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Toxicity of crude oil and chemically dispersed crude oil on Shatt Al-Arab River *Melanoides tuberculata* and *Corbicula fluminalis* under laboratory tidal regime at two controlled temperatures

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Abstract. This study describes the acute toxicity of Iraqi crude oil, Nahran Omar (light) from which 12-14% had been lost by evaporation, and chemically dispersed crude oil with tow commercial oil dispersants (Corexit 9500 and Corexit 9527). Static-renewal toxicity tests were performed using two Shatt Al-Arab River test species, the snail *Melanoides tuberculata* and bivalve *Corbicula fluminalis* under laboratory tidal regime at two controlled temperatures (10°C and 32°C). The tidal regime was maintained for 32 hours to *M. tuberculata* and for 71 hours to *C. fluminalis*. Crude oil plus dispersants were immediately toxic to snails and no recovery took place during the rest of the tidal regime. A slower but progressive toxic effect of crude oil alone was recorded. The snails LT_{50} values of crude oil alone (3 to 12 hours) suggested that there was no significant effect of the volume of crude oil lost by evaporation on its toxicity toward the snails. However, highly significant effect of temperature was demonstrated. Although the snails from crude oil and crude oil plus dispersants recovered faster, but those from crude oil plus dispersants needed longer recovery period. The snails RT_{50} values (12 to 85 hours) confirmed that the recovery from crude oil plus dispersants was more rapid at 10ºC than at 32ºC. Neither the temperature, nor the volume of crude oil lost appeared to affect significantly the recovery from crude oil alone. No toxic effects of crude oil alone to bivalves were observed. Crude oil plus Corexit 9527, however, showed an initial toxicity at 10ºC followed by partial recovery and in turn by progressive toxicity. This effect was not seen from crude oil plus Corexit 9527 at 32ºC, although after 71 hours of tidal regime there were fewer survivors at 32ºC than at 10ºC. The previous toxic effect was also not seen from crude oil plus Corexit 9500 at either temperature. No recovery took place of bivalves from crude oil plus dispersants. The bivalves from crude oil alone showed progressive recovery (TR_{50} value, 17-20 hours), but those from 13.5% evaporated crude oil recovered somewhat more slowly in the earlier period than those from 14% evaporated crude oil.

Key Words: Shatt Al-Arab River, crude oil, dispersants, oil toxicity, molluscs.

Introduction. The increasing demand for petroleum and its products in the last decades has led to an increase in oil pollution in the environment. Many sources of oil pollution, such as routine tanker and shipping operations, industrial wastewater, tanker accidents, terrestrial runoff, atmospheric fallout, natural seepage, and offshore well disasters still contaminate the aquatic environment. The incidences of oil spillage had profound negative effects on the aquatic flora and fauna of the oil producing areas. Some aquatic species have become endangered while others have gone into extinction (NRC 2003).

One of the methods used in oil clean-up is the application of dispersants. Most dispersants are proprietary, and the complete composition is not often public knowledge. Nalco, BP, Shell and Total Special Fluids are the manufactures of a variety of chemical dispersants, and 19 miscellaneous dispersants are manufactured by others (Wise & Wise 2011). Dispersants are chemical substances composed of surface active agents (surfactants), solvents (like water and hydrocarbons) and stabilizing agents. Surfactants are a specific chemical compounds which contain anionic and non-ionic molecules. Owing to the presence within the molecule of both hydrophilic and hydrophobic characteristics, the surfactants can reduce the interfacial tension between oil and water, so forming fine mixed oil-surfactant micelles dispersed into the water column (NRC 2005). The use of dispersants would help to accelerate dilution of the oil layer and consequently increasing the rate of biodegradation of the oil (Couillard et al 2005). Dispersants are probably the only effective solution to remove the oil spill from the aquatic environment, especially when the mechanical removal is not possible. However, the use of dispersants often counteracted with their toxicity. The toxicity of dispersants to aquatic organisms has been well documented (Hall et al 1989). Dispersants are mainly used to enhance oil mobilization into water column, so they will increase the biological stresses in the affected areas due to increased concentrations of oil (Fuller et al 2004). Numerous studies have shown that dispersants can increase the concentration of crude oil in the water column (Kanga et al 1997). However, many other studies have shown that the use of dispersants also reduces the crude oil toxicity (Fuller et al 1999; Page et al 2000; Harris et al 2002; Mueller et al 2003). The toxicity of dispersants is a matter which is attended by some degree of ambiguity. In fact, it be confusing and based on at least five elements; the dispersant, the dispersed oil, the nature of the exposure, and the organism type and stage of their life. The subscription between these and other factors will determine the final effects of dispersants to the ecosystem or specific living resource (Singer et al 1999). A work which considers both the advantages and toxicity of dispersants use is required in order to establish a comprehensive framework for dispersants use policies for affected areas. In an attempt to do so, the past studies have evaluated the acute toxicity of single dispersants. More recent studies have taken into consideration the toxicity of the petroleum and dispersants interaction.

