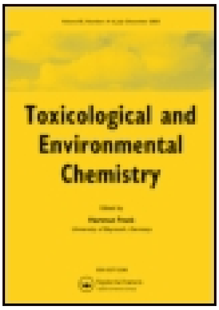

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Comparative toxicity of crude oil, dispersant and oil-dispersant mixture to prawn, Palaemon elegans

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COMPARATIVE TOXICITY OF CRUDE OIL, DISPERSANT AND OIL-DISPERSANT MIXTURE TO PRAWN, *PALAEMON ELEGANS*

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INTRODUCTION

Petroleum contamination of the marine environment can occur from a variety of sources; discharge from near shore ship operations, via urban and industrial sewage effluents, discharge of ballast waters by tankers and other vessels and accidental spills from offshore wells and shipping mishaps.[1]

The Mediterranean is a semi-enclosed sea and subject, as a whole, to a heavy tanker traffic from middle east to western countries in all seasons of the year. Therefore, a large number of minor accidental or deliberate spills occur each year in connection with oil transport activities within the region.[20]

The annual inputs of petroleum hydrocarbons from different sources into the Mediterranean were estimated to be 635×10^3 tonnes and more than half of this input (330×10^3) comes from tankers, ballasting and loading operations.[20]

In the northeastern part of the Mediterranean, on the southern coasts of Turkey, there are two very busy ports (İskenderun and İçel-Mersin), two oil loading terminals (Dörtyol and Botaş), a refinery (ATAŞ-İçel) and many industrial complexes.

The detailed studies carried out by the Institute of Marine Sciences of Middle East Technical University[21] on the physical oceanography of the Cilician Basin have revealed the general circulation pattern of the region. According to these studies, some eddies and gyres occur along the southern coasts of Turkey and the substances, including pertoleum hydroxarbons, introduced into the area may have relatively long residence time due to entrapment by the eddies and peripheral features of the Asia Minor current .[21] It is well known that, the accidents occurred in bays and estuaries where the spilled oil was not diluted sufficiently.[20] This is the case in northeastern part of the Mediterranean.

On the other hand, the toxicity of crude oils of different origins to marine organisms has been the subject of several studies.[1,5–6,8–14]

Dispersants as surface-active agents are being used more widely around the world for the control and elimination of oil spills at the surface of the sea. They cause the oil to break up into fine droplets that are more readily dispersed and degraded by natural chemical or biological processes.[11] Many dispersants are commercially available. The problem with dispersants is that, they may be toxic to marine life themselves or they may increase the toxicity of the oil involved.[3] Therefore, a great deal of investigations have been carried out concerning the lethal and sublethal effects of dispersants and oil-dispersant mixtures.[7,9,16,17,18,23]

On the other hand, the Crustanceans were classified among the most sensitive organism group to crude oils and dispersants.[9,12]

Although many investigations have been performed on the toxicity of different crude oils, dispersants and oil-dispersant mixtures to different classis of marine organisms, including *Palmaemonidae* family, the only data found in the litterature on the toxicity of oil and oil dispersants to *Palaemon elegans*. was that of Axisk.[4] The present investigation aims therefore, at evaluating the sensitivity of this species to crude oil, disperstant and oil-dispersant mixture.

MATERIAL AND METHOD

All studies were conducted in a temperature-constant room at the Institute of Marine Sciences of Middle East Technical University.

Test Animals

A rockpool prawn, *Palaemon elegans* was used as the test organism in this study. They were collected from uncontaminated areas and as far as possible they were of similar size. Test organisms were transported to the laboratory and transferred to shock tanks of 140 l as soon as possible after capture. They were acclimatized for at least 14 days to laboratory conditions. During this period, the organisms were gradually acclimated to the temperature of the experiment provided that temperature change does not exceed 1 °C per day. A photoperiod of 14 h light, 10 h dark was employed. During the quarantine period, the animals were fed on fresh sea urchin eggs.

Test Vessels

Glass jars measuring approximately 24 cm high, 13 cm in diam and 9 cm in mouth diam (approximately 3 l size) were utilized as the test vessel.

Test Compounds

The samples of crude oil and dispersant (Spillwash L.T.-Emkem International Ltd. U.K.) were supplied by a local refinery (ATAŞ) in İçel (Mersin). This refinery refines mostly the middle east crude oils. The composition of the dispersant could not be provided.

Water soluble fractions (WSF) of crude oil, dispersant and oil-dispersant mixture were used as the test substance.

