Effect of crude petroleum oil on the intestinal tissue of the fish *Oreochromis niloticus.*

Saber, A., Sakr ; Hamed, M. A., Mutwally and Adnan, M., Hijji.

Biology Department, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, P.O. Box 3711, Saudi Arabia.

ABSTRACT

The histological effect of crude petroleum oil on the intestine of the fish *Oreochromis niloticus* was investigated. Exposing fish to 50 and 100 p.p.m of crude oil induced many histopathological altarations. The epithelium of the mucosal folds showed marked discontinuity with many eroded tips and the cell boundaries were ill-defined. The muscular layer exhibited severe fragmentation and degeneration. Moreover, the general carbohydrates showed apparant decrease in the mucosal epithelial cells of the treated fish. It is speculated that one or more of the crude oil hydrocarbons are responsible for the observed histopatholgical effects.

INTRODUCTION

The effects of crude oil on aquatic organisms has increased in recent years due to the incidence of accidental oil spills. The toxicity of crude oil or refined petroleum to fishes has received much attention by many investigators. Mitrovic (1972) found that oil and oil products at 0.001 ml /L accelerated the death of the fishes *Sargus annularis; Crenilabus tinca* and *Mugil saliens*. Anderson, et al. (1974) mentioned that the two crude oils, a Bunker C oil and No.2 feul oil were toxic to *Cyprindon variegatus, Fundulus similus* and *Menidia beryllina.* Ghatak and Konar (1990) reported that the petroleum product n-heptane was toxic to *Tilapia mossambica*. Moreover, oil and oil products in sublethal doses were reported to produce histopathological changes in the organs of different fishes (Vishnevetsku, 1961; Mitrovic, et al., 1968; Mazhar, et al., 1987; Hijji & Saleh , 1994). The present work was aimed to study the effect of crude petroleum oil on the intestinal tissue of the fish *Oreochromis niloticus* .

MATERIALS AND METHODS

Fish *Oreochromis niloticus* (300 ±5g each) were obtained from breeding pond of fishing farm near Makkah. They were transported to the laboratory and were keept in especially equipped aquaria which were continuously aerated by air pumps. Fish were provided twice daily with tetramin (Tropical fish food, Tetraweke, Germany). They were acclimated for one week in the aquaria before the beginning of the experiment.

The crude petroleum oil used in this study was taken from Jeddah field Red Sea Station, Saudi Arabia.

Fish were divided into 3 groups each containing 25 fish. The first group was exposed to 50 p.p.m crude oil for 10 days while the second group was exposed to 100 p.p.m oil for 5 days. The third group was used as control. For histological study, specimens were taken from control and experimental groups and dissected. After dissecsion, small pieces of the intestine were removed and fixed in Bouin's fluid. The tissue was then embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. For the detection of general corbohydrates, portion of the intestine was fixed in Carnoy's fluid and stained by periodic acid Schiff's method (Hotchkiss, 1948).

RESULTS

a) Normal Intestine :

The intestine of *O. nilotica* is a long coild tube histologically, it consists of two layers, mucosa and muscularis. The mucosa is thrown into numerous folds which protruding into the lumen. The intestinal mucosal epithelium composed of two majar cell types: the columnar cells and goblet clls which are interspersed between the columnar cells. The crypts or glands of Lieberkuhn were not observed. The columnar cells showed a striated apical border with a basophilic cytoplasm and a basal nucleus. At the base of the mucosal folds, an irregular layer of smooth muscle was found surrounding the epithelium and extends into

the connective tissue core of the mucosal folds. The muscularis composed of circular and longitudinal muscle fibers followed by the lamina propria layer which contain blood capilleries (Figures 1&2).

b) Intestine of Treated Fish :

Intestine of fish exposed to 50 p.p.m of crude oil showed certain histopathological changes. The connective tissue core of the mucosal folds became enlarged in comparison with that in the control fish. The lining epithelium of the mucosal folds has exhibited marked discontinuity with many eroded tips. The cell boundaries were ill-defined being almost undetectable in many parts (Figure 3). Moreover, the muscularis was found to contain large vacuoles separating the circular muscle fibers from the longitudinal one (Figure..4).

These signs of deterioration appeared to have been more enhanced in fish exposed to 100 p.p.m crude oil. The core of mucosal fold became very large, deteriorated and structureless. The lining epithelium showed distinct signs of destruction. Considerable parts of the epithelium have broken off and the great majority of the mucosal cells were no more having distinct bounderies being mostly replaced by a syncytial structure. The rest of the cells showed clear symptoms of necrosis (Figure 5). The muscle fibers manifested severe fragmentation degeneration and became highly separated from each other leaving large vacuoles in the muscularis layer (Figure 5).

Concerning, the general carbohydrates, using the periodic acid Schiff 's method (PAS), carbohydrate inclusions were appeared as magenta coloured components. In the intestine of the control fish, the striated borders of the columnar cells and the goblet cells gave a positive PAS reactivity (Figure 6). Exposing fish to 50 p.p.m of crude oil induced slight reduction in PAS-positive materials of the striated borders of the columnar cells and goblet cells (Figure 7). However, the muscularis layer showed a marked reduction in PAS-positive

materials. This reduction of PAS-positive materials became more conspicous in fish exposed to 100 p.p.m of crude oil (Figure 8).