The data's reported in the present study concern the toxicity of Iraqi crude oil (Nahran Omar) and crude oil plus dispersants (Corexit 9500 and Corexit 9527) on two Shatt Al-Arab River species at two controlled temperatures. Since, the most toxicity tests were generally done with straight dilutions of pollutants and such toxicity tests may not accurately reflect the reactions of organisms that live in the intertidal zone because the effects of alternate exposure and immersion during a tidal cycle cannot be taken into account, the recent toxicity tests was done under laboratory tidal regime. The two species used are widely distributed in the study area: a snail, *Melanoides tuberculata* and a bivalve, *Corbicula fluminalis*.

The dispersants, Corexit 9500 and Corexit 9527 have had the large field application. The first large application of Corexit 9500 and Corexit 9527 was during the BP Deepwater Horizon oil crisis. The Corexit 9527 was also used during the Exxon-Valdez spill in Alaska in 1989 (Wise & Wise 2011). A lot of studies considered the toxicity of Corexit 9500 and Corexit 9527 to marine organisms (Ordzie & Garofalo 1981; Hartwick et al 1982; Wells et al 1982; Bobra et al 1989; Singer et al 1991; Law 1995; Singer et al 1996; George-Ares et al 1999; Wheelock et al 1999; Negri & Heyward 2000; Pollino & Holdway 2002; Hamoutene et al 2004; Fuller et al 2004; Scarlett et al 2005; Jung et al 2009; Venn et al 2009; Duarte et al 2010). The US EPA (2011) has posted a full list of the chemical components of Corexit 9500 and Corexit 9527: 1,2-Propanediol; Ethanol, 2-butoxy- (not included in the composition of Corexit 9500); Butanedioic acid, 2-sulfo-,1,4-bis (2-ethylhexyl) ester, sodium salt (1:1); Sorbitan, mono-(9Z)-9-octadecenoate; Sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivatives; Sorbitan, tri-(9Z) -9-octadecenoate, poly(oxy-1,2-ethanediyl) derivatives; 2-Propanol, 1-(2-butoxy-1-methylethoxy)-; Distillates (petroleum), hydro treated light.

Material and Method

Crude oil. Nahran Omar crude oil (light- API gravity >34) was used in the recent experiments. It was obtained from Iraqi South Oil Company with properties shown in Table 1, according of Ali et al (2013). The oil was transferred to the laboratory by dark glass bottle closed tightly and stored in laboratory temperature (20±2 ºC) in dark place prior to use.

Two liter of crude oil was stirred for 24 hours by a magnetic stirrer at laboratory temperature to allow evaporation. This was done to minimize the changes in toxicity that would have taken place during the experimental period due to evaporation of the more volatile fractions and to simulate the condition of an actual oil spill at water. This procedure caused the loss of 12-14% of the original volume of the crude oil.

Table 1

Properties of Nahran-Omar crude oil

Index	*Value*
Density by 20°C, kg/m^3	856
Sulfur content, % of mass in crude oil	0.73
fraction (i.b.t.-180°C)	0.029
fraction (180-360°C)	0.64
Water content, % of mass	Absence
Content of mechanical impurities, % of mass	Absence
Concentration of chloride salts, mg/dm^3	23.01
Content of paraffin in crude oil, % mass	3.0
Temperature, °C	
freezing point of kerosene fraction	-59°C
pour point of diesel fraction	-12°C
Content of fractions boiling, % Mass	
up to 200°C	34.35
up to 350°C	59.87

Dispersants. The dispersants used were Corexit 9500 and Corexit 9527 (Nalco Energy Services Company, USA). Corexit 9500 is contains propylene glycol 1-5%, w/w; distillates, petroleum, hydrogenated light 10-30%, w/w; organic sulfonic acid salt (proprietary) 10-30%, w/w. This dispersant is harmful by inhalation on repeated or prolonged exposure and may cause irritation of respiratory tract eyes and skin. If swallowed, it may cause nausea and vomiting and CNS depression. The organic portion of this preparation is expected to be biodegradable and component substances have a potential to bioconcentrate. The physical and chemical properties of this product are physical state = liquid, appearance = clear hazy amber, odor = hydrocarbon, specific gravity = 0.95 at 60°F/15.6°C, density = 7.91 lb/gal, solubility in water = miscible, pH = (100%) 6.2, viscosity = 177 cst at 32°F/0°C, 70 cst at 60°F/15.6°C, 22.5 cst at 104°F/40°C, pour point = < -71°F/< -57°C, boiling point = 296 °F/147°C, vapor pressure = 15.5 mm Hg at 100°F/37.8°C.

