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Toxicity of oil and dispersant mixtures to several species of molluscs collected from Shatt Al – Arab river

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Abstract

The present toxicity experiments show that the oil and dispersant (Corexit 8666) mixtures are more toxic to all species than either net crude oil or dispersant , net or diluted . In the case of BP 1100X and BP 1100 dispersants , however , the oil and dispersant mixtures are more toxic to *M . tuberculata* , *C . fluminea* and *C . fluminalis* than the oil alone and just as toxic as the dispersant alone when undiluted , when the dispersant in the mixtures is diluted (i.e. with river water) , however , then the mixtures are relatively very low in toxicity . For *L . auricularia* , *T . jordani* , *P . acuta* and *M . nodosa* with BP 1100X and BP 1100 , the oil and dispersant mixtures are more toxic than oil alone and just as toxic as the net or diluted dispersant . For all species , the lower the concentration of dispersant in river water (without any oil) , the lower the mortalities ; the exception to this is the Corexit 8666 which resulted in a lower mortalities when net than when diluted for all species of molluscs . The order of sensitivity of species of molluscs tested are as follows : *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

Fresh water snail *L. auricularia* belong to the phylum Mollusca , class An estimated input of 8.8 million metric tons of petroleum pollutants from both natural and anthropogenic sources may be discharged into the world's oceans annually (NRC, 2003). These inputs may occur in remote reaches of the oceans or near centers of petrochemical industries . Because of the physical properties of crude oil and transport mechanisms such as ocean and wind currents, releases from offshore as well as inshore spills may impact sensitive habitats. The sensitive ecosystems most at risk to petroleum contamination include estuaries , costal reefs , and shorelines . These shallow areas can receive greater oil loading per unit volume of seawater than deeper offshore waters (Birtwell and McAllister , 2000). Thus , shallow areas may be susceptible to considerable environmental harm from accidental petroleum discharges.

Dispersants are one class of chemical countermeasures that have been developed to reduce the a mount of oil that may impact sensitive nearshore and inshore habitats .

Oil spill dispersants , which are typically chemical surfactants , may reduce the severity of damage to fragile ecosystems when applied to oil spills under favorable environmental conditions . However , dispersants use is not hazard free . Several scientific investigations have found surfactants to be toxic to marine and aquatic organisms (Tjeerdema *et al* . , 1990 ; Singer *et al* . , 1996 ; Singer *et al* ., 2001) . Additionally , dispersant use may cause increased biological stresses in affected areas because high exposure concentrations of the spilled product may occur (Lewis and Aurand , 1997) .

The objective of present paper was to determine the comparative toxicities of oil and dispersant mixtures to several species of molluscs common in Shatt Al – Arab river . These species of molluscs are *Lymnaea auricularia* , *Theodoxus jordani* , *Physa acuta*, *Melanopsis nodosa*, *Melanoides tuberculata* (snails), *Corbicula fluminea* and *Corbicula fluminalis* (bivalves) .

2- Materials and Methods

Chemicals :

BP1100X and BP1100 dispersants (both non–soluble) were supplied from BP Trading Ltd., Great Britain, and Corexit 8666 dispersant (non–soluble) from Esso chemicals .

Apparatus :

<u>Thermometer</u>

A simple thermometer with range from 0 to 100 °C graduated at 0.2 °C made by Hanna company was used to measure temperature .

Dissolved oxygen meter

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

pH - meter

A pH – meter type HI 8915 made by Hanna company was used to determine pH .

Salinometer

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

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 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 ± 2 °C with light / dark cycle (12 : 12) under aerated conditions .

Toxicity experiments **:**

These experiments were designed to investigate the toxicity of mixtures of crude oil and dispersants compared with that of (1) the net crude oil and (2) the net dispersants alone . Mixtures (500 ml) of Rumella crude oil and three different dispersants were made by shaking together manually different proportions of oil , dispersants and the river water for 5 - second . The relative proportions of each liquid in each mixture are shown in (Tables 1 - 6) . The dispersants used were Corexit 8666 , BP1100X and BP1100 . The net crude oil alone and net dispersants alone were also tested simultaneously for toxicity .

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 liquid being tested for toxicity for a period of 24 - and 48 - hours in glass aquarium (40 × 22 × 15 cm^3 in size) , followed by the 5 – days recovery period in clean aerated river water . The aquariums were covered with glass lids to reduce evaporation of hydrocarbons . The animals were left without food during the exposure period . 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 in June and July , 2004 .

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 ± 2°C .

The dissolved oxygen ranged was 8.5 to 10.1 mg/l. As to the other characteristics of tests solutions , pH was 7.1 to 7.8 and salinity was 1.6 to 1.8 ppt .

