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Toxicity of Water Soluble Fractions of Petroleum Crude Oil and its Histopathological Alterations Effects on Red Tilapia Fish

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ABSTRACT

Five treatments including control and four concentrations of water soluble fraction (WSFs) crude oil (5 ppm, 6 ppm, 8 ppm and 10 ppm) were made. Triplicate and ten fish of red tilapia with an average weight of 8g ± 1.5 were incubated for 96 hours. The 96 hours LC_{50} was determined (5.25ppm). Histopathological examination of the fish was made for liver, gills, kidney, male gonad and female gonad. Liver showed congestion of blood vessels and hepatic sinusoids with focal necrotic changes of hepatocytes. Gills showed congestion of blood vessels, hyperplasia and adhesion of secondary gill lamellae. Kidney showed congestion of blood vessels and degeneration of renal tubules. Male gonad showed degeneration and necrosis of some seminiferous tubules, edema and severe reduction in all spermatogenic stages and degeneration and Female gonad showed severe necrosis of developmental stages of oocytes along with degenerated mature ripped oocytes in WSFs treated fish compared to control group.

Keywords: Water soluble fractions, crude oil, short term toxicity, histopathology

INTRODUCTION

Fish are considered as the most successful group of vertebrates and their number is bigger than all terrestrial vertebrates together. Over 25 thousand of living species their abundance is related directly to their adaptive diversity, and they occupy a wide range of ecological environments from fresh to saltwater, environmental extremes and some even emerge onto land. A remarkably small number of fish have been studied scientifically, and even fewer are exploited by humans in aquaculture (Karin *et al*., 2013).

The excessive contamination of aquatic ecosystems has evoked major environmental and health concerns worldwide (McNeil and Fredberg, 2011). Environmental pollution, especially water pollution, is a serious issue that include all world countries. Water pollution not only affects the survival and reproduction of aquatic organisms but also adversely harms human health through bioconcentration (Yu-Jie *et al.*, 2014).

Water soluble fractions of crude oil are a complex and toxic mixture of hydrocarbons that aquatic organisms directly encounter in oil spills. It plays an important role in the toxicity of crude oil to aquatic organisms (Lari *et al.*, 2015). The fractions of oil that are most bioavailable to marine biota such as teleosts are the dissolved hydrocarbons, which include the polycyclic aromatic hydrocarbons (PAHs). Incardona *et al.* (2004) showed that immunotoxicity and carcinogenicity are both hallmarks of PAHs toxicity in teleosts. Moreover, individual PAHs have distinct and specific developmental consequences when fish are exposed at early life-history stages. Hematological and histopathological changes in fish exposed to pollutants have been proposed and used as sensitive biomarkers for assessing the effects of several environmental contaminants, including petroleum hydrocarbons (Gabriel *et al.*, 2007).

Among hydrocarbons, Polycyclic aromatic hydrocarbons (PAHs) are a wide spread class of environmental pollutants that are carcinogenic and mutagenic (Bob-Manuel, 2012). PAHs are ubiquitous organic contaminants found in air, water, sediment, and soil. The United States Environmental Protection Agency (USEPA) identified 16 PAHs as priority environmental pollutants. PAHs are important pollutants due to their capability of bioaccumulation in invertebrate species (Meador *et al.*, 1995), and their known toxic effects (Moore *et al.*, 1989). Studies investigated that PAHs have primarily focused on aquatic ecosystems (Kannan and Perrotta, 2008).

Determination of median lethal concentration (LC_{50}) is one of the basic tests which provides a better understanding regarding the sensitivity of animals to crude oils. The LC_{50} values of water soluble fractions (WSFs) of different crude oils reported previously by Anderson *et al.* (1974) for three species of shrimp and three species of fish, and Neff and Anderson (1981) on several life stages of four species of marine shrimp, appeared to be different from each other.

The current study is aimed to investigate the acute toxicological effect of the water soluble fractions of crude oil on red tilapia (*Oreochromis mossambicus*× *Oreochromis niloticus)* that obtained from (Hatchery of Elkilo 21 (Alexandria), Fish wealth authority).

MATERIALS AND METHODS

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;

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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 litre each of the five concentrations levels of the water soluble fractions (WSFs). Fish were observed for 96 hours.

Water quality

Water quality parameters had been determined and it was salinity 34 ppt, pH 7.5, dissolved oxygen DO 12 mg/l and temperature 28°C.