Corexit 9527 is contains 2-butoxyethanol 30-60%, w/w; propylene glycol 1-5%, w/w; Organic sulfonic acid salt (proprietary) 10-30%, w/w. This product is harmful by inhalation, in contact with skin and if swallowed, irritating to eyes and skin. The organic portion of this preparation is expected to be readily biodegradable and component substances have a low potential to bioconcentrate. The product have the following physical and chemical properties; physical state = liquid, appearance = clear amber, odor = mild, specific gravity = 0.98-1.02, density = 8.2-8.5 lb/gal, solubility in water = complete, pH = (100%) 6.1, viscosity = 160 cst at 32°F/0°C, pour point = < -40°F/< -40°C, boiling point = 340°F/171°C, vapor pressure = < 5 mm Hg at 100°F/38°C same as water, evaporation rate = 0.1. (USEPA Oil Program Center 1994; Nalco EC9500A, Material Safety Data Sheet 2005).

Test water. The natural river water obtained from Shatt Al-Arab River which was filtered through 0.45 μm Whatman sterile membrane filter and boiled was used for the preparation dilutions in bioassays. Water quality measurements of control/dilution water used in the tests during the tests period are dissolved oxygen = 6.8-6.9 mg/L, pH = 7.5-7.9, temperature = 23.3-27.5°C, salinity = 5.1-5.3 ‰ , conductivity = 1155-1187 μ mos/cm.

Test animals. The biological material used in the tests were the bivalve *C. fluminalis* (Müller) [Molluscs: Bivalia: Eulamellibranchiata: Corbiculidae] and the snail *M. tuberculata* (Müller) [Molluscs: Gastropoda: Monotocardia: Melaiidae]. Specimens of adult and uniform size individuals of the both species were collected freshly from Shatt Al-Arab River for experiments. The animals were transferred to laboratory for acclimation period of 24 hours prior to the toxicity experiments, under laboratory temperature of 20±2 ºC with light/dark cycle (12 hours: 12 hours) under aerated conditions. After acclimatization, aeration was removed.

Toxicity experiments. Toxicity experiments were conducted at two controlled temperatures (10°C and 32°C). Ten organisms were placed in three glass containers ($50 \times 50 \times 35$ cm^3 in size) each one containing 6 liter of clean river water. 500 mL of crude oil was added in each of two of the containers and to one of them about 50 mL of dispersant was also added. The mixtures were then agitated for about 2 minutes by a glass rod. The third container served as a control. The containers were then drained, so that the crude oil or crude oil plus dispersant settled onto the organisms. Eight hours after draining the containers were refilled with 6 liter of clean river water and the aeration was resumed. Six hours after refilling the containers were drained again. The tidal regime was maintained for 32 hours with *M. tuberculata* and for 71 hours with *C. fluminalis*. The containers were kept without cover and the organisms were examined at intervals. At the end of experiments, the organisms in the test containers were rinsed in river water to remove the oil. The organisms were then transferred into other containers contained clean river water and maintained at the same temperature. The containers were covered and aerated, and the organisms were kept under observation for 84 hours for *M. tuberculata* and 46 hours for *C. fluminalis*. The snails were considered healthy when they were able to adhere to the walls of the containers or when they withdrew into the shell when touched with a glass rod. They were classed as moribund or dead if they were not movable when touched with a glass rod. The bivalves were classed as healthy when their shell closed and gave a solid thump sound when tapped with a glass rod. They were considered moribund or dead if the shell was agape and unclosed even when the soft inner parts were prodded, or if it appeared to be closed but gave a hallow noise when tapped with a glass rod.

Statistical analysis and calculation of median lethal time (LT_{50}) (time causing 50% mortality) and median recovery time (RT_{50}) (time to 50% recovery). The LT_{50} and RT_{50} values were calculated using the method described by UNEP (1989) analysis of variance (ANOVA) was used to test for significant differences ($p>0.05$) of these values.

Results and Discussion. Oil dispersants play an important role in environmental technologies aiming to enhance the dissolving of oil in the water column by converting oil spills into chemically dispersed droplets. Potential ecotoxicological effects of this change must be taken into consideration because benthic organisms that initially are not affected by oil may be exposed to the harmful impacts of dispersants (Singer et al 1996). Mollusca are for example on these benthic organisms which are exposed to the harmful impacts when dealing with the devastating agents of oil and oil dispersants.

