

Marsh Bulletin 4(1)(2009)1-20



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# Comparative toxicities of crude oil and refined oil products to several species of molluscs from Shatt Al-Arab river

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### **Abstract**

The present study includes toxicity experiments carried out under laboratory conditions for 24 - and 48 - hours periods by using renewal toxicity test system to determine the comparative toxicities of crude oil (Basrah regular crude oil) and refined oil products (diesel oil, gas oil, gasoline, grease oil, kerosene, motor oil, paraffin wax and automatic transmission oil) to several species of molluscs collected from Shatt Al – Arab river. These species of molluscs are snails, *Lymnaea auricularia*, *Theodoxus jordani*, *Physa acuta*, *Melanopsis nodosa*, and *Melanoides tuberculata*, and bivalves, *Corbicula fluminea* and *Corbicula fluminalis*. The toxicity experiments showed that the different types of oils vary widely in their toxicities to the species tested. The gasoline is clearly the most toxic fraction, while Kerosene is the next most toxic fraction, and is slightly less toxic than Basrah regular crude oil. The remaining fractions diesel oil and gas oil are relatively less toxic to the species tested, while the grease oil is almost non – toxic. Paraffin wax is non – toxic to all species, while the motor oil and automatic transmission oil are toxic to different species in varying degrees. There are some variation in the pattern of recovery rates of each species of snails from exposure to the oils.

The order of sensitivity of molluscs species tested were: L. auricularia > P. acuta > M. nodosa > T. jordani > M. tuberculata > C. fluminalis > C. fluminea. The overall acute effects of hydrocarbons on the species of molluscs tested are abnormal activities, narcosis and anesthesia, the loss of ability to react to the external cue, rapture the tissues and die.

#### 1-Introduction

Man's technological and scientific advances have caused environmental changes that are impossible to evaluate and fully comprehend. NRC (2003) reported that our ability to change the environment has increased faster than the ability to predict the effect of that change .

Rapid increase in the use of crude oil and its by-products has increased the levels of oil contamination in the marine environment (SenGupta *et al.*, 1993; API, 2001; Mai *et al.*, 2003). Dicks (1998) estimate that the influx of oil into the marine environment is approximately three million tons/years. The majority of this oil pollution is occurring in estuaries and near – shore waters.

The water of Shatt Al-Arab river receives the oil contaminants from varying origin including; accidental spills, normal operations of boating activities, runoff from land and introduction via sewage outfalls and disposal of oil waste material. In addition, some oil originates from atmospheric transport which includes hydrocarbons from combustion engines (Al-Saad, 1995). Oil pollution causes a serious threat to the river environment. High concentrations of oil kill the river plants and animals, even at very low levels, there is slow accumulation of oil by the river organisms (Farid, 1998).

Crude oils and refined oil products vary widely in chemical composition and physical properties . The toxicity of different types of oils has found to vary in experiments carried out on several species of organisms, as reported in a previous papers (Pace *et al.*, 1995; Singer *et al.*, 2001; NRC, 2003). From these and other experiments it is, evident that the light oils were more toxic than heavy ones. The most volatile fractions of oils are the most toxic, and that fresh oils are more toxic than residue.

This paper describes toxicity tests carried out in the laboratory to determine the comparative toxicities of crude oil (Basrah regular crude oil) and refined oil products (diesel oil, gas oil, gasoline, grease oil, kerosene, motor oil, paraffin wax, and automatic transmission oil) to several species of molluscs common in Shatt Al-Arab river. These ware Corbicula fluminea, Corbicula fluminalis (bivalves), Lymnae aurticularia, Theodoxus jordani, Physa acuta, Melanopsis nodosa and Melanoides tuberculata (gastropods).

## 2- Materials and Methods

## Chemicals:

Crude oil (Basrah regular - medium - API gravity between 28 – 34) and refined oil products (diesel oil, gas oil, gasoline, grease oil, kerosene, motor oil, paraffin wax) were supplied by Iraqi South Oil Company. They were transferred to laboratory by dark glass bottle closed tightly and kept in a cold and dark place prior to use. Table (1) represents the specifications of the refined oil products.

