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Laboratory acute toxicity test of water soluble fractions on *Liza carinata* fingerlings to assess vulnerability field data of the Gulf of Suez

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ABSTRACT

Median lethal effect of the water soluble fraction (WSF) of crude oil on the survival of the fingerlings of mullets (*Liza carinata*) was investigated under laboratory conditions for 96 hours. The tested concentrations of WSF of crude oil were (2.5, 5, 10, 15, 20, 30, 40 and 50 %), which correspond to 0.195, 0.39, 0.78, 1.17, 1.56, 2.34, 3.12 and 3.9 ppm of total petroleum hydrocarbons content (TPH), respectively of the test water. The study was prepared in two groups (A and B). No mortality was recorded in 2.5-10 % of toxicant, while 12.5 % mortality was recorded in the 15 % of the toxicant. In 20 % of toxicant 37.5 % mortality was recorded, while in 30 % of toxicant, 50 % mortality was recorded and 87.5 % mortality was recorded in the 40 % of toxicant. 100 % mortality was recorded in 50 % of toxicant at 96 hours. The 96 hours LC50 for both batches was 27.81 % (2.17 ppm). It was observed that mortality was concentration-dependent: the higher the concentration, the higher the mortality. However, it was observed that the field concentration of TPH ranged between (0.009-0.39 ppm) from (0.5-3km) near petroleum refineries to end point of the offshore. This range of TPH in the field studied doesn't reach to the range of lethal concentration, but still at sub lethal concentration.

Key words: LC_{50} test, water soluble fraction, *Liza carinata*, fingerlings, TPH, Gulf of Suez.

INTRODUCTION

Crude oil is a complex mixture containing many types of compounds. Global demand for crude oil and associated products has increased dramatically over the last half of this century, and the demand is growing still. Therefore, oil companies have maintained a firm holds on the developed world's need for energy. This increased demand means more extraction; transport and manufacturing of crude oil in future which are inevitable, and serves an additional source of stress for aquatic organisms.The degree of hazardous effect of crude oil products is dependent on their concentration, chemical components and solubility in water. These

products have been recognized as a potential environmental contaminant shortly after the beginning of 20th century (Albers, 1995).

Water soluble fraction (WSF) of petroleum is that small fraction of oil containing components which are fully or sparingly soluble in water (Kavanu, 1964). WSF is produced during a long period of oil water contact which may result from delay in cleaning operations after an oil spill (Baker, 1970). It can be incorporated in the food chain and exert toxic effects on aquatic biota (Venkatessan *et al.,* 1980). This is so because this fraction comprises toxic components such as the polycyclic aromatic hydrocarbons (PAHs), mono-aromatic hydrocarbons like benzene, toluene, ethylbenzene, xylene (BTEX); phenols, heterocyclic compounds and heavy metals (Rodrigues *et al.,* 2010). The aliphatic hydrocarbons once regarded as non-toxic have now been recognized as significantly toxic (Manahan, 1992). Exposure of aquatic organisms to crude and refined oils, water soluble and water accommodated fractions of crude oil have been shown to impact on various aspects of fish physiology and sometimes leading to large scale mortality (George *et al.,* 2014). Fish have been the most popular test organisms because they are presumed to be the test understood organisms in the aquatic environment. Also, fish have an elemental position in relation to man and his food chain. So, fish can be used as bio-indicators to evaluate the environmental contamination levels of hydrocarbons (Anyakora *et al.,* 2005).