The results of the present study have reconfirmed that the crude oil plus corexit 9500 and crude oil plus Corexit 9527 were immediately toxic to almost all the test snails. No more than one of them were healthy at the end of the first hour of low tide, and no recovery took place among snails during the rest of the tidal regime (Table 2). Singer et al (1996) reported the ability of Corexit 9500, Corexit 9527, and Corexit 9554 to induce death in molluscs, specifically in red abalone embryos (*Haliotis rufescens*). A slower but progressive toxic effect was seen among the *M. tuberculata* subjected to crude oil alone. According to Committee (2006) 75% of researchers of the recent toxicity studies found that chemically dispersed oil was more toxic than dispersant or oil alone. More of them found that the cause for this was the increased polycyclic aromatic hydrocarbons - PAHs (typically about 5 to 10 times) in the water column. Others noted the increased amount of total oil in the water column and little researchers noted the damage to fish gills caused by the increased amount of droplets.

Table 2

Number of survived *Melanoides tuberculata* subjected to crude oil and crude oil plus dispersants during tidal regime and LT_{50} values

Toxicant	*Temperature (°C)*	*Draining the containers*			*Refilling the containers (6 hrs) and draining them again*		*Refilling the containers again (6 hrs) and draining them again*		*LT_{50} (hour)*
		1 h after draining	*3 hrs after draining*	*8 hrs after draining*	*Through draining*	*9 hrs after draining*	*Through draining*	*3 hrs after draining*	
12% evaporated crude oil + Corexit 9500	10	1	0	0	0	0	0	0	1
	32	0	0	0	0	0	0	0	1
12% evaporated crude oil	10	10	8	7	2	1	0	0	11
	32	6	5	1	1	0	0	0	3
13% evaporated crude oil + Corexit 9527	10	1	0	0	0	0	0	0	1
	32	1	0	0	0	0	0	0	1
13% evaporated crude oil	10	9	8	7	4	2	2	2	12
	32	7	6	3	2	1	1	0	5
Control	10	10	10	10	9	9	9	9	-
	32	10	10	10	9	9	8	8	-
Total time (hour)		1	3	8	14	23	29	32	-

Among the numerous examples in literature related to toxicity testing of crude oil, dispersant and dispersed oil are the researches conducted by Guleg et al (1997), Singer et al (1997), Singer et al (1998), Fuller et al (1999), Epstein et al (2000), Fuller & Bonner (2001), Bhattacharyya et al (2003), Koyama & Kakuno (2004), Couillard et al (2005), Khan & Payne (2005), Smith et al (2005), and Chukwu & Odunzeh (2006). However, Less than 25% of researchers noted that chemically dispersed oil was equivalent or less toxic than that of the oil or dispersant separately such as the works of Adams et al (1999), Long & Holdway (2002) and Fuller et al (2004). The snails LT_{50} value at 10ºC was 11 hours for 12% evaporated crude oil and 12 hours for 13% evaporated crude oil, but was 3 hours for 12% evaporated crude oil and 5 hours for 13% evaporated crude oil at 32ºC (Figure 1). Obviously, a small difference in snails LT_{50} values is observed between those snails subjected to 12% evaporated crude oil and those subjected to 13% evaporated crude oil. The difference is 1 hour at 10ºC and 2 hours at 32ºC, and is not significant, particularly because there was only a 1% difference in volume loss on the original crude oil. The 8 hours and 7 hours difference in the LT_{50} values of snails subjected to 12% evaporated crude oil and 13% evaporated crude oil respectively between 10ºC and 32ºC is high significant. The effects of temperature on the toxicity of oils and other toxicants had been reported by numerous studies (NRC 2003; NAS 2005; Committee 2006). Throughout the tidal regime, snails that had become unattached to the substratum of the containers, some within the shell and others extended. The same observations were noted by Farid (2007) on snails exposed to crude oil and its products.