Automatic transmission oil (Fuchs Titan ATF) was supplied from Alhamrani-Fuchs petroleum Ltd., Saudi Arabia.

## Apparatus:

## **Thermometer**

A simple thermometer with range from 0 to  $100~^{\circ}\text{C}$  graduated at  $0.2~^{\circ}\text{C}$  made by Hanna company was used to measure water temperature .

# Dissolved oxygen meter

A dissolved oxygen meter type YSI 556 MPS (USA) was used to determine dissolved oxygen in water .

## pH - meter

A pH-meter type HI 8915 made by Hanna company was used to determine pH of water .

#### Salinometer

A digital salinometer E-202 type Tsurumi Seiki (Japan) was used to determine salinity of water .

## **Collection of molluscs samples:**

The specimens of molluscs, *Lymnaea* auricularia, *Theodoxus jordani*, *Physa acuta*, *Melanopsis nodosa*, *Melanoides tuberculata* (gastropods), *Corbicula fluminea* and *Corbicula fluminalis* (bivalves) were collected from Shatt Al-Arab river (along the region extended from Abu – Al - Khasib to Garmat - Ali ) during low tide in 2004 and 2005 (Figure 1). Each species consisted of at least 350 adult and of uniform size individuals.

## Acclimation of the test species (molluscs):

The species of molluscs were transferred to an aquarium for acclimation period of ten days prior to the toxicity experiments , under laboratory temperature of 20  $\pm$  2 °C with light / dark cycle ( 12:12 ) under aerated conditions .

## **Toxicity experiments:**

Experiments were carried out to determine the toxicity of several types of refined oil products compared with Basrah regular crude oil. The refined oil products investigated were diesel oil, gas oil, gasoline, grease oil and kerosene.

The three types of oil products were also investigated. They are motor oil , paraffin wax, and high quality paraffin automatic transmission oil (Fuchs Titan ATF). The toxicity of all these fractions was tested simultaneously with that of Basrah regular crude oil for comparison .

The experimental procedure adopted for toxicity determination was based on the method established by Pace *et al* . (1995). Batches of 50 animals of each species were exposed to each type of oil for a period of 24 - and 48 - hours in aquariums ( 40 × 22 × 15 cm³ in size ), followed by the 5-days recovery period in clean aerated river wate . The aquariums were covered with glass lids to reduce evaporation of hydrocarbons. The concentration of test solution of each type of oil consists of manually mixing 20 ml of oil with each liter of river water. The animals were left without food

during the exposure period . The recovery rate of each species of gastropod was recorded by noting the number of animals which crawled out of the water and stick to the sides of glass aquariums. Mortalities in species of gastropods were taken as the number of animals still immobile and remaining in the water after 5- days , in the case of bivalves , mortalities were

taken as the number of individuals showed no signs of life and with permanently gaping shells. These tests were set up in three replicates together with three control (untreated). All experiments were carried out in the renewal toxicity system for test from January to March, 2004.

Table (1): Specifications of Iraqi Refined Oil Products (Reference, Iraqi South Oil Company)

	Refined oil								
Property	Diesel oil	Gas oil	Gasoline	Lubricatin g oil	Kerosen e	Paraffin wax			
Specific gravity @ 15.6 °C maximum	0.8398	0.8398	0.7310 (Premium)		0.8017				
Viscosity CST. @ 37.8 °C maximum	12.5 – 18.5	6		65 – 500					
Aromatic Content volume % maximum					20				
Lead content gm / l. maximum			0.8						
Sulpher % weight maximum	2.5	0.5	0.2		0.1				
Oil content % maximum						0.2 - 0.9			
Melting point °C						60 - 68			

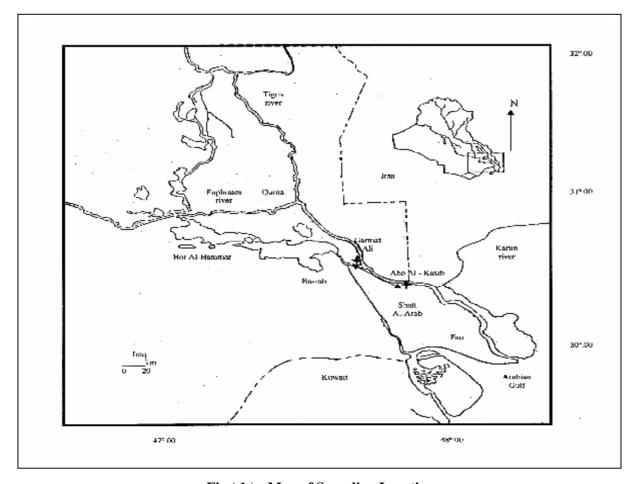


Fig.(1): Map of Sampling Location.