Toxicity can be defined as the negative effects on organisms caused by exposure to a chemical or substance. These negative effects may be lethal or sub lethal. Toxicity tests are designed to determine the specific concentrations of chemicals that induce a measured effect on a target organism. A common approach for acute toxicity testing is the LC_{50} test; which determines the concentration at which exposure to a substance causes mortality to half of the test population under standard laboratory conditions. The use of acute toxicity for testing the potential hazards of chemical contaminant to aquatic animals was studied by Hutchinson *et al.* (2006). However, few researches about the toxicological effect of petroleum hydrocarbons compounds in the environment of the Gulf of Suez are found. Therefore, the aim of this study is to determine LC50 of water soluble fractions (WSF) of crude oil on *Liza carinata* fingerlings in toxicity test and compared with the studied total dissolved petroleum hydrocarbons field data. In order to assess the vulnerability of these compounds on fish life and to take the required necessary precaution to protect them. Also, this study will include examination of the effect of changes in physico-chemical parameters of the water tested, i.e. water temperature, salinity, pH, DO and nutrient salts.

MATERIALS AND METHODS

1. Sources of test compounds:

The test compound (crude oil) was obtained from oil company (Suez Company of Petroleum Refinery) in stoppered can and transported to the laboratory.

2. Sources of test organisms:

Liza carinata fingerlings were obtained from Suez farm in Suez city, and were brought to the laboratory for acclimatization. *L. carinata* (Mullets) belongs to family (Muglidae) is a Red Sea species (Masuda *et al.*, 1984) which is selected as suitable test species due to commercially important, easily maintained under laboratory conditions and provide sufficient numbers of an appropriate size and age. They were collected in the morning between 8.00 am and 9.00 am, when the temperature was low enough to prevent heat stress. The number of animals collected at the experimental period was about 300. The fingerlings were (3.9 ± 0.5 cm) in length and (0.51 ± 0.06 gm) wet weight.

3. Acclimatization:

In the laboratory, fingerlings of *L. caranata* were acclimatized in glass aquaria for 7 days to be accustomed to the laboratory environment. During the acclimatization period they were fed with fish commercial food, and aerated with air stone connected to electrically powered aquarium pumps.

4. Preparation of water soluble fractions (WSF):

The WSF was prepared according to Anderson *et al.* (1974). A sample of crude oil (500 ml.) was slowly mixed with seawater (500 ml.) with which the fingerlings were cultured; the crude oil-water mixture was stirred slowly for 24 hours with a magnetic stirrer. This was to enhance the dissolution in the water of the water-soluble components of the crude. The mixture was made to stand for 3 hours before it was poured into the separating funnel and allowed to stand overnight so as to obtain a clear oil-water interphase. The lower layer of water, containing the WSF of crude oil was decanted into a clean round bottom flask with stopper, and was used as 100% strength for sample "lower phase" (WSF). This process was repeated several times until sufficient quantity of the WSF was obtained to carry out the study. The total petroleum hydrocarbons content (TPH) was measured by

Digital Spectrofluorometer (Sequoi- Turner corporation, made in USA.) model 450 with NB 360 for excitation filter and SC 415 for emission filter, where NB 360 is a narrow band excitation filter, 360 nm peak and SC 415 is a sharp cut emission filter, transmits > 415nm. Both of these filters are used for heavy petroleum fraction.

5. Exposure of test organisms:

Glass tanks of about 30 liters capacity were used as the holding tank, and glass bowls of about 2 liters capacity served as the LC_{50} test containers. Healthy fingerlings of *L. caranata* with similar sizes were taken from the holding tanks to LC_{50} test containers using hand net. Experiment started with eight fingerlings in duplicate test containers were introduced to the following range of WSF concentration: (2.5, 5, 10, 15, 20, 30, 40 and 50 %), and the total hydrocarbons content of the test water were (0.195, 0.39, 0.78, 1.17, 1.56, 2.34, 3.12, and3.9 ppm, respectively) and the control one. Mortality was noticed once in every 24 hrs over till end the 96 hrs (4 days period). The numbers of fish's deaths recorded during the test period with different concentrations.

6. Measuring of water quality parameters:

Water quality parameter was measured according to APHA (2005) and Parsons *et al.* (1984) before start of the experiment. This includes water temperature, salinity, pH, DO, nitrite (NO_2-N), nitrate (NO_3-N) and ammonia (NH_3-N).

