytes, cytoplasmic vacuolation and inflammation as histological alterations in juvenile *C. gariepinus* after exposure to a refined petroleum product (petrol). In the present study multiple foci of hepatocellular coagulation necrosis was

observed in samples of *C. gariepinus* exposed to high concentration of WSF of the crude oil. Necrosis in the liver is a typical lesion in fish exposed to contaminants. It decreases the number of functional cells in the hepatic tissue with deleterious consequences to the proper functioning of the organ of the marine pejerrey *Odontesthes argentinensis* larvae exposed to petroleum water-soluble fraction (Gusmao *et al.*, 2012).

Gills: In the present investigation, gills of juvenile *C. gariepinus* exposed to WSF of crude oil exhibited moderate lamellae atrophy, diffuse lamellae hyperplasia and moderate diffuse lamellae hyperplasia. The alterations in the gills of fish exposed to WSF of crude oil falls within the general responses of fish gills to environmental pollutants. Gills have been noted to be very sensitive to crude oil or its WSF even at relatively low concentrations (Al-Kindi *et al.*, 2000). The pathological changes in the gills were very pronounced and produced convincing evidence that WSF of crude oil could pose a serious health challenge to fishes. Significant pathological alterations such as lamellae atrophy, diffuse lamellae hyperplasia and moderate diffuse lamellae hyperplasia observed in this study could lead to an overall reduction in the efficiency of gill filaments to aid in diffusion of oxygen across the gill lamellae resulting in the development of a hypoxic condition within the fish (Elahee and Bhagwant, 2007). Apart from the impairment of respiratory functions, observable lesions in the gills also have the tendencies of impairing osmo-regulatory functions (Tang and Au, 2004).

Various alterations of gill morphology have been reported by different authors on a variety of fish species exposed to crude oil (Ayoola and Alajabo, 2012; George *et al.*, 2014). There is also wide disparity between the current study and the histopathological responses that have been reported previously in the liver of various fish species exposed petroleum products. Gabriel *et al.* (2007) reported similar changes in *C. gariepinus* exposed to petroleum oil and kerosene. Mild and severe congestion as well as severe inflammation and calcification were also been reported by Ayoola and Alajabo (2012) in the gill tissues of *Sarotherodon melanotheron* after exposure to engine oil. Fish are able to develop several defense mechanisms, which could prevent the toxicant negative effects. These mechanisms are expressed in different morphological changes including edema, proliferation of epithelium and fusion (Yancheva *et al.*, 2016).

Brain: The fish brain is a fundamental part of the nervous system. It is the master organ of all living organisms as it is the controlling centre for all other receptor and effector organs. Because of its structural complexity and functional diversity, brain performs a number of complex biological functions that are essential for survival. Therefore, alterations in the structure of the brain could result in various forms of neurological disorders (Summerfield *et al.*, 2005). In the present study, histopathological alterations on the brain of *C. gariepinus* were mild congestion of cerebral blood vessels, vascular congestion and atrophy of neurons, neuronal necrosis and gliosis as well as loss of neurons and gliosis. The pathological changes observed in the brain of exposed fish may be attributed to the hypoxic condition developed within the fish as a result of the inability of the gill to aid sufficient oxygen diffusion across the gill epithelium due to degenerative changes in gill structure. This is because of the fact that brain cells are very sensitive to hypoxia and neuronal degeneration may occur in the absence of oxygen (Adeogun *et al.*, 2012). These findings agreed with those of Omitoyin *et al.* (2006) that reported severe lesions in the brain of juvenile *C. gariepinus* exposed to Gramoxone (paraquat).

Conclusion: Result of the present study unveiled the effects of WSF of crude oil on water quality parameters as well as its implication on histopathological components in juveniles of *C. gariepinus.* These results can therefore provide a reasonable and reliable basis for comparison of the effects oil spillage on aquatic organisms.

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