# **Acute effects of oils on test species (molluscs)**

Some acute effects of oils on species of molluscs tested are observed by monitoring the animals closely during the exposure periods.

## Test solutions:

The tests solutions of all experiments were monitored for temperature, dissolved oxygen, pH and salinity at regular intervals . The temperature was at 20  $\pm$  2°C . The dissolved oxygen ranged from 8.5 to 10.1 mg /1

. As to the other characteristics of tests solutions , pH from 7.1 to 7.8 and salinity from 1.6 to 1.8 ppt .

# 3- Results and Discussion

Tables ( 2 and 3 ) represent the mortalities percentage of the tested species after exposure to different types of oils for 24 - and 48 - hours followed by the 5 - days recovery period in the river water . It can be seen that the different

types of oils vary widely in their toxicity to the species tested . The order of toxicity of the oils tested is not exactly the same for all species . Considering Basrah regular crude oil and the refined oil products (diesel oil, gas oil, gasoline, grease oil and kerosene), gasoline is clearly the most toxic fraction. This particular gasoline was leaded, however, which may have contributed to its high toxicity. Kerosene is the next most toxic fraction, and is slightly less toxic than Basrah regular crude oil . The remaining fractions diesel oil and gas oil are relatively less toxic to the species tested , while the grease oil is almost non – toxic .

The three oil fractions investigated similarly vary in their toxicity to different species. Paraffin wax, as one might expect, is non – toxic to all species, while the motor oil and automatic transmission oil are toxic to different species to various degrees. Both motor oil and automatic transmission oil undoubtedly contain various additives, while the latter is distilled to remove the majority of the more toxic aromatic components which occur in commercial kerosene.

Figures (2-6) illustrated recovery rates of L. auricularia, T. jordani, P. acuta, M. nodosa and M. tuberculata during the 5 – days recovery period following exposure for 48 – hours to crude oil and various oil fractions.

The variation in the pattern of the recovery rates of each species of the snails following exposure to the oils, can be seen. The toxicities vary between oils, which is to be expected because the concentration composition of individual hydrocarbons within the oil vary. The refined oils are generally considered to be more toxic than crude oils, since smaller volumes of refined oils are needed to kill 50 % of the test animals in a given time. The increased toxicity of refined oils, as measured on a volume added basis, is primarily caused by two factors: (1) refined oils often have concentrations of aromatic hydrocarbons, (2) refined oils are usually less viscous than crude oils thus requiring less mixing energy for toxic concentrations to be mixed into the water (Boelsterli, 2000; NRC, 2003).

The order of toxicity of oils tested in the present study is similar to that found by other studies carried out using some or all of these fractions to different species of organisms . McDonald et al. (1991) tested the toxicity of petroleum products to fishes, and found that the fractions of tested gasoline were the most toxic, fuel oil somewhat the less toxic and bunker oil the least Peters and Scheuerman (1992) investigated the effects of oil mixed with sand on aquatic animals, and found in experiments on the hydrozoa and fish that crude oil was the most toxic and the lubricating oil the least toxic while fuel oil and diesel oil were of intermediate toxicity. In their experiments on the oyster they showed that diesel oil to be slightly less toxic than Russian crude oil, while heavy fuel oil was non - toxic to the intertidal gastropods of the Black sea . SenGupta et al .

(1993) found Kuwait residue to be less toxic than Kuwait crude oil to a number of intertidal species of molluscs. Al-Aabbawy (1999) found in her study on the acute toxicity of kerosene, gas oil and lubricating oil on two species of gastropods from Shatt Al - Arab river (M. nodosa and T. jordani) that kerosene was more toxic to snails followed by gas oil and lubricating oil respectively. Farid (2002) investigated the lethal toxicity of kerosene and gasoline on three species of snails from Shatt Al - Arab river, T. jordani, M. nodosa, and M. tuberculata. It is found that the gasoline was more toxic than kerosene . Altte (1988 ) studied the effect of kerosene and crude oils on the survival of crab and found that the kerosene was more toxic than crude oils.