7. Statistical analysis:

Median lethal concentration (LC_{50}) was calculated according to Arithmeke method (Karber, 1977) (adapted by Dede, 1992). The numbers of dead organisms between control and experimental group were analyzed using R square (R^2).

$LC_{50} = LC_{100} –((\Sigma$ Mean death X Conc. Diff) / No. of organisms per group))

RESULTS AND DISCUSSION

Laboratory studies on oil toxicity have shown to be complementary to research conducted in the field, where the field studies allow for less control of environmental variables but allow for investigations that may not be possible in most laboratory experiments.

In the present study water quality parameters were measured before start of the experiment. The values of water temperature, salinity, pH, DO, nitrite (NO_2-N), nitrate (NO_3-N) and ammonia (NH_3-N) were 40 ^{0}C; 35 ‰; 8.4; 6.1 mgO_2l^{-1}; 5.9 µmoll^{-1}; 11.91 µmoll^{-1} and 37.53 µmoll^{-1}, respectively. Also, their respective values measured during 96 hrs of LC_{50} test ranged

between (40.10-39.90 ^{0}C); (35.20-34.90 $\%_{0)}$; (8.50-7.80) ; (6.20-5.78 mgO_2l^{-1}); (6.10- 5.72 $\mu moll^{-1}$); (13.78-12.10 $\mu moll^{-1}$) and (38.45-37.67 $\mu moll^{-1}$). So, it was observed that concentrations of the investigated water parameters were not significantly different from those likely to have no role in the toxicological effect.

It was noticed that no mortality occurred at the concentrations of WSF at 2.5, 5, and 10% with TPH 0.195, 0.39, and 0.78 ppm, respectively. Figure (1) shows that the mortality percentage was 12.5 %, at concentration 15 % of WSF at 1.17 ppm TPH after 96 hrs. On the other hand, at concentration of 20 % WSF (TPH 1.56 ppm) the mortality reached to 25% for two days (after 24 hrs and 48 hrs), while, it was 37.5% after 72 and 96 hrs (Fig. 2). Moreover, Figure (3) represented the acute toxicity of WSF at concentration of 30% (TPH 2.34 ppm) after exposure of 96 hrs and the mortality reached to 50 %. The concentration of 40 % WSF (TPH 3.12ppm) was more than acute toxicity where the mortality recorded 50% at 24hrs and after 96hrs the mortality reached to 87.5 % (Fig. 4). It was obvious from Figure (5) that the WSF concentration 50% (3.9 ppm) was more dangerous and it recorded mortality percentage about 62.5 % after exposure to 24 hrs and reached to 100% after 96 hrs.

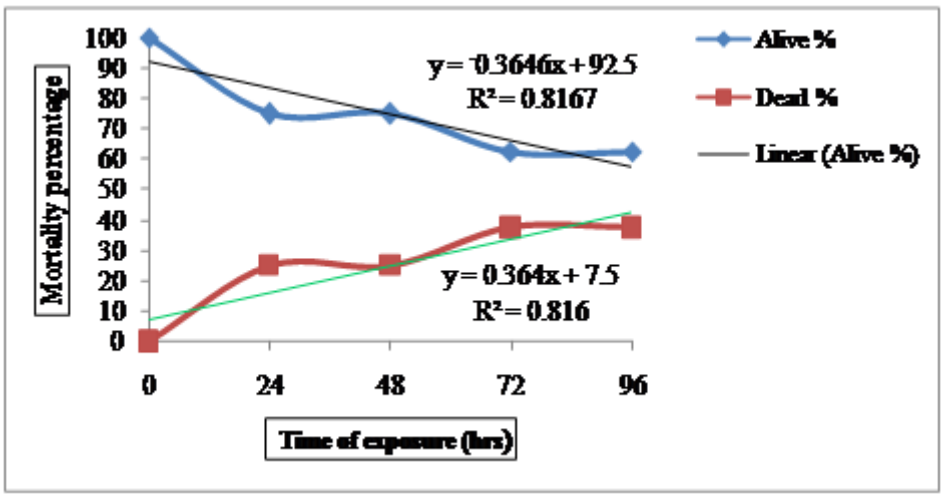

Fig. (1):LC_{50} test of WSF 15 % (TPH 1.17 ppm) on *L. carinata* fingerlings during 96 hrs.

