Lethality And Histopathological Alterations Of The Red Tilapia Fish (*Oreochromis Niloticus*) Raised In Water Soluble Fraction Polluted Culture

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ABSTRACT

The lethal concentration and histopathological damages of crude oil water soluble fractions (WSFs) on the liver and gills damages of the Red Tilapia (*Oreochromis niloticus*) was investigated. Water soluble fractions of the crude oil was prepared according to Anderson *et al.* method and was made into five concentrations; Toxicity of WSFs on Red Tilapia was investigated at 0 ppm control, 5 ppm, 6 ppm, 8 ppm and 10 ppm. The dilutions were made with the control water (habitat of which fish were cultured). Twenty fish per group (8.5± 1.5g) were exposed to 5 liter each of the five concentrations levels of the WSFs and the fishes were observed for 96 hours. Water quality parameters were determined and showed that salinity was 34 ppt, pH was 7.5, dissolved oxygen (DO) was 12 mg/l and temperature was 28°C. The number of dead fishes per group was recorded against the time of their death. The obtained data were used to determine the median lethal concentration (LC50) of the WSFs of the crude oil using arithmetic method. The preserved liver and gill organs from the control and highest concentration group were processed according to Mohammed method, fixed in boiling solution of 75% saturated picric acid, 25% formalin and 5% glacial acetic acid and dehydrated in an Ethyl alcohol series in ascending concentrations (70, 80, 90, 95, 100%) after which were maintained in Methyl Benzoite overnight and then embedded in paraffin wax, blocked and sectioned at 5-6 µm. The tissue sections were stained with haematoxylin-eosin (H×E) and examined under electron microscope. Three sections of each tissue were examined and photographed as appropriate. Results showed that Liver from the control group fish showed normal histological picture of hexagonal hepatic lobules with centrally located central veins from the central veins radiating cords of polyhedral hepatocytes. Each hepatocyte showed eosinophilia homogeneous cytoplasm and a large central nucleus with prominent nuclei. On the other hand**,** liver treated with WSFs showed congested and severely dilated central veins, multifocal degeneration and necrosis of the hepatocytes. Gills from the control group fish showed normal primary and secondary lamellae with normal mucous production by gill epithelial cells and no congestion of blood vessels while gills treated with WSFs showed severely congested blood vessels, hyperplasia and adhesion of secondary gill lamellae. Kidneys from the control group fish showed normal glomeruli, renal tubular epithelium and resting inter-tubular capillaries with no inflammatory reaction. Kidneys treated with WSFs showed congested blood vessels and focal hemorrhages, degeneration of renal tubular epithelial cells and focal necrosis of some renal tubules. It was therefore concluded that

crude oil spillage is lethal at very low concentration on Red Tilapia and causes significant and severe damages to liver and gills and recommended the prevention of crude oil spills into aquatic bodies to reduce the negative impact on the histopathology of aquatic organisms.

Keywords: Water soluble fractions, Lethal Concentration, Tilapia, histopathology,

biochemical parameters

1.1 Introduction

The evaluation and prediction of the effects of oil pollution on water environment have become a very urgent and important issue (Engelhardt, 2000, Khan *et al.*, 2001). This spilled crude oil if left uncleaned could have terrible adverse effects on aquatic life. Tilapia fish (*Oreochromis niloticus*) is one of the most economically significant fishes in the aquatic environment.

Petroleum exploration, exploitation and refining as well as transportation, storage, marketing and use of petroleum products have all created pollution problems in the various aquatic habitats in various part of the world including Nigeria (NEST, 1991). During these processes, accidental spill and discharges of petrol, lubricating oil, gasoline as well as sludge and bitumen slops from tank cleaning processes and operation are commonly discharged onto the land and eventually into the various water bodies through runoff and erosion of contaminated soils (Ogbeibu and Omoigberele, 2005). The formation of a film of oil on water bodies effectively prevents natural aeration, leading to the death of aquatic organisms (NEST, 1994).