The oil products, with low boiling points, thus appear to be considerably more toxic than the heavier fuel oils and bunker oils, while crude oils are intermediate with respect to toxicity (Talley, 2000). Pearson et al. (1995) found that the damaging influence of petroleum fractions on the marine organisms decreased with increasing boiling points of these fractions, while the phenomenon was partially inverted when the fraction had been emulsified. The low-boiling hydrocarbons present in oil include aromatics such as benzene, toluene, xylene, naphthalene and phenanthrene, all of which are water - soluble to some extent, and which are known to be toxic to a wide variety of marine organisms

(Baussant et al., 2001). Among the higherboiling crude oil fractions, Sterling et al. (2004) considered the polycyclic aromatic hydrocarbons to be seriously damaging long-term activity, poisons, inducing carcinogenic although the significance of this in the marine environment is still disputed. These polynuclear aromatic hydrocarbons, however, are present at least in minute concentrations in all marine waters because they are formed by aqueous flora (Mai et al., 2003), and it is unlikely that they would be present sufficient in concentration in marine waters to cause very much biological damage, except in the event of an oil spill in a very confined body of water. In addition to toxicity, the physical properties of refined oil products may cause biological damage. The degree of persistence of an oil depend on the physical nature of the oil . " Persistent "oils were defined by the API (2001) as crude, residual fuel and lubricating oils, also tar oils and creosote etc., while non persistent oils include all other distilled hydrocarbon oils, such as motor spirit, kerosene and gas oil, and also animal and vegetable oils. The persistent type of oils are more likely to cause biological damage by virtue of their physical properties, possibly for a considerable length of time, while the non-persistent types of oils are more likely to cause biological damage by virtue of their toxic nature for a relatively short period of time. Exposure of oil by spillage at sea results in fairly rapid disappearance of non-persistent oils by evaporation, while persistent oils become weathered in characteristic ways. The physical and chemical changes undergone by crude oil when exposed to weathering include an increase in asphaltene content, a slight increase in wax melting point, a decrease in sulphur content and increase in both viscosity and specific gravity ( Crawford et al., 2002; NRC, 2003). Ghio et al. (2002) investigated the biological effects of Kuwait atmospheric residue and certain residue blend, similar in physical properties to weathered crude oils. These residues were found to be effectively non-toxic, but their relatively high viscosity renders them physically dangerous to marine life. The more persistent types of oils may also be toxic to marine animals, especially when in the form of an emulsion. Dicks (1998) found that large quantities of Bunker C oil are taken up by a variety of invertebrates and distributed through their tissues and spectrofluorometric methods revealed the initial accumulation of this oil in the guts of flounders and lobsters.

Refined oil products thus vary widely in toxicity. In general, the low – boiling fractions are more toxic than crude oil, which in turn more toxic than the heavier, high – boiling residual fractions, some degree of reversal of this order of toxicity is apparent, however, when the oil is in the form of an emulsion. From the review of some of the major spillages in recent years it appears that the biological damage resulting from spillages of refined oil products is determined more by the degree of

persistence of the oil concerned than the absolute toxicity of the oil when fresh.

When assessing the potential impact of spill oil, it is crucial to know if some species or life stages are more sensitive than other to oil toxicity (Zakaria *et al.*, 2000; Vernberg and Vernberg, 2001).

In the present study, the order of sensitivity of species of molluscs tested are not exactly the same for all petroleum toxicants, but some trends are clearly evident that the sensitivity of animals are as the following trend; L. auricularia > P. acuta > M. nodosa > T. jordani > M. tuberculata > C. fluminalis > C. fluminea ( Tables 1 and 2).

The difference in sensitivity to petroleum toxicants among the species of molluscs may be due to the difference in the membrane structure of their bodies, their ability on the metabolism, excretion and storage of the toxicants and/ or the difference in the transportation of the toxicants into the site of action.