LC_{50} Determination

The number of dead fishes per group had been 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 (LC_{50}) of the WSFs of the crude oil on using arithmatic method of Dede and Kaglo (2001**).**

Histopathology of liver, gills, kidney, male gonad and female gonad

The preserved organs from the control and highest concentration group had been processed according to Mohamed, (2009) method, fixed in boin 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 Methyle 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 "Zeiss" microscope. Three sections of each tissue had been examined. Sections of examined organs were photographed as requested.

RESULTS

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 had been 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

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

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

Table (4): Toxicity Testing observations at 96 hours

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

Table (5): 96 HOURS LC50 DETERMINATION: Using Arithmetic method of Karber adapted by Dede 1997), the LC50 value was determined as follows $LC_{50=} LC_{100} – \Sigma$ [(Mean death x Conc. Diff.) / No. of organisms per group] = 10 - (95/20) = 5.25 ppm

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- Pervious concentration, Mean death = Sum of dead of two aquariums / 2
Mean death dose difference = concentration difference* mean death

Histopathological findings in the liver, gills, kidney, male and female gonad of red tilapia following exposure to the WSFs of crude oil

Livers 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 eosinophilic homogeneous cytoplasm and a large central nucleus with prominent nuclei (Fig. 1a). On the other hand**,** livers treated with WSFs showed congested and severely dilated central veins, multifocal degeneration and necrosis of some hepatocytes (Fig. 1b).

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 (Fig. 2a). Gills treated with WSFs showed severely congested blood vessels, hyperplasia and adhesion of secondary gill lamellae (Fig. 2b). Kidneys from the control group fish showed normal glomeruli, renal tubular epithelium and resting inter-tubular capillaries with no inflammatory reaction (Fig. 3a). Kidneys treated with WSFs showed congested blood vessels and focal hemorrhages, degeneration of renal tubular epithelial cells and focal necrosis of some renal tubules (Fig. 3b).

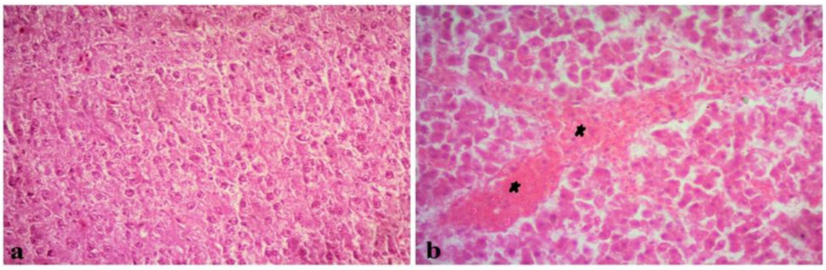

Figure (1): Liver of *O. niloticus X O. mossambicus* showing normal arrangement hepatic cords with normal polyhedral hepatocytes in normal control fish (a) congestion of blood vessels and hepatic sinusoids with focal necrotic changes of hepatocytes in WSFs treated fish (b) H&E. X 400, Congestion (starstick), H&E. X 40.

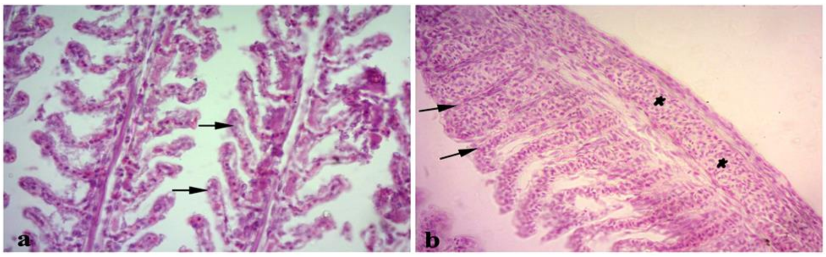

Figure (2): Gills of O. *niloticus X O. mossambicus* showing normal histology of both primary and secondary lamellae in normal control fish (a) congestion of blood vessels, hyperplasia and adhesion of secondary gill lamellae in WSFs treated fish (b) H&E. X 400, Gill lamellae (arrows), congestion (starstick). H&E. X 400.