Most fish species exhibit cumulative characteristics to the pollution effects of petroleum and its derivatives in the short term. These pollutants in the long term may become disastrous to the various developmental stages of these fish species including other biotic and fauna lives Lindgren and Lindblom, 2004). Their effect may be grievous to species under culture conditions causing expressed mortalities to the population (Akpan *et al.,* 1998; Baker; 1981; Guthricao and Porry 1980; Adeniji *et al.,* 1986).

In aquaculture operations, emphasis is always laid on the water quality of the culture medium for the successful production of the animals. Fish being the cheapest source of protein to man is usually one of the cultured species in aquaculture. As such a good quality and pollution – free water is highly demanded.

If such culture medium is polluted by petroleum or any of its derivatives, high mortality of the cultured species is likely to occur. Crude oil being one of the derivatives of petroleum could cause high mortalities in such cultured population if accidentally or purposefully allowed to enter the culture medium, therefore impacting negatively on the affected species, hence on the subsequent reduction in the available fish protein available to man.

The tilapia (*Oreochromis niloticus*) is a species of tilapia, a cichlid fish native to the northern half of Africa and the Levante area, including Jordan, Palestine, and Lebanon. Numerous introduced populations exist outside its natural range, it is also commercially known as mango fish, nilotica, or boulti. The first name leads to easy confusion with another tilapia (*Sarothrodon galilaeus*).

Crude oil is a naturally occurring petroleum produce composed of hydrocarbon deposits and other organic materials. A type of fossil fuel crude oil is refined to produce usable products including gasoline disel, and various other forms of petrochemicals. It is a nonrenewable resource, which means that it can’t be replaced naturally at the rate we consume it and is, therefore, a limited resources.

Crude oil is the raw natural resource that is extracted from the earth and refined into products such as gasoline, jet fuel, and other petroleum products.

The soluble fraction of crude oil is a complex and toxic mixture of hydrocarbons that aquatic organisms directly encounter in oil spills. WSF plays an important role in the toxicity of crude oil to aquatic organisms. Acute exposure to WSF increases the respiration rate and decreases activity of fish. Acute exposure to WSF changes the gas pressure of O_2 and Co_2 in fish blood. Egression of the volatile components causes underestimating the toxicity of WSF

Based on the premise that petroleum and its derivatives are toxic to both biotic and faunal live, it is important that studies on the toxic effect of a particular derivative such as crude oil of petroleum on the early life stages of particular fish species especially those under culture be undertaken. This is to determine particularly the tolerance limits of the fish species, the dose response of the species and the medium lethal concentration (L_{c50}) at which half of the individual affected organisms are expected to die within the experimental period.

MATERIALS AND METHODS

2.1 Preparation of water soluble fraction

Water soluble fractions of the crude oil had been prepared according to Anderson *et al.* (1974) method and the WSFs was made into five concentrations; Toxicity of Water Soluble Fractions of Crude Oil on Red Tilapia control 0 ppm, 5 ppm, 6 ppm, 8 ppm and 10 ppm. The dilutions were made with the control water (habitat of which fish were cultured). Twenty fish per group (8.5± 1.5g) were exposed to 5 liter each of the five concentrations levels of the water soluble fractions (WSFs) and the fishes were observed for 96 hours.

2.2 Water quality

Water quality parameters were determined and it showed that salinity was 34 ppt, pH was 7.5, dissolved oxygen (DO) was 12 mg/l and temperature was 28°C.