Preparation of water-soluble fractions

The WSFs of crude oil and dispersant were obtained using the procedure described by Anderson:[2] 100 ml of oil or dispersant was placed over 900 ml of sea water in a flask. The WSF of oil-dispersant mixture was obtained by adding 100 ml of oil and 10 ml of dispersant to 900 ml of sea water in a flask. The flasks, without stopper, were then slowly stirred with a teflon-covered magnetic bar for 20 h at room temperature (23 ± 2 °C). The stirring speed was adjusted so that the vortex in the flask does not exceed more than 25% of the distance from the top of the fluid to the bottom of the flask. After mixing, the compound (oil or dispersant or oil-dispersant mixture) and water phases were allowed to separate for 1/2 to 1 h before the water phase was siphoned off and then the WSF in the lower level was siphoned through glass tubing into a one-liter flask. A stopper was placed on the flask until required for the bioassay.

Experimental Procedure

Ten test animals weighing 0.18 ± 0.5 g in average were placed in 3 l glass jars containing 2 l of filtered sea water and appropriate amount of WSFs. The sea water used was collected from offshore at 35.5 ± 0.4 ‰S and 8.2 ± 0.05 pH. The temperature was maintained at 20 ± 1 °C and the air was supplied such that 50 ± 10 bubbles per minute were produced from the tip of a 1 ml disposable pipette with 1 mm ID, as was proposed by La Roche.[13] The mouth of the jars was covered with aluminium foil to prevent the evaporation. The test concentrations followed a roughly logarithmic series as much as possible. All toxicants were evluated at a minimum of five test concentrations plus the control. Over the 24 h test period, the organisms were examined and the mortality was recorded. Criteria for death were the complete cessation of respiration and absence of a response to prodding with a glass rod. The test animals were not fed for 48 h before the start of experiment and during the test period.

The 24 h LC_{50} values, their 95% confidence limits and slope functions were calculated by the graphical method of Litchfield and Wilcoxon.[15]

RESULTS

The percentage mortalities were plotted against the concentration of test substances and the LC_{16}, LC_{50} and LC_{84} values were obtained from graphs (Figures 1 to 3).

Table 1 presents the mortalities observed for *Palaemon elegans* exposed to the WSFs of crude oil, dispersant and oil-dispersant mixture during the 24 h test period and the expected mortalities obtained from the Figures 1 to 3. The contributions to Chi^2 were also calculated using the method of[15] and shown in Table 1.

It is apparent from the data in Table 2 that, the LC_{50} values are lowest for dispersant and highest for crude oil which imply that the dispersant is the most

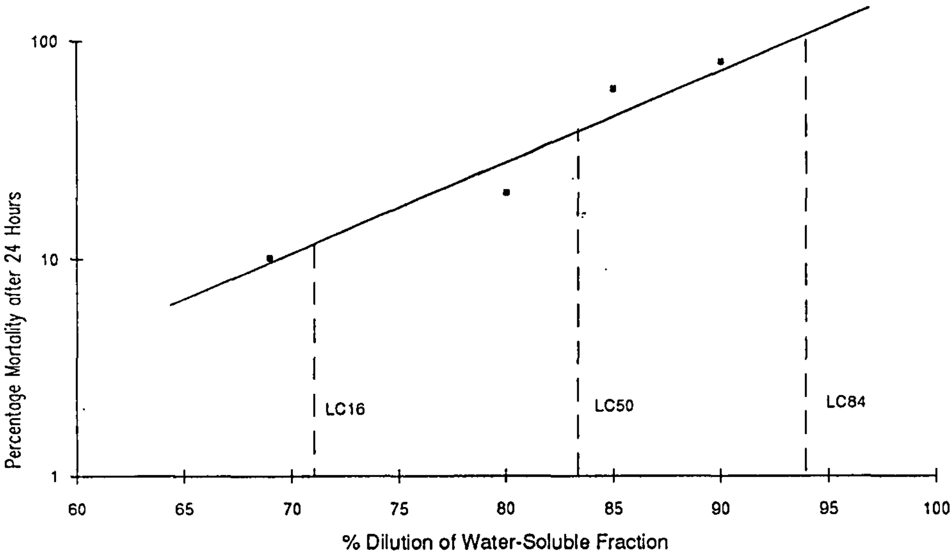

Figure 1 Estimation of 24 hour LC_{50} values for oil.