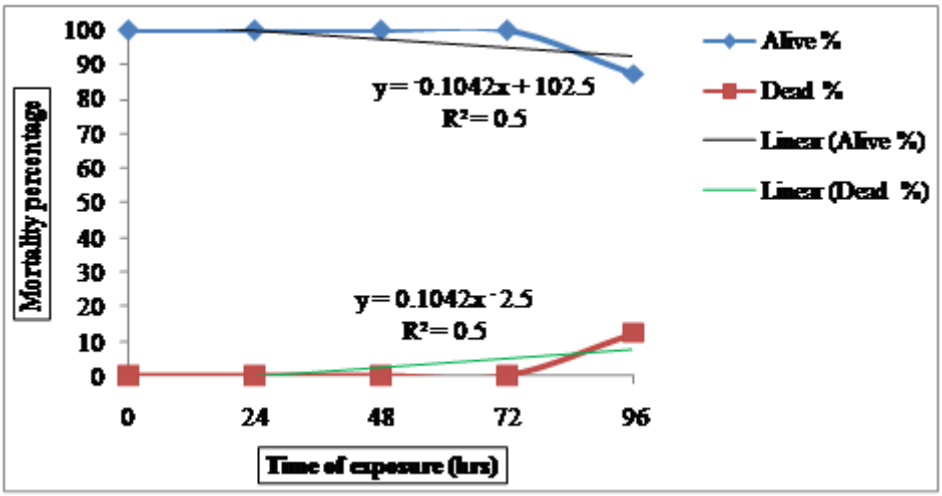

Fig. (2): LC_{50} test of WSF 20 % (TPH 1.56 ppm) on *L. carinata* fingerlings during 96 hrs.

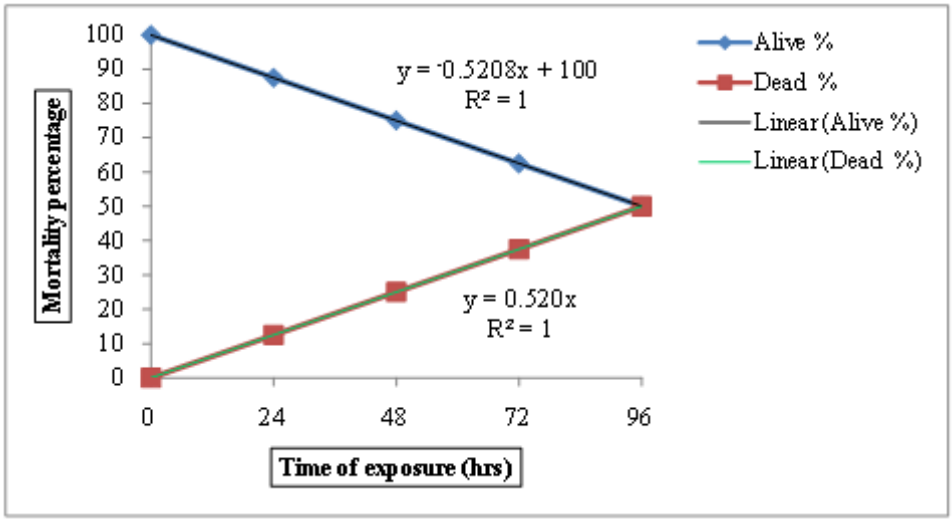

Fig. (3): LC_{50} test of WSF 30 % (TPH 2.34 ppm) on *L. carinata* fingerlings during 96 hrs.