2.3 LC_{50} Determination

The number of dead fishes per group was recorded against the time of their death according to method specified by Sprague (1972). The obtained data had been used to calculate the median lethal concentration (LC50) of the WSFs of the crude oil on using arithmetic method of Dede and Kaglo (2001**).**

2.4 Histopathology of liver and gills of Tilapia fishes

The preserved organs from the control and highest concentration group had been processed according to (Mohamed, 2009) method, fixed in boiling solution (75% saturated picric acid, 25% formalin and 5% glacial acetic acid), tissues had been dehydrated in an ethyl alcohol series of ascending concentrations (70, 80, 90, 95, 100%) after that maintained in Methyl Benzoite overnight and then embedded in paraffin wax, blocked and sectioned at 5-6 μm. The tissue sections had been stained with haematoxylin-eosin (H×E) and examined by electron microscope. Three sections of each tissue were examined and were photographed as appropriate.

RESULTS

3.1 1-Toxicity test

Results of Toxicity test of Water Soluble fractions (WSFs) of crude oil on red tilapia within 24, 48, 72 and 96 hours were observed and presented in table 1, 2, 3 and 4.

TABLE 1

Toxicity test observations at 24 hours

Conc. (ppm)	No surviving	% alive	% dead
Control	20	100	0
5	17	85	15
6	19	95	5
8	14	70	30
10	15	75	25

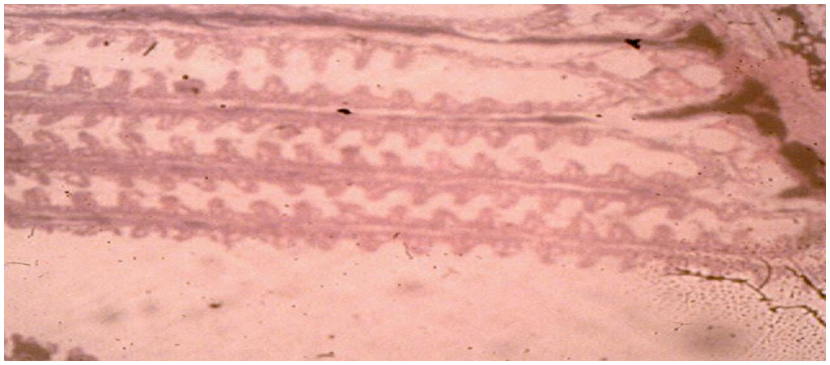

Plate 1: Normal Gills in Control

TABLE 2

Toxicity Test observations at 48 hours

Conc (ppm)	No surviving	% alive	% dead
Control	20	100	0
5	14	70	30
6	19	95	5
8	13	65	35
10	9	45	55

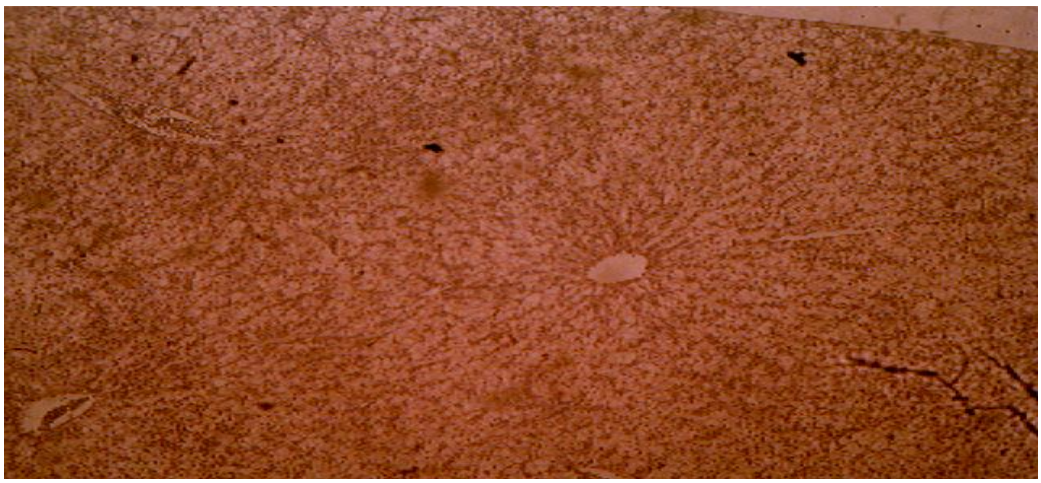

Plate 2: Normal fish liver in control

TABLE 3

Toxicity test observations at 72 hours

Conc (ppm)	No surviving	% alive	% dead
Control	20	100	0
5	13	65	35
6	18	90	10
8	11	55	45
10	7	35	75