DISCUSSION

The present results revealed that exposing fish *Oreochromis nilotica* to crude oil induced histopathological changes in the intestine. The lining epithelium of the mucosal folds were degenerated with many eroded tips and the muscle fibers became highly separated from each other leaving large vacuoles in the muscularis layer. Similar results were observed in the intestine of *Clarias lazera* exposed to crude oil from Balayim field, Sinai, Egypt (Mazhar, et al., 1987). DiMichele and Taylor (1978) reported that naphthalene, a component of many petroleum mixtures, has been shown to induce extensive necrosis of the gut mucosa in *Fundulus heteroclius*. Reichenbach- Klinke (1965) studied the effect of low phenol concentration (0.02 to 0.07mg /L) on 14 fish species cought a live in the Rhine and Elbe. He found serious lesions in the gills and inflammatory and destructive changes in the skin, intestine and liver . Several other investigators gave reported histopathological changes in various fish organs exposed to oil and oil derivatives. Hepatic damage, local haematomas and occluded blood vessels were produced in fish exposed to crude oil (Vishnevetsku, 1961). Mitrovic, et al (1968) observed that in subacute poisoning by phenol , a general intoxication of fish organism occurs followed by inflammatory and necrotic changes of vital organs, gills, brain, liver, Kidneys, spleen and gonads. Exposing fish *Oreochromis nilotica* to crude oil was found to induce many histopathological changes in the liver and gills (Hijji & Saleh, 1994).

Exposing fish to crude oil, in the present work, induced depletion of general carbohydrate contents of the intestinal epitheliuum. Similarly, glycogen and lipid depletion has been reported in *Fundulus heteroclitus* collected near an oil spill (Sabo, et al., 1975). Hawkes (1977) observed depletion of glycogen in the liver of rainbow trout that received prudhoe Bay crude oil in their diets.

Carbohydrate depletion observed in the intestinal epithelium under the effect of crude oil is similar to those observed by treating fish with different pesticides. El-banhawy, et al. (1986) reported a decrease of carbohydrate contents in the ileal epithelial cells of *Clarias lazera* exposed to the insecticide , cyolane. The same results were obtained by Sastry and Malik (1979) in the fish *Channa punctatus* exposed to dimecron . Exposure of the catfish *Macrones keletius* to gradual sublethal concentrations of dimethoate induce inhibition of amylase activity in the intestine (Hameed & Vadamali , 1986). Asztalos, et al . (1990) showed that treatment with copper sulphate, paraquate and methidation caused tissue damage and stress in fish *Cyprinus carpio* indicated by the increase of LDH and GOT enzyme activity and elevated blood sugar level .

It is well known that the effect of crude oil on marine life is attributed to the presence of hydrocarbons (Blumer, 1969). In the present work, it is speculated that one or more hydracarbons or its metabolites are responsible for the histopathological effects recorded in the intestine of O. *nilotica* .

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EXPLAINATION OF FIGURES

Figure 1. Section of intestine of intestine of a control fish showing the muscularis (m), mucosal fold (F) and the lumen (L), x 120 .

Figure 2. Enlarged mucosal fold of a contral fish showing the columnar epithelial cells (EP) and the core of the mucosal fold (C), x 300.

Figure 3. Section of intestine of a fish exposed to 50 p.p.m crude oil showing enlarged mucosal core (C) and degnerated mucosa with eroded tips (ET), x 200.

Figure 4. Enlarged portion of intestine of a fish exposed to 50 p.p.m crude oil showing large vacuoles (V) in the muscularis, x 300.

Figure 5. Section of intestine of a fish exposed to 100 p.p.m crude oil showing large and deteriorated core (C); broken epithelium (arrows) and large vacuoles (V) in the muscularis (m), x 200 .

Figure 6. Section of intestine of a control fish showing marked PAS-positive materials in the goblet cells (g) and striated borders of the columnar cells (sb) and muscularis, (m), x 120.

Figure 7. Section of intestine of a fish exposed to 50 p.p.m crude oil showing reduction in PAS-positive materials in the goblet cells (g) and the striated borders of the columnar cells (sb), the muscularis showed a marked reduction in PAS-postive materials, x 200 .

Figure 8. Section of intestine of a fish exposed to 100 p.p.m crude oil showing obvious reduction of carbohydrate inclusions, x 200.

تأثير التلوث بزيت البترول الخام على انسجة أمعاء أسماك البلطى النيلى

صابر عبدالرحمن صقر ، حامد محمد متولى ، عدنان محمد حجى

قسم الأحياء-كلية العلوم التطبيقية-جامعة أم القرى-مكة المكرمة-ص. ب.-3711 - المملكة العربية السعودية .

الملخص:

درس هذا البحث تأثير التلوث بزيت البترول الخام على أمعاء أسماك البلطى النيلى. عند تعرض الأسماك لجرعة مقدارها 50 ، 100 جزء في المليون من زيت البترول الخام أظهرت الفحوص الهستولوجية تهدم حواف الخلايا العمادية في الطبقة المخاطية ، وأنفصلت الطبقة العضلية عن باقى طبقات الأمعاء وظهرت بها فجوات كبيرة . كذلك بينت النتائج أن تعرض الأسماك لزيت البترول ا لخام قد أدى إلى نقصان واضح فى المحتوى الكلى للكربوهيدرات في خلايا الطبقة المخاطية . افترض في هذا البحث أن التغيرات الهستوباثولوجية التى حدثت فىالامعاء قد ترجع إلى واحد أو أكثر من المركبات الهيدروكربونية الموجودة في زيت البترول الخام.