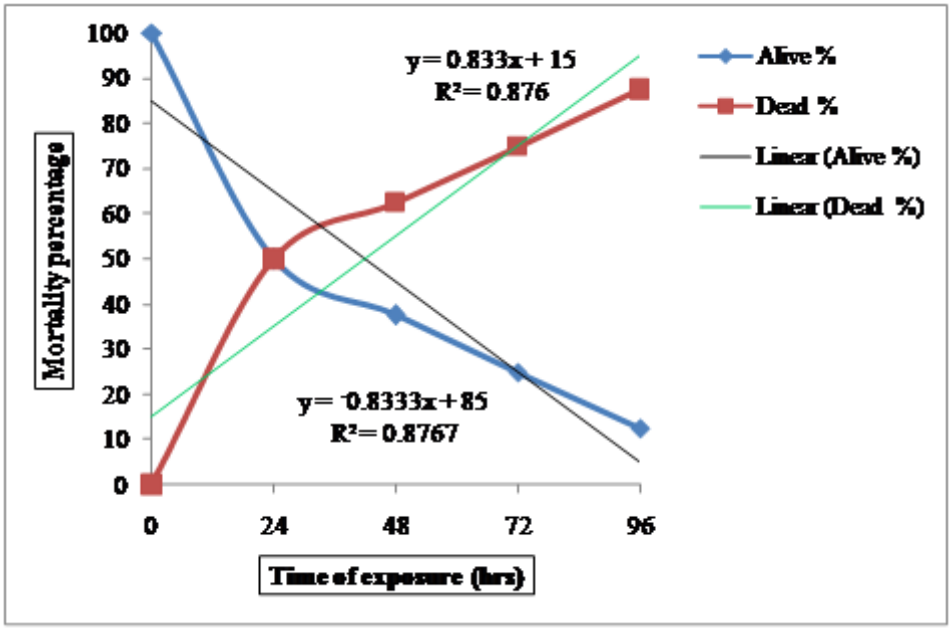

Fig. (4): LC_{50} test of WSF 40 % (TPH 3.12 ppm) on *L. carinata* fingerlings during 96 hrs.

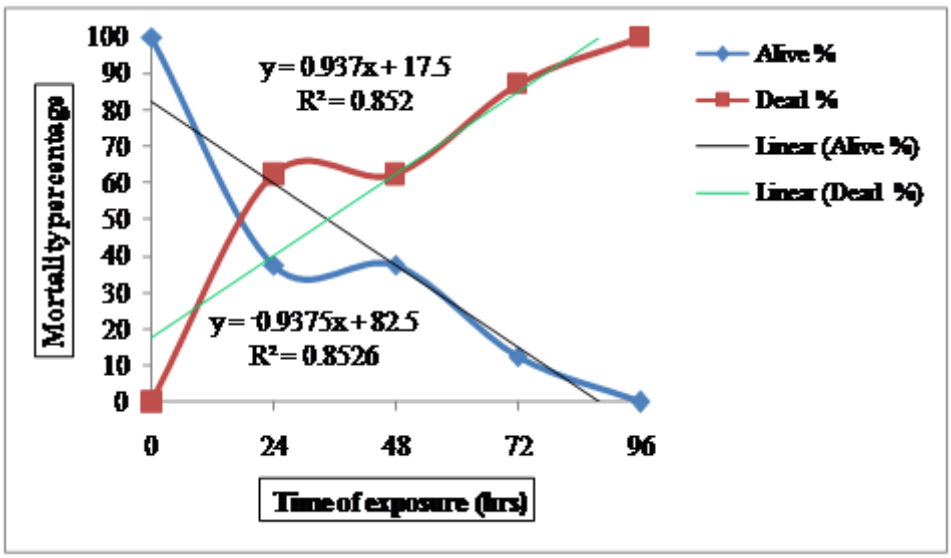

Fig. (5): LC_{50} test of WSF 50 % (TPH 3.9 ppm) on *L. carinata* fingerlings during 96 hrs.