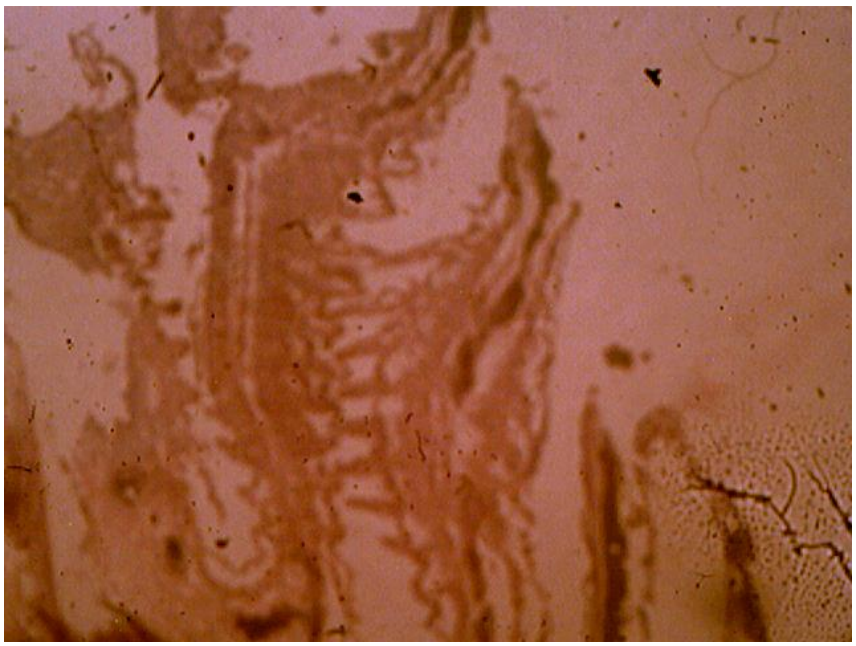

Plate 3: Damaged Gills after 72hrs of fish exposure

TABLE 4

Toxicity Test observations at 48 hours

Conc (ppm)	No surviving	% alive	% dead
Control	20	100	0
5	2	10	90
6	18	90	10
8	11	55	45
10	9	9	100