3-Results

Tables (1-6) represent the percentage mortalities of the tested species after 24-and 48-hours periods of exposure to crude oil , emulsifiers and mixtures of these in various concentrations and dilutions , followed by the 5 – days recovery period .

It can be seen that the more recently developed " low – toxicity " dispersant , differs in its toxic effects from the other two dispersants investigated . In the case of Corexit 8666 the oil and dispersant mixtures are more toxic to all species than either net crude oil or dispersant , net or diluted . In the case of BP 1100X and BP 1100 , however , the oil and dispersant mixtures are more toxic to *M . tuberculata* ,*C . fluminea and C . fluminalis* than the oil alone and just as toxic as the dispersant alone when undiluted , when the dispersant in the mixtures is diluted (i . e . with the river water) . However then the mixtures are relatively very low in toxicity . For *L . auricularia* , *T . jordani* , *P . acuta* and *M . nodosa* BP 1100X and BP 1100 , the oil and dispersant mixtures are more toxic than oil alone and just as toxic as the net or diluted dispersant . For all species , the lower the concentration of dispersant in river water (without any oil) , the lower the mortalities ; an exception to this , however , the Corexit 8666 which results in a lower mortalities when net than when diluted for all species of molluscs.

Table (1) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al – Arab river after periods of 24 - hours of exposure to crude oil (Rumella) , emulsifier (Corexit 8666) and mixtures of these in various concentrations and dilutions , followed by the 5–days recovery period .

Emulsifier	Concentration of toxic liquid			Percentage mortalities						
	Rumella Crude oil ml.	Emulsifier ml .	River water ml .	*L . aurcularia* (n = 50)	*T . jordani* (n = 50)	*P . acuta* (n = 50)	*M . nodosa* (n = 50)	*M . tuberculata* (n = 50)	*C . fluminea* (n = 50)	*C . fluminalis* (n = 50)
Corexit 8666	---	500 (Net)	---	54	15	49	35	9	0	0
	100	400	---	80	66	76	75	62	38	44
	100	200	200	94	66	89	81	53	26	50
	100	100	300	76	58	62	58	56	42	46
	---	250	250	76	38	57	51	27	10	19
	---	125	375	46	17	41	30	12	4	6
	500 (Net)	---	---	45	38	53	45	23	12	18
	---	---	500 (Control)	2	0	3	1	0	0	3

Table (2) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al – Arab river after periods of 48 - hours of exposure to crude oil (Rumella) , emulsifier (Corexit 8666) and mixtures of these in various concentrations and dilutions , followed by the 5 – days recovery period .

Emulsifier	Concentration of toxic liquid			Percentage mortalities						
	Rumella Crude oil ml.	Emulsifier ml .	River water ml .	*L . aurcularia* (n = 50)	*T . jordani* (n = 50)	*P . acuta* (n = 50)	*M . nodosa* (n = 50)	*M . tuberculata* (n = 50)	*C . fluminea* (n = 50)	*C . fluminalis* (n = 50)
Corexit 8666	---	500 (Net)	---	58	20	53	38	13	2	4
	100	400	---	83	61	85	78	56	42	55
	100	200	200	98	68	92	72	60	31	49
	100	100	300	80	64	68	65	62	28	49
	---	250	250	78	42	61	54	30	14	22
	---	125	375	58	23	46	33	15	9	11
	500 (Net)	---	---	67	42	56	48	29	15	22
	---	---	500 (Control)	0	0	3	3	3	2	3

Table (3) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al – Arabriver after periods of 24 - hours of exposure to crude oil (Rumella) , emulsifier (BP 1100X) and mixtures of these in various concentrations and dilutions , followed by the 5 – days recovery period .

Emulsifier	Concentration of toxic liquid			Percentage mortalities						
	Rumella Crude oil ml.	Emulsifier ml .	River water ml .	*L . aurcularia* (n = 50)	*T . jordani* (n = 50)	*P . acuta* (n = 50)	*M . nodosa* (n = 50)	*M . tuberculata* (n = 50)	*C . fluminea* (n = 50)	*C . fluminalis* (n = 50)
BP 1100X	---	500 (Net)	---	100	97	100	99	69	26	33
	100	400	---	100	95	98	99	68	24	29
	100	200	200	96	87	90	89	4	0	3
	100	100	300	92	86	88	88	6	2	4
	---	250	250	100	99	100	100	11	8	8
	---	125	375	82	80	81	80	3	2	2
	500 (Net)	---	---	64	38	53	45	23	12	18
	---	---	500 (Control)	2	0	4	1	0	0	3

Table (4) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al – Arabriver after periods of 48 - hours of exposure to crude oil (Rumella) , emulsifier (BP 1100X) and mixtures of these in various concentrations and dilutions , followed by the 5 – days recovery period .