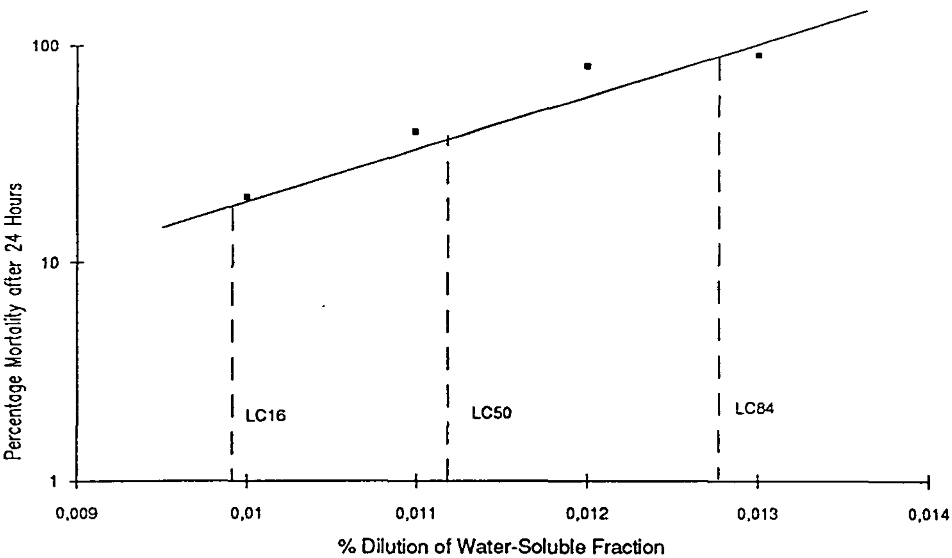

Figure 2 Estimation of 24 hour LC_{50} values for dispersant.

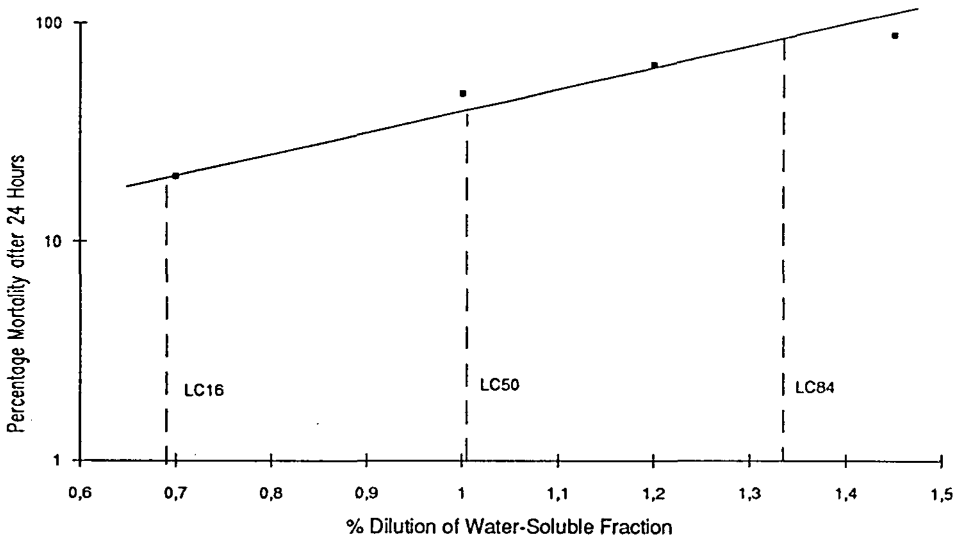

Figure 3 Estimation of 24 hour LC_{50} values for oil-dispersant-mixture.

toxic and the crude oil is the least toxic of three substances tested on *Palaemon elegans.*

The toxicity of oil-dispersant mixture was also quite significant to the same species. Its toxic effect resulted mainly from the dispersant, since the comparison of the results obtained for crude oil and dispersant confirm this pattern.

DISCUSSION

The comparative toxicities of the WSFs of crude oil, dispersant and oil-dispersant mixture to prawn, *Palaemon elegans* were studied. This species was chosen, because it meets the required characteristics recommended by La Roche:[13] local abundance, wide distribution, ease of collection, adaptability to laboratory conditions.

The results of the acute toxicity tests on *P. elegans* using the WSFs of three substances have shown that, the dispersant is the most toxic, the crude oil is the least toxic and the oil-dispersant mixture is also significantly toxic to this species (Table 2). Same order of toxicity was obtained for *Palaemon pacificus* by Eisler[9] for the same test substances but of different origin. This author exposed ten marine species to crude oils of different sources, to a chemical dispersant and to the oil-dispersant mixtures and obtained the following LC_{50} values for *P. pacificus* after 24 h exposure period: >30 ml/l for crude oils, 0.012 ml/l for dispersant and 0.090 ml/l for oil-dispersant mixtures.