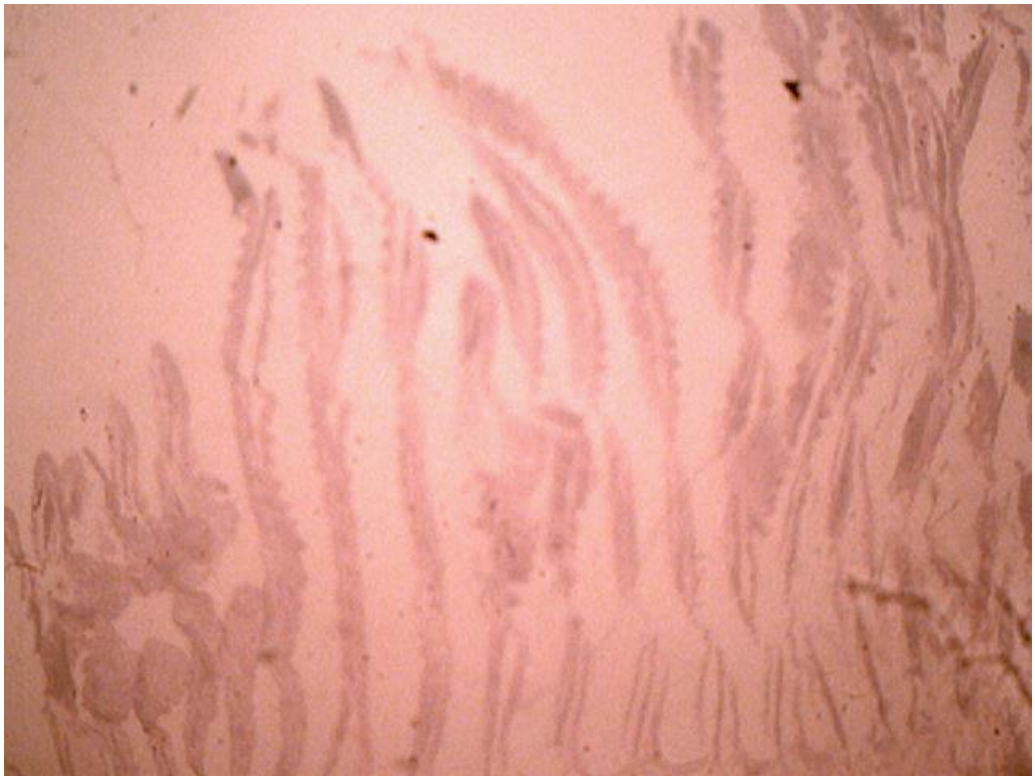

Plate 4: Fusion of fish gills after 72 hours of fish exposure

TABLE 5

96 Hours LC50 determination: Using arithmetic method of Karber adapted by Dede (1997), the LC50 value was determined as follows LC_{50}=LC_{100}-Σ (Mean death x Conc. Diff)/ No of organism per group = 10 (95/20) =5.25ppm

Conc (ppm)	Conc. Difference	No alive	No dead	Mean death	Mean death dose diff
Control	-	20	0	-	0
5	5	2	18	9	45
6	1	18	2	10	10
8	2	11	9	5.5	11
10	2	0	20	14.5	29
	Sum				95

Concentration difference = Used concentration, Mean death-Sum of dead of two aquarium/2