The present study exhibited the LC_{50} at each concentration percentage test occurs at different times depend on the dose of exposure and bioavailability of the bio-organism to uptake the toxin. Where oils and most of the refined products as water soluble fraction have a narcotic effect on fish; acute symptoms are effects on the nervous system and respiratory activity. The main clinical symptoms include an initial increased activity and respiratory rate followed by a loss of balance, loss of response to stimuli, reduced activity, shallow respiratory movements, loss scales and ultimately death (Zdenka *et al.*, 1993). As well as the fraction of petroleum PAHs cause toxic as increased binding affinity to receptors could be related to their enhanced toxicity (Turcotte *et al.* 2011). There are also large differences between oil and its different products as to their toxicity to fish; most of them have 48h LC_{50} values within the range of petroleum hydrocarbons 0.5 to 200 mg per liter. The toxicity varies according to the chemical composition of the different products, with the water solubility of the different petroleum hydrocarbons.

The Median lethal concentration (LC_{50}) was calculated according to Arithmetic method (Karber, 1977) (adapted by Dede, 1992). Table (1) represented the data required to calculate the LC_{50} theoretically as follow:

$LC_{50} = LC_{100} -((\Sigma$ Mean death X Conc. Diff)/ No. of organisms per group))

$LC_{50} = 50 - (177.5 / 8)$

$LC_{50} = 50 - 22.1875 = 27.8125$ %

LC_{50} represents 2.17 ppm or mgl^{-1} of WSF concentrations in this experiment.

Table (1): Median lethal concentration of WSF during toxicity test.

Conc. %	Conc. Difference	No. of live	No. of dead	Mean death	Mean death (dose diff.)
0	0	8	0	0	0
2.5	2.5	8	0	0	0
5	2.5	8	0	0	0
10	5	8	0	0	0
15	5	6	2	1	5
20	5	5	3	2.5	12.5
30	10	4	4	3.5	35
40	10	2	6	5	50
50	10	0	8	7.5	75
					177.5

Laboratory acute toxicity test of water soluble fractions on *Liza carinata* fingerlings to assess vulnerability field data of the Gulf of Suez

The present results of laboratory toxicity test of water soluble fraction as petroleum product, reveal that the LC_{50} 96-hr according to The Median lethal concentration (LC_{50}) was calculated according to Arithmetic method (Karber, 1977) (adapted by Dede, 1992) was at concentration 2.17ppm.
Figures (6) and (7) compare with the field distribution of TPH started from near shore petroleum refineries to end point distance that far away from these petroleum activities and the concentration of LC_{50} red color column laboratory studied. It is discern that the field concentration of TPH don't reach to the range of lethal concentration, but still at sub lethal concentrations.

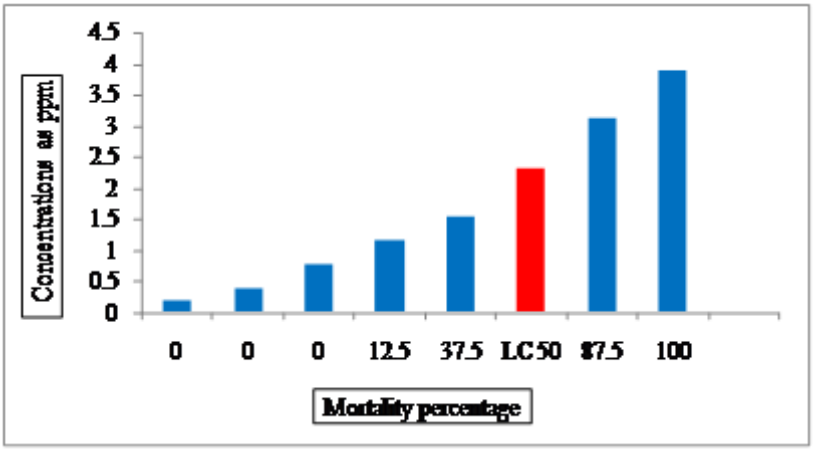

Fig. (6): Distribution of TPH in field studies from near petroleum refineries to end point of offshore.