The difference in sensitivity to varying environmental conditions between different species is well known. Zakaria *et al*. (2000) reported that the tolerance of marine fauna exposed to oil pollutants increases in the series from fish, to higher vertebrates then to lower invertebrates. Tolerance also increases in the marine habitat series from pelagic animals to subtidal benthic animals and intertidal animals. Rice *et al*. (1976) tested 27 species of marine fish and invertebrates, and permitted the best comparisons of species sensitivities since

methods, temperature, ect., were all similar. Fish and shrimp were usually among the more sensitive species tested, while intertidal animals tolerant . Intertidal were generally more animals are probably more tolerant to static exposure because they can temporarily insulate themselves from the exposures, at least until the concentration in the static exposure declined to sublethal levels. The intertidal limpets and chitons were sensitive than the other intertidal animals, but this might be due to damage occurring when they were collected. Vernberg and Vernberg (2001) reported that sensitivities of cold-water species appear greater than sensitivities of similar species from warmer climates. Talley (2000) speculated that the cold - water species may appear more sensitive because lower temperatures increase the persistence of toxic aromatic hydrocarbons, even though there are differences in oils and species between the studies. Jaweir and Habash (1987) in their experiments on the toxicity of water-soluble hydrocarbons (WSF) of kerosene to two species of polychaetes from Shatt Al river (Namalycastic Dendronereides heteropoda), found that D. heteropoda was slightly more sensitive to WSF of kerosene than N . indica . The 96 - hours  $LC_{50}$  values recorded were 1.5 ml / 1 for D. heteropoda and 4 ml/l for N . indica . Laboratory studies on the snails (M. nodosa, T . jordani , L . aurcularia and M . tuberculata ) and the bivalves  $(C ext{ . } fluminalis ext{ and } C ext{ . }$ 

fluminea), a common inhabitant in the Shatt Al-Arab river estuary area, were made with crude oils refined products and oil fractions (Farid, 1998, Al-Aabbawy 1999, Farid, 2002, Farid and Farid, 2002, Farid  $et\ al.$ , 2002; Farid, 2003). These studies showed that the sensitivity of molluscs to oil pollutants were very different as following trends; L. aurcularia>M. nodosa>T. jordani>M. tuberculata>C. fluminalis>C. fluminea.

In the present study, some acute effects of oil hydrocarbons on the species of molluscs were observed by monitoring the animals closely during exposure periods. These effects are; restrict their normal activities, prevent their adhering ability to test vessels (including the snails only), bring narcosis and anesthesia, loss their ability to reach the external cue, rapture their tissues, leave their shells and finally die. Such effects are due to physical and /or chemical toxic effects of hydrocarbons which produce the abnormal activities in molluscs and die.

Boelsterli, (2000) reported the toxicity significance of the various fractions of hydrocarbons, the low boiling saturated hydrocarbons have been showed to produce anesthesia and narcosis at low concentrations, and at higher concentrations caused cell damage to a wide variety of animals. Higher – boiling saturated hydrocarbons may not be directly toxic, although it is suggested that they may interfere with nutrition. The aromatic

hydrocarbons are the most dangerous fractions. The low – boiling aromatics represent the acute toxic hazard , while the higher molecular weight polynuclear species may well be of significance in their long-term effects. Similar toxic effects of hydrocarbons on other species of aquatic organisms were also reported by (Kenchington , 1996 ; Dicks, 1998 ; Singer *et al.*, 2001 ; Birwell and McAllister, 2000 ; Farid , 2005 ) .

The tests of the this study were performed under laboratory conditions and therefore

inevitably did not provide a complete description of the undoubtedly more complex processes which took place in the natural environment . The results indicate , however , a number of toxic effects on an ecologically important species and that these may occur at comparatively high levels of oil pollution in the waters of Shatt Al – Arab river .