Mean death dose difference = concentration difference* mean death

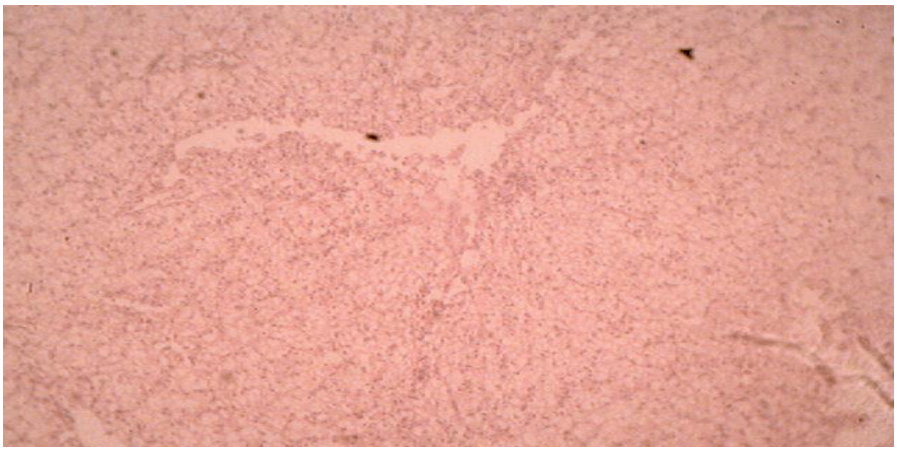

Plate 5: Necrotic Cells of the Liver after 72 hours of fish exposure

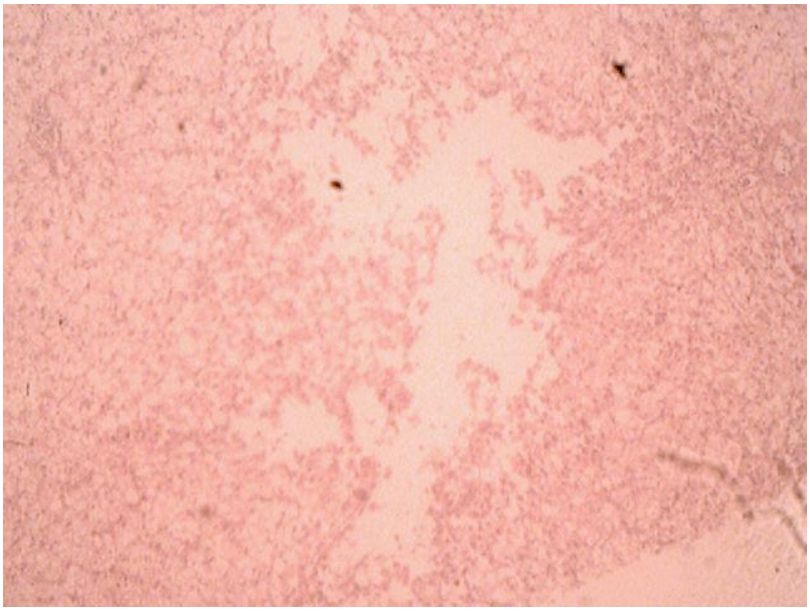

Plate 6: Severe Necrosis of the liver of Tilapia after 72 hours of fish exposure

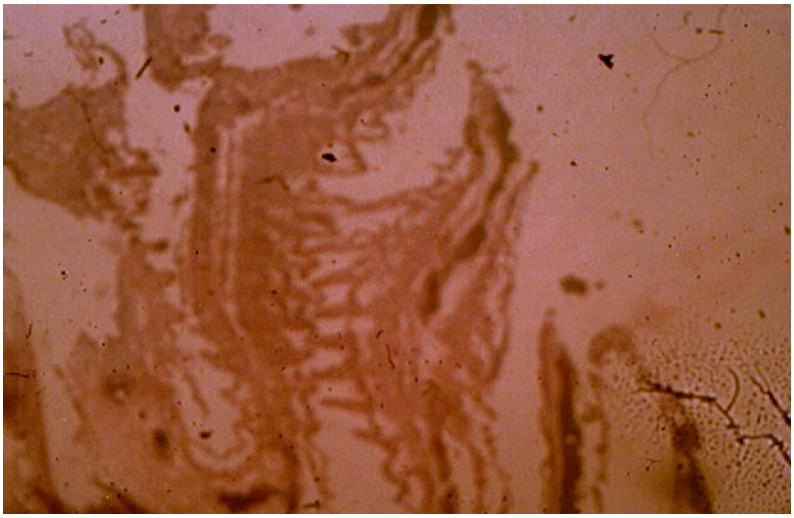

Plate 7: Severe fusion of liver cells after 72 hours of necrotic

fish exposure

3.2 Histopathological finding in the liver and gills of *Orechromis niloticus* exposed to WSFs

Liver from the control group, fish showed normal histological picture of hexagonal hepatic lobules with centrally located central veins from the central veins radiating cords of polyhedral hepatocytes. Each hepatocyte showed eosinophilia homogeneous cytoplasm and a large central nucleus with prominent nuclei. On the other hand**,** livers treated with WSFs showed congested and severely dilated central veins, multifocal degeneration and necrosis of some hepatocytes.

Gill from the control group fish showed normal primary and secondary lamellae with normal mucous production by gill epithelial cells and no congestion of blood vessels. Gills treated with WSFs showed severely congested blood vessels, hyperplasia and adhesion of secondary gill lamellae.

Kidney from the control group fish showed normal glomeruli, renal tubular epithelium and resting inter-tubular capillaries with no inflammatory reaction. Kidneys treated with WSFs showed congested blood vessels and focal hemorrhages, degeneration of renal tubular epithelial cells and focal necrosis of some renal tubules.