Table 1 Experiments of twenty-four hours testing WSFs of crude oil, dispersant and oil-dispersant mixture on prawn, *Palaemon elegans*

Test substances	*Conc. of extract %*	*No. of test animals*	*Observ. mortality %*	*Expect. mortality %*	*Contribution to Chi²*
Crude oil	Cont.	10	0	0	Not plotted
	70	10	10	9	0.0012
	80	10	20	36	0.11
	85	10	60	56	0.0053
	90	10	80	74	0.02
	100	10	100	—	Not plotted
				Total	0.1365
Dispersant.	Cont.	10	0	0	Not plotted
	0.010	10	20	18	0.003
	0.011	10	40	42	0.0018
	0.012	10	80	71	0.04
	0.013	10	90	90	0
	0.014	10	100	—	Not plotted
				Total	0.0448
Oil-dispersant mixture	Cont.	10	0	0	Not plotted
	0.7	10	20	18	0.003
	1.0	10	50	50	0.0
	1.2	10	70	72	0.0022
	1.4	10	90	89	0.0012
	1.5	10	100	—	Not plotted
				Total	0.0064

Table 2 LC_{50} values obtained for the test species and the statistical analysis of the test results by the method of Litchfield and Wilcoxon (1949). Slope functions (S) and 95% confidence limits are given for each LC_{50} value

Test substances	*LC_{50} %*	*Upper conf. limit*	*Lower conf. limit*	*S*	*Total Chi² of line*	*Goodness of fit*
Crude oil	83.5	88.7	78.6	1.13	1.365	Best fit
Dispersant	0.0112	0.0117	0.0107	1.10	0.448	Best fit
Oil-Dispersant mixture	1.10	1.31	0.92	1.42	0.064	Best fit

In the present study, the 24 h LC_{50} values found for crude oil, dispersant and oil-dispersant mixture have been 83.5% (83.5 ml/l), 0.0112% (0.0112 ml/l) and 1.10% (1.10 ml/l) respectively. Thus, with the exception of the value obtained for crude oil, there is a consistency between our results and those found by Eisler.[9]

Different LC_{50} values were found for the WSFs of different crude oils even for the same species,[8] since the relative toxicities of different oils will be dependent on both hydrocarbon concentration and hydrocarbon composition.[2] According to Anderson,[2] this difference in LC_{50} values is due to the fact that, the toxic fractions of a crude oil, (e.g. aromatics) may be more volatile than that of the other one. Since, the soluble aromatics of an oil produce the majority of its toxic effects in marine environment.

Sunay,[19] pointed out that the middle east crude oil, which is still refining in the local refinery (ATAŞ) from where our crude oil sample was provided, comprises more *n*-paraffins than aromatics. So, the low LC_{50} values of the crude oil obtained in the present study is probably due to its low aromatic content.

Although our low LC_{50} values obtained for dispersant and oil-dispersant mixture are similar to those found by Eisler[9] for *P. pacificus*, the high LC_{50} values were also found by other investigators for different dispersants. These differences resulted not only from the chemical composition of the dispersant but also from the species itself. Thus, Wilson[23] studied the acute toxicity of several oil dispersants to fish larvae and obtained the 100 h LC_{50} values ranging from 4 to 35 parts/10^6.

Likewise Nagell,[16] studied the toxicity of four oil dispersants to some marine animals, including crustaceans, and obtained the 96 h LC_{50} values ranging also from >150 to 10 000 ppm. So, our LC_{50} values were lower than those found by Nagell[16] and Wilson.[23] In other words, the dispersant (Spillwash L.T.) used in the present study is more toxic than several other dispersant. Unfortunately, because its formulation could not be provided, it is difficult to make any statement on the mode of toxic action of the present dispersant. Because as it is stated by Wilson[23] the mode or modes of toxic action of dispersants have been attributed to such properties as the ability to lower surface tension and the effects of water/lipid solubility. Some authors found a correlation between aromatic content of dispersants and their toxic effects.[22,23] They stated that, the high toxicity of dispersants was due to their high aromatic content. But Eisler and Nagell[9,16] found that, the most toxic component of the dispersants they studied is surfactant. It is therefore necessary to know the chemical composition of any dispersant to be tested.

The test organisms, exposed to lethal and sublethal concentrations of pollutants, exhibit some abnormal behavioral patterns before dying[9] or towards the end of chronic tests.[1]

In the present study, test organisms exposed to the WSFs of crude oil, especially of dispersant and oil-dispersant mixture exhibited high swimming activity at the beginning of the exposure and before dying they lied on their back, beat their pleopods and then died. We suggest that the death was caused by asphyxia, since several studies showed the effects of oils and dispersants on the respiration of test organisms exposed to these substances.[6,16,18,23]

In summary, the effets of WSFs of a crude oil, dispersant (Spillwash L.T.) and an oil-dispersant mixture were evaluated on a rockpool prawn, *Palaemon elegans* after 24 h test period. Crude oil was found to be the least toxic and dispersant as the most. The oil-dispersant mixture of 10 part oil to 1 part dispersant (v/v) was also significantly toxic to the same species.

The dispersant to be applied should therefore be used with caution taking into account the directions and/or recommendations as was described, for example, by EPS (1984) on the use and acceptability of oil spill dispersants.

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