Table (1): Specifications of Iraqi Refined Oil Products (Reference, Iraqi South Oil Company)

	Refined oil								
Property	Diesel oil	Gas oil	Gasoline	Lubricating oil	Kerosene	Paraffin wax			
Specific gravity @ 15.6 °C maximum	0.8398	0.8398	0.7310 (Premium)		0.8017				
Viscosity CST. @ 37.8 °C maximum	12.5 – 18.5	6		65 – 500					
Aromatic Content volume % maximum					20				
Lead content gm / l. maximum			0.8						
Sulpher % weight maximum	2.5	0.5	0.2		0.1				
Oil content % maximum						0.2 - 0.9			
Melting point °C						60 - 68			

Table (2): Mortalities percentage of several species of intertidal molluscs from the Shatt Al - Arab river after 24 - hours exposure to different types of oils, followed by the 5 - days recovery period in the river water.

Species	Type of oil									
	Number	Busrah regular erode oil	Diesel oil	Gas oil	Gasoline	Greuse gil	Kerosene	Motor oil	Paratlin wax	Automatic transmission oil
L , aurcularia	50	58	15	11	73	5	67	33	3	83
F. jordani	50	16	3	5	29	3	11	3	0	20
P. acida	50	31	7	7	65	5	25	11	3	77
M . nodosa	50	19	7	7	52	6	21	11	0	47
M . Inberculata	50	14	3	5	25	U	5		0	21
C. floodiaer	50	4	0	3	*	11	11	0	ď	8
C floniaalis	50	6	0	5	11	n	3	0	0	3

Table (3): Mortalities percentage of several species of intertidal molluscs from the Shatt Al - Arab river after 48 - hours exposure to different types of oils, followed by the 5 - days recovery period in the river water.

Species	Type of oil									
	Number	Basrah regular crude oil	Diesel oil	Gas oil	Gasoline	Grease oil	Kerosene	Motor oil	Paratiin wax	Automatic transmission oil
L , $aurcularia$	50	63	18	19	78	9	72	36	8	87
T. $jordani$	50	20	8	12	34	6	16	10	6	27
P , $aona$	50	33	10	12	69	10	29	17	6	82
M , $nodoso$	50	24	9	11	57	7	26	16	6	50
M. tuberculata	50	20	6	9	29	4	9	7	5	31
C. flaminea	50	8	4	7	7	9	4	5	5	11
C. fluorinalis	50	11	4	8	18	3	6	7	4	16

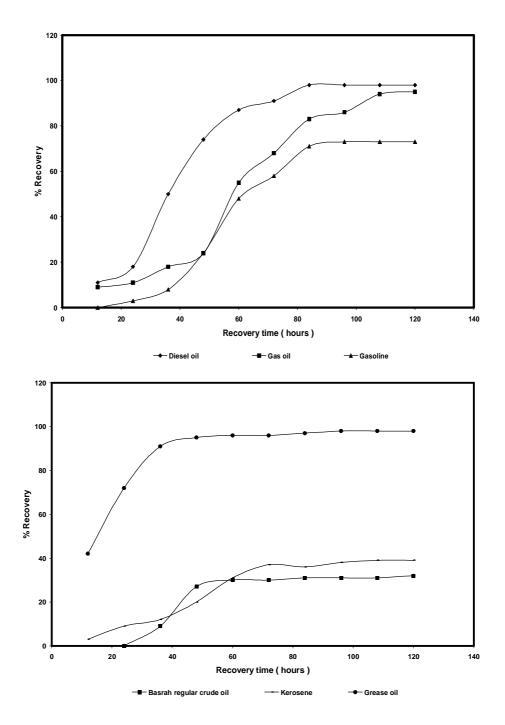


Fig. (2): Recovery of L. auricularia (n=50) during the 5- days recovery period following exposure of 48- hours to crude oil and various fractions