Discussion

Exposure of Tilapia fish to water soluble fractions of crude oil showed mortality even at low concentrations. Toxicity of substances based on their median lethal concentration (LC50) showed that water soluble fractions of crude oil is slightly toxic to tilapia and this agree with previous reports on the toxic effect of water soluble components of hydrocarbon on aquatic life. The results of Akbari *et al.* (2004) on fish, *(Lutjanus argentimaculatus)* and shrimp *(Penaeus monodon*) showed that the 96 h LC50 values of the WSFs of crude oil for fish and shrimp were 3.24 ± 0.21 and 8.52 ± 0.89 ppm of WSFs of crude oil, respectively. In this investigation, the fish were more sensitive to crude oil than the shrimp, with respect to the similarity in their habitats.

Ayoola and Alajabo (2012) reported that the lethal concentration Lc50 that caused 50% mortality was approximately 1.12mg/l of engine oil on Black Chin Tilapia (*Sarotherodon melanotheron*). It was observed in the study of (Seiyaboh *et al.,* 2013) that with the highest concentration of bonny light crude oil (0.02%) all the fishes died. This is an indication that 0.02% of bonny light crude oil is the most toxic (100% mortality rate). Lc50 which is the lethal concentration was observed in the study to start from 0.01%. Bonny light crude oil is toxic to (*Sarotherodon melanotheron).*

The most studied and reported pathological effect of polycyclic aromatic hydrocarbons (PAHs) is cancer. Other diseases connected with PAHs pollution are various skin and liver lesions and cataracts (blindness) in fish. The liver of oil-treated cod also showed histological alterations, characterized by the formation of microvesicles within hepatocytes. The hydrocarbons present in the crude oils might, therefore, have been responsible for the changes reported herein. Fish can accumulate, metabolize, and secrete hydro-carbon components into bile (McCain *et al*., 1978). Compared to the control specimens, various histological changes were identified in the livers of juvenile fish exposed to the oil water soluble fraction (WSFs). Dispersed oil and blood vessel congestion were observed early in all treatments, although fish exposed to dispersant showed less significant effect. Similarly, exposure to dispersant did not

result in significant blood sinusoid dilation in contrast to WSFs and dispersed oil effect. Similar lesions were also described following exposure to toxicants (Van Dyk *et al.*, 2007). Dessouki *at al.* (2013) showed that gills of *Tilapia zillii* gills received oil revealed mild congestion in the gill lamellae and mild atrophy and shortening in the epithelial lining of the secondary lamellae.

Study of Abo Elnaga *et al.* (2005); Rodrigues *et al.,* (2010) and Doherty *et al.,* (2013) had been shown that section through the gill of the exposed fish to diesel had moderate area of lession necrosis, malignancy, pigment, inclusion bodies, separation of epithelium from gill lamellae, space filled with eosinophilic material and fusion of the second lamella were observed in fish gill on Sehi, Tilapia and catfish respectively. Fishes exposed to water contamination had tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman´s space (Takashima and Hibiya, 1995). The histological study of Ebonwu and Ugwu, (2016) showed that crude oil water soluble fraction (WSFs) resulted in cytoplasmic vacuolation of the kidney tissue of *Tilapia guineensis* fingerlings compared with the normal. The kidney micro-photo scope showed swelling of the renal tubules and clear vacuolation of the epithelial cells leading to degeneration of the cytoplasm, enlarged tubule and shrinkage had observed as concentration increased. WSF also caused gradual cell tissue disintegration of the kidney.

Conclusion

Results from the present study indicated that the toxicity values of water soluble fractions "WSFs" could be varied according to many factors including age, species and environmental conditions and it's has different histological alteration in fish organs. Liver, gill and kidney showed severe necrotic lesions on exposure to 96 hours of crude oil water soluble fractions when compared to the control where no WSFs was used as culture solution.

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