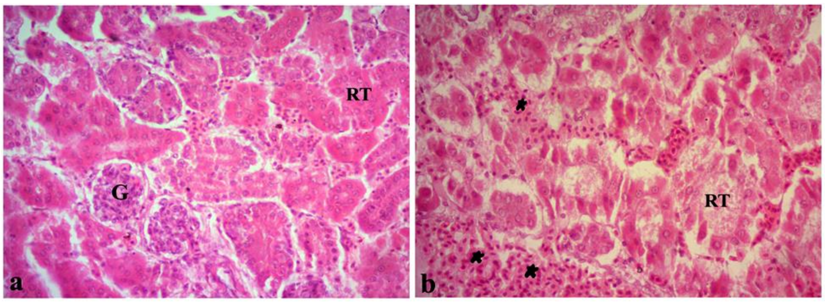

Figure (3): Kidney of *O. niloticus X O. mossambicus* showing normal histology of both glomeruli and renal tubules in normal control fish (a), congestion of blood vessels and degeneration of renal tubules in WSFs treated fish (b). H&E. X 400, Glomeruli (G), renal tubules (RT), congestion (starstick), H&E. X 400.

Testes from the control group fish showed normal seminiferous tubules with abundant number of the diff-errent developmental stages of spermatogonia, sper-matids and mature spermatozoa in their lumenae (Fig. 4a). The testes treated with WSFs revealed focal dege-neration and necrosis of some seminiferous tubules that had either degenerated spermatogonial cells or complete depletion of all spermatogeneic stages (Fig. 4b).

Ovaries from the control group fish showed normal ovarian histological structure with presence of normal developmental stages of ovarian oocytes, chromatin nucleolar oocyte, perinucleolar oocytes, cortical alve-olar oocyte and mature ripped oocyte (Fig. 5a).

Ovaries treated with WSFs showed degeneration, edema and severe necrosis of most of the developmental stages (Fig. 5b).

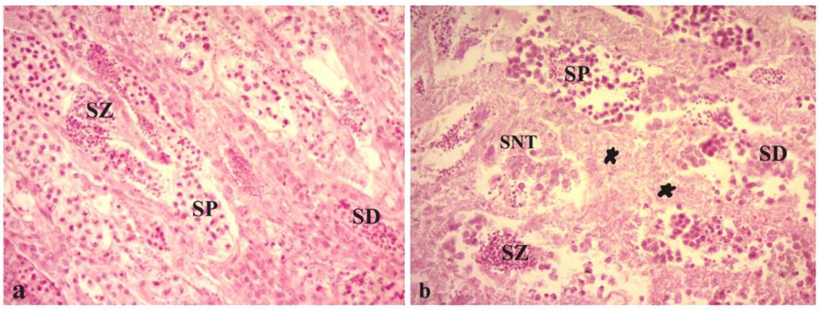

Figure (4): Testes of *O. niloticus X O. mossambicus* showing normal seminiferous tubules that having normal number of spermatocytes (SP), spermatids (SD) and spermatozoa (SZ) in control fish (a) degeneration and necrosis of some seminiferous tubules (SNT), edema (starstick) and severe reduction in all spermatogenic stages in WSF treated fish (b) H&E. X 400.

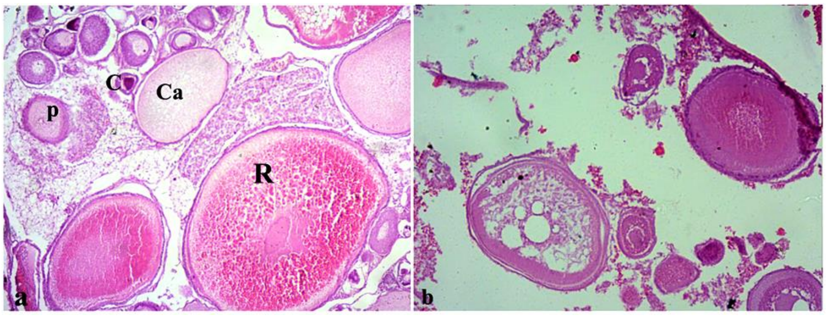

Figure (5): Ovary of *O. niloticus* showing normal stages of ovarian follicles, chromatin nucleolar oocyte (C) perinucleolar oocytes (P) cortical alveolar oocyte (Ca) and mature ripped oocyte (R) in control fish (a) edema, degeneration and severe necrosis of developmental stages of oocytes along with degenerated mature ripped oocytes in WSF treated fish (b) H&E. X 400.

DISCUSSION

Exposure of Red Tilapia fish to water soluble fractions of crude oil showed mortality even at low concentrations. Toxicity of substances based on their median lethal concentration (LC_{50}) the 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 LC_{50} 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 Lc_{50} 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). Lc_{50} which is the lethal concentration was observed in this study start from 0.01%. Bonny light crude oil is toxic to (*Sarotherodon melanotheron)*.