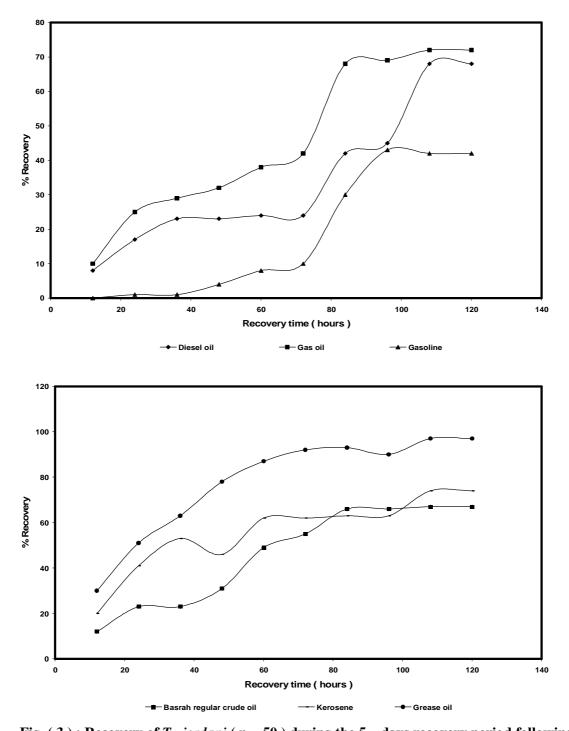


Fig. (3): Recovery of T. jordani (n=50) during the 5- days recovery period following exposure of 48- hours to crude oil and various fractions.

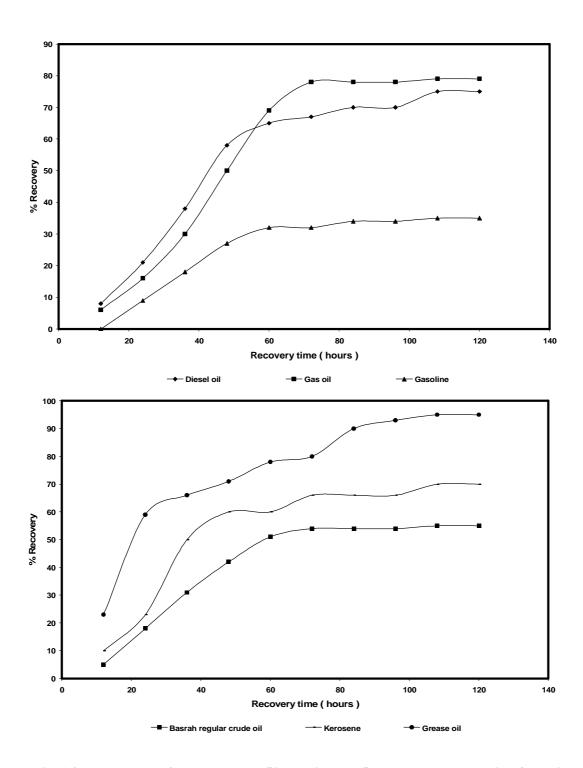


Fig. (4): Recovery of P. acuta (n=50) during the 5-days recovery period following exposure of 48-hours to crude oil and various fractions.

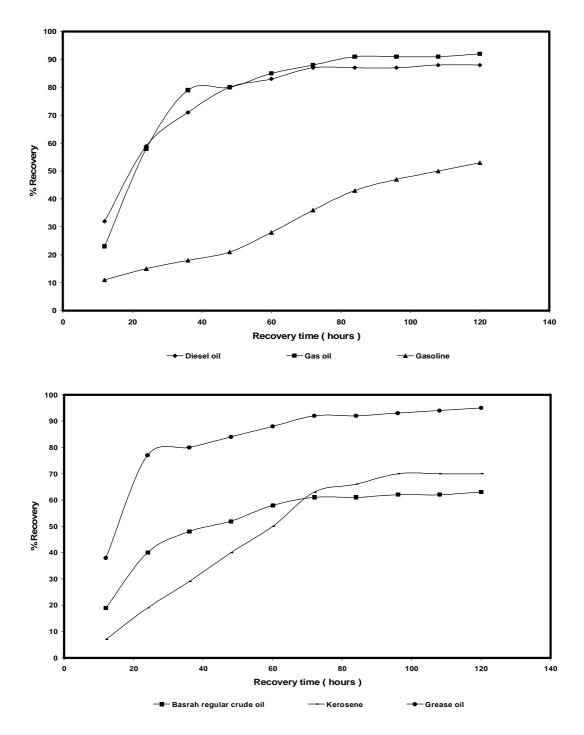


Fig.(5): Recovery of M . nodosa (n = 50) during the 5 – days recovery period following exposure of 48 - hours to crude oil and various fractions .