Emulsifier	Concentration of toxic liquid			Percentage mortalities						
	Rumella Crude oil ml.	Emulsifier ml .	River water ml .	*L . aurcularia* (n = 50)	*T . jordani* (n = 50)	*P . acuta* (n = 50)	*M . nodosa* (n = 50)	*M . tuberculata* (n = 50)	*C . fluminea* (n = 50)	*C . fluminalis* (n = 50)
BP 1100X	---	500 (Net)	---	100	100	100	100	74	30	38
	100	400	---	100	99	100	99	70	30	36
	100	200	200	100	93	98	95	6	2	4
	100	100	300	97	91	94	94	10	8	7
	---	250	250	100	100	100	100	15	13	13
	---	125	375	90	88	90	92	7	2	4
	500 (Net)	---	---	67	42	56	48	29	15	22
	---	---	500 (Control)	0	0	3	3	0	2	3

Table (5) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al – Arab river after periods of 24 - hours of exposure to crude oil (Rumella) , emulsifier (BP 1100) and mixtures of these in various concentrations and dilutions , followed by the 5 – days recovery period .

Emulsifier	Concentration of toxic liquid			Percentage mortalities						
	Rumella Crude oil ml.	Emulsifier ml .	River water ml .	*L . aurcularia* (n = 50)	*T . jordani* (n = 50)	*P . acuta* (n = 50)	*M . nodosa* (n = 50)	*M . tuberculata* (n = 50)	*C . fluminea* (n = 50)	*C . fluminalis* (n = 50)
BP 1100	---	500 (Net)	---	100	100	100	100	100	100	100
	100	400	---	100	100	100	100	99	96	99
	100	200	200	96	90	95	92	6	4	5
	100	100	300	92	88	92	89	7	6	6
	---	250	250	100	95	99	96	7	6	5
	---	125	375	82	79	79	78	3	2	0
	500 (Net)	---	---	64	38	53	45	23	12	18
	---	---	500 (Control)	2	0	4	1	0	0	3

Table (6) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al – Arab river after periods of 48 - hours of exposure to crude oil (Rumella) , emulsifier (BP 1100) and mixtures of these in various concentrations and dilutions , followed by the 5 – days recovery period .

Emulsifier	Concentration of toxic liquid			Percentage mortalities						
	Rumella Crude oil ml.	Emulsifier ml .	River water ml .	*L . aurcularia* (n = 50)	*T . jordani* (n = 50)	*P . acuta* (n = 50)	*M . nodosa* (n = 50)	*M . tuberculata* (n = 50)	*C . fluminea* (n = 50)	*C . fluminalis* (n = 50)
BP 1100	---	500 (Net)	---	100	100	100	100	100	100	100
	100	400	---	100	100	100	100	100	100	100
	100	200	200	99	97	99	99	9	9	10
	100	100	300	97	96	96	97	12	7	10
	---	250	250	100	98	100	100	14	6	8
	---	125	375	90	84	89	88	8	4	4
	500 (Net)	---	---	67	42	56	48	29	15	22
	---	---	500 (Control)	0	0	3	3	0	2	3

Discussion

From the results of present experiments it can be seen that mixtures of crude oil and dispersants are often more toxic than the oil by itself , and that , in the presence of oil , dilution of these dispersants is vital for reduction of toxicity . The small percentage mortalities of the tested animals were recorded in the control treatment during the experiments . These mortalities may due to the competition between the individual of tested animals .

Naturally , the behavior of spilled oil in water surface can be markedly altered by the application of chemical dispersants (surfactants) to form emulsions . The formation of emulsions have been used to control oil spills , and thereby alleviate some of the known adverse effects . However , the use of chemical dispersants have been extremely toxic to many marine organisms (NRC , 2003 ; Sterling , 2004) .