By far the most studied pathological effect of 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 hydrocarbon 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 accommodated fraction (WAFs), dispersed oil, Blood vessel congestion was 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 WAFs 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 vascuolation of the kidney tissue of *Tilapia guineensis* fingerlings compared with the normal. The kidney micro-photoscope 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.

PAHs have been found to disrupt the endocrine system and affect reproductive function and growth of fish (Horng *et al.,* 2010). Exposure to PAHs in fish has been linked to reduced investment in gonadal tissues (Booc *et al.*, 2014) and interference with steroid metabolism (Monteiro *et al.,* 2000). In maturing female fish, exposure to PAHs has been found to impair oocyte developpment, increase prevalence of atresia and decrease steroid plasma levels of estradiol-17β (E2) and testosterone (T) (Arukwe and Goksøyr, 2003). In males, PAHs exposure has been found to suppress spermatogenesis in clams (Frouin *et al*., 2007) and increase testosterone (T) production in goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) by promoting testicular steroidogenesis (Evanson and Van Der Kraak, 2001). Chronic pollution may lead to a decrease in quality of gametes, thereby impairing reproductive success and posing a significant threat to the sustainability of fish population (Doherty *et al.,* 2013).

Vignet *et al.*, (2016), investigated the effects of the polluted water from El-Salam Canal in *Oreochromis niloticus*, *Tilapia zillii* and *Synodoniis schall* and determined the seminiferous tubular epithelial degeneration in low dose group and significant tubular atrophy as well as germ cell degeneration in high dose group. As reported that in the testis, degenerative and necrotic changes in the cellular elements of seminiferous tubules, with inhibition of spermatogenesis.

Oocyte atresia is a degenerative and resorptive process, most often of vitellogenic eggs is a normal physiological event. A number of investigators have described atresia in teleost ovaries (Mytilineou 2000).

Atresia is characterized by the disintegration of the nucleus, vitelline envelope breakdown and increase in number and size of follicular (granulosa) cells; liquefaction of yolk globules with follicular cells entering the oocyte to phagocytize degenerating material; degeneration of the follicular cells once yolk resorption is complete and eventually fibroblast-like cells around yellowish-brown material (lipofuscin/ceroid) remain, Long term exposure in the study of Stott *et al.* (1983) showed that atretic follicles were seen in all developmental stages and were most prominent in growing and mature follicles. Collapsed zona pellucida of atretic mature follicles formed some bizarre shapes.

The only time atresia was especially prominent was in the ovaries of plaice fish, Histological defects similar to those found in the present study have already been described before by other authors (Dutta and Maxwell, 2003; Dutta and Dalal, 2008; Pieterse *et al.*, 2010).

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 has different histological alteration in fish organs.

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سمية الأجزاء الذائبة في الماء من زيت البترول الخام و تأثيراتها التغيرية النسيجية على سمكة البلطي النيلي

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الملخص العربي

تم إجراء خمسة معاملات مشتملة على المجموعة الضابطة و أربعة تركيزات من الأجزاء الذائبة من زيت البترول الخام (5، 6، 8، 10 جزء في المليون) و كذلك تم استخدام ثلاث مكررات و عشرة أسماك من البلطي الأحمر بمتوسط وزن 8جم ±1.5 لكل معاملة وذلك لمدة 96 ساعة. قيست الجرعة المميتة لخمسين بالمئة من حيوانات التجربة بعد 96 ساعة وكانت (5.25 جزء في المليون). أجريت الإختبارات النسيجية على كل من الكبد و الخياشيم و الكلى و المناسل الذكرية و الأنثوية. أوضحت قطاعات الكبد إحتقانا في الأوعية الدموية وبعض التجاويف وكذلك تغيرات بؤرية نخرية في خلايا الكبد. أظهرت الخياشيم إحتقانا في الأوعية الدموية و كذلك تضخما و إلتصاقا في الصفيحة الخيشومية الثانية. أظهرت الكُلي إحتقانا في الأوعية الدموية و تدهورا في الأنابيب الكُلوية. أظهرت المناسل الذكرية تدهورا و نخرا في بعض الأنيبيبات المنوية و استسقاءا و إنخفاضا و تدهورا في مراحل تكوين الخلايا المنوية. أظهرت المناسل الأنثوية نخرا شديدا في مراحل تطور البويضة مع تدهور واضح في البويضات الناضجة وذلك في المجاميع المعاملة مقارنة بالمجموعة الضابطة.

الكلمات الدالة: الأجزاء الذائبة في الماء- زيت البترول الخام- السمية قصيرة المدى- التشريح المرضي.