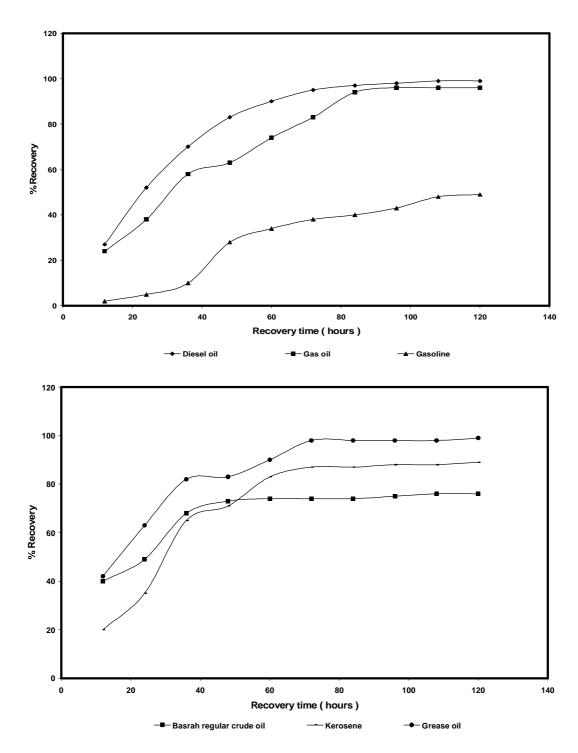


Fig. (6): Recovery of M. tuberculata (n = 50) during the 5 – days recovery period following exposure of 48 - hours to crude oil and various fractions.

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## الملخص

تضمنت الدراسة الحالية تجارب للسمية أجريت تحت الظروف المختبرية في نظام متجدد للاختبار لمدة 24 و 48 ساعة لمقارنة سميات النفط الخام ( نفط خام البصرة الاعتبادي ) ومنتجات تكرير النفط ( زيت الديزل و زيت الغاز والكازولين و زيت التربت والكيروسين و وزيت القل الحركة الاتوماتيكي) اتجاه عدة أنواع من النواعم ( كاتنات اختبار ) المتواجدة في نهر شط العرب و في خمسة أنواع من القواقع ( Dhysa acuta و Eymnaea auricularia و Melanopsis nodosa و Physa acuta و Melanopsis nodosa و في خمسة أنواع من القواقع ( Corbicula fluminalis و Corbicula fluminea ) ونوعين من المحار ( Melanoides tuberculata و Corbicula fluminalis ) ونوعين من المحار ( المختبار كان الجازولين اكثر النفوط سمية اتجاه كاتنات الاختبار يليه الكيروسين الذي بدوره كان اقل سمية بقليل من نفط خام البصرة الاعتبادي أما زيت الديزل و زيت الغاز فكانا اقل سمية نسبيا اتجاه كاتنات الاختبار المحرك و زيت القل الحركة الأتوماتيكي و شمع البرافين في سميتها اتجاه كائنات الاختبار على حد سواء كما نتنوع منتجات التكرير زيبت الاختبار بينما كانا زيت المحرك و زيت ناقل الحركة الأتوماتيكي سامين اتجاه كاتنات الاختبار بدرجات مختلفة كما بينت التجارب بان الاختبار بينما كانا زيت المحرك و زيت ناقل الحركة الأتوماتيكي سامين اتجاه كاتنات الاختبار بدرجات مختلفة كما بينت التجارب بان هناك تنوع في نماذج معدلات الاسترجاع لكل نوع من أنواع القواقع المدروسة بعد التعرض إلى النفوط المختلفة .

 $M. < P. \ acuta < L. \ auricularia$  كان ترتيب حساسية كائنات الاختبار المدروسة اتجاه جميع السموم النفطية كما يلي كانت مجمل التأثيرات الـسامة الحــادة  $C. \ fluminea < C. \ fluminealis < M. \ tuberculata < T. \ jordani < nodosa للهيدروكاربونات النفطية اتجاه كائنات الاختبار المدروسة ( النواعم ) هي عرقلة الفعالية الطبيعية وأصابتها بالخدر والخمول و فقدانها القدرة على الاستجابة للحوافز الخارجية وتمزيق أجزائها الجسمية ( أنسجتها ) واخيرا موتها$