Investigations made by other worker on the toxicity of oil and dispersant mixtures have given somewhat similar or conflicting results of the present study . Tjeerdema *et al* . (1990) found that the median tolerance level of the shore crab (*Carcinus maenas*) to mixtures of BP 1002 and crude oil at the 96 hours LC_{50} was 15.0 ml / l , compared with 29.0 ml / l for BP 1002 alone , indicating that the addition of oil increases the toxicity of the dispersant . NRC (2003) reported that mixtures of Kuwait crude oil and the dispersant Corexit were more toxic to barnacles and mussels than Kuwait crude oil alone . Singer and Tjeerdema (1995) found that both bunker C , Kuwait crude oil , and Corexit caused very low mortalities in mussels after a 20 hours of exposure , while mixtures of these oils with Corexit caused no increase in toxicity over that of each oil alone . Wolfe *et al* . (1997) tested that the toxic effects of BP light diesel and the oil dispersants , BP1100X , BP1100WD and Shell Oil Herder to green algae and found that the Shell Oil Herder , either alone or in combination with BP light diesel , were most toxic among the three oil dispersants tested . In general , mixtures of BP light diesel and any one of the three oil dispersants were more toxic than the diesel oil or the oil dispersants alone . Fuller *et al* . (2004) found that emulsions of Finasol were just as toxic to shrimp and guppy whether or not crude oil was added . Two types of Finasol and four crude oils were investigated , of which the dispersants alone were far more toxic than any of the crude oils alone . Field experiments with Kuwait crude oil and BP1002 showed that for a number of rocky shore species the oil and dispersant mixtures (or " runoff " from areas oiled and cleaned on the shore) were more toxic than oil alone or oil cleaned by dispersant 30 minute after application (API , 2001) . Singer and Tjeerdema (1995) used behavioral parameters of Caribbean coral species to compare the effects of the dispersants Corexit 7664 and Shell LTX , respectively , with crude oil extracts . They found that dispersants were more toxic than the oil alone .

Mixtures of oil and dispersants on the open sea are likely to cause relatively little biological damage since such emulsions appear to disperse and become degraded fairly rapidly . In the event of spilt oil coming ashore and then being treated with dispersants , however , severe biological effects may occur . In such case , oil and dispersant mixtures would become dispersed in rock pools which would otherwise have only a surface covering of oil until the next tide washed this away . The toxicity of such mixtures accounts for the number of barren pools reported on Cornish shores following their treatment with dispersant after the Torrey Canyon spill (NRC , 2003) , empty limpet seats , or " scars " were especially conspicuous in pools , and extensive mortalities were suffered by several species of rock pool algae .

It appears that no simple relationship between the toxicity of mixtures of oil and dispersants in one hand and on the other the toxicity of oil or dispersant alone . The conflicting results described above indicate that a considerable number of factors are of importance in determining the overall toxicity of such mixtures ; such factors include the type of dispersant and oil , the relative proportions and dilution of these and the degree of emulsification .

Although there are certain differences between laboratory conditions and the ecosystem of potential oil spill area , nevertheless , laboratory findings on oil and oil dispersant toxicity can provide useful information on choosing a suitable oil dispersants when considering the effectiveness of the oil dispersant on the one hand and effects of this dispersant on the aquatic life on the other .

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 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 6) .

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 were generally more tolerant . Intertidal 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. Laboratory studies on the snails (*M . nodosa* , *T . jordani* , *L . aurcularia* and *M . tuberculata*) and the bivalves (*C . fluminalis* and *C . 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 to the external cue , rapture their tissues , lead them to leave their shells and finally lead them to die . Such effects are due to physical and / or chemical toxic effects of hydrocarbons which produce the abnormal activities in molluscs and die .

Boelsterli (2002) 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 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 .

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الخلاصة

بينت تجارب السمية الحالية بان خلائط النفط الخام – المشتت (الكوريكسيت 8666) اكثر سمية اتجاه جميع كائنات الاختبار من النفط الخام وحده (صافي) او المشتت وحده في حالة تخفيفه أو عدم تخففه (صافي) بماء النهر . أما في حالة البي بي 1100 اكس و البي بي 1100 فان خلائط النفط الخام – المشتت كانت اكثر سمية اتجاه القوقع *M . tuberculata* و المحار *C . fluminea.* و *C . fluminalis* من النفط الخام وحده (صافي) وتقارب في سميتها سمية المشتت وحده عندما يكون غير مخفف (صافي) ، وفي حالة تخففيه بماء النهر فان سمية الخليط عند إذن تكون واطئة جدا . أما بالنسبة للقواقع *L . auricularia* و *T. jordani* و *P . acuta* و *M . nodosa* فان خلائط النفط الخام – المشتت (البي بي 1100 اكس و البي بي 1100) كانت اكثر سمية من النفط الخام وحده (صافي) وتقارب في سميتها سمية المشتت وحده في حالة تخففه بماء النهر او عدم تخففه (صافي) . يسبب التركيز الواطئ من المشتت مع ماء النهر عموما (دون النفط الخام) وفيات اقل اتجاه جميع كائنات الاختبار . ماعدا في حالة الكوريكسيت 8666 الذي يسبب وفيات اقل اتجاه جميع كائنات الاختبار عندما يكون غير مخفف بماء النهر (صافي) مقارنة عند تخففه بالماء . كان ترتيب حساسية كائنات الاختبار المدروسة اتجاه جميع السموم النفطية كما يلي : *L . auricularia* > *P . acuta* > *M . nodosa* > *T. jordani* > *M . tuberculata* > *C . fluminealis* > *C . fluminea* . كانت مجمل التأثيرات السامة الحادة 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 .