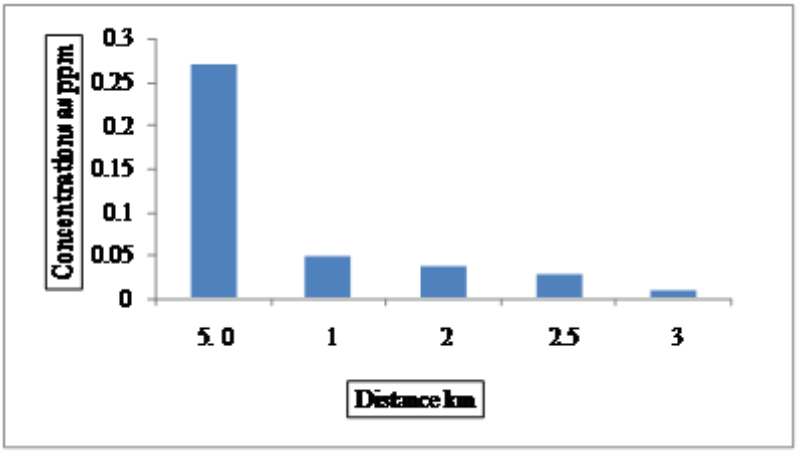

Fig. (7): Distribution of TPH in toxicity test of laboratory studies after 96 hrs.

The present field results indicated that the concentrations of TPH of the surface water of Suez Gulf didn't reach to this level of toxicity near or far from the source of pollution that ranges from 0.39 to 0.009 ppm.

Although, the chronic low levels of hydrocarbons in water in the field were under sublethal effects, yet they may produce biological responses in fish. Therefore, it is important to assess the toxicological significance of these sublethal effects or to define genuine harmful effects in contrast with effects parse.

Conclusion:

The results of this study indicated that the WSF of crude oil from (Suez Company for Petroleum Refineries) was slightly toxic to fingerlings of *Liza carinata* even at median (moderate) concentrations, although, the concentrations in the field studied along the surface water of the Gulf didn't reach to this level of toxicity near or far from the source of pollution. However, the continuous increase of crude oil activities and their discharge to the marine environment would cause adverse effects to aquatic environments, food chain and human at the long term. So, companies refining these products should be adhered to modern techniques of waste management and disposal.

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اختبار السمية المعملي للأجزاء الذائبة في الماء من النفط على أصبعيات سمكة السهلية للتحقق من مخاطر البيانات الحقلية في خليج السويس

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1 - معمل الكيمياء البحرية ، المعهد القومي لعلوم البحار والمصايد فرع خليجي السويس والعقبة

2 - قسم علوم البحار ، كلية العلوم ، جامعة قناة السويس.

المستخلص

تهدف الدراسة لتحديد التركيز النصف مميت للأجزاء الذائبة في الماء المستخرجة من النفط على أصبعيات سمكة السهلية *Liza carinata* . لوحظ عدم وجود وفيات لاصبعيات سمكة *Liza carinata* في تركيزات (2.5,5,10%) (0.195, 0.39, 0.78 جزء من مليون) خلال مدة الدراسة 96 ساعة. بينما ظهرت وفيات في تركيز (15%) (1.17 جزء من مليون) بنسبة (12.5, 25%) خلال 96 ساعة. وسجلت أعلى نسبة وفيات (100%) في تركيز(50%) (3.9 جزء من مليون) خلال 96 ساعة. وهذا يعني ان زيادة نسبة وفيات اصبعيات السمكه تزداد بزيادة تركيز المادة. سجل التأثير المميت لنصف كمية السمك 27.81% (2.17 جزء من مليون). بينما سجل تركيز المادة في مياه خليج السويس بالقرب من معامل تكرير البترول (0.39 جزء من مليون) وهذا يدل على أن تركيز المادة في مياه الخليج لم يصل الى التركيز النصف المميت لاصبعيات سمكة السهلية.