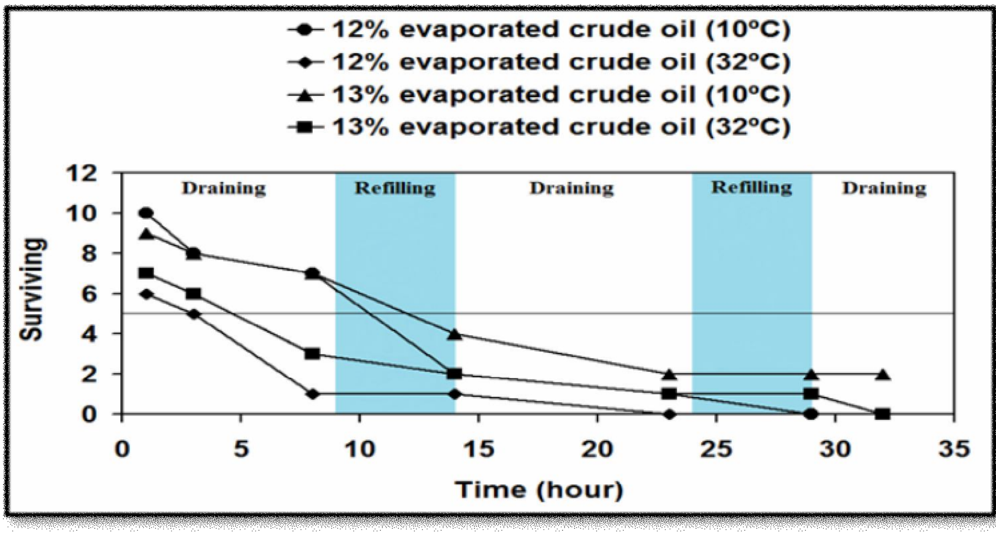

Figure 1. Tidal regime of *Melanoides tuberculata* subjected to crude oil.

No toxic effects were seen among the *C. fluminalis* subjected to crude oil alone during the tidal regime. 14% evaporated crude oil plus Corexit 9527, however, showed an initial toxicity to bivalves at 10ºC which was followed by partial recovery and in turn by progressive mortality. The effect was not seen among the bivalves subjected to the 14% evaporated crude oil plus Corexit 9527 at 32ºC, although after 71 hours of tidal regime there were fewer survivors of bivalves at 32ºC than at 10ºC (Table 3 & Figure 2). This initially acute reversible toxic effect of 14% evaporated crude oil plus Corexit 9527 to bivalves at 10ºC may be as a result of a slower evaporational loss of the solvent of the dispersant at the lower experimental temperature, where this solvent fraction of the dispersant would not have persisted for the long time. Fuller & Bonner (2001) found a great different in toxicity of dispersants between the earlier types and those which have been developed subsequently. The examination of relationship between the toxicity and composition of dispersants shows that this resides primarily in the nature of the solvent. Wilson (1968) have demonstrated a loss of toxicity of BP 1002 due to evaporation. The author showed that *Sabellaria spinulosa* larvae apparently recovered from 1 ppm BP 1002 after 2 days, but 4 weeks later they were moribund on the bottom of the tank.

Table 3

Number of survived *Corbicula fluminalis* subjected to crude oil and crude oil plus dispersants during tidal regime

Toxicant	*Temp. (ºC)*	*Draining the containers*			*Refilling the containers (6 hrs) and draining them again*		*Refilling the containers again (6 hrs) and draining them again*		*Refilling the containers (12 hrs) and draining them again*		*Refilling the containers (24 hrs) and draining them*
		1 h after draining	*3 hrs after draining*	*8 hrs after draining*	*Through draining*	*9 hrs after draining*	*Through draining*	*3 hrs after draining*	*Through draining*	*3 hrs after draining*	*Through draining*
13.5% evaporated crude oil + Corexit 9500	10	10	10	9	8	8	8	8	8	8	8
	32	10	10	10	10	10	10	10	9	9	8
13.5% evaporated crude oil	10	10	10	10	10	10	10	10	10	10	10
	32	10	10	10	10	10	10	10	10	10	10
14% evaporated crude oil + Corexit 9527	10	3	3	3	8	8	8	8	8	7	5
	32	8	8	8	8	8	8	8	7	7	1
14% evaporated crude oil	10	10	10	10	10	10	10	10	10	10	10
	32	10	10	10	10	10	10	10	10	10	10
Control	10	10	10	10	10	10	10	10	10	10	10
	32	10	10	10	10	10	10	10	10	10	10
Total time (hour)		1	3	8	14	23	29	32	44	47	71

The *C. fluminalis* subjected to 14% evaporated crude oil plus Corexit 9527 at 10ºC behaved in a fashion similar to *S. spinulosa* larvae (although over a much shorter period). In both instances one may conclude that the initial toxicity is caused by the evaporate solvent of the dispersant. 13.5% evaporated crude oil plus Corexit 9500 was less toxic toward *C. fluminalis* at either temperature than 14% evaporated crude oil plus Corexit 9527. Out of a total of 10 bivalves used in the present tests no more than 2 individuals at any time showed any adverse effects. The difference in dispersants toxicity to aquatic organisms has been well documented (Committee 2006). Mitchell & Holdway (2000) found that the acute toxicity of Corexit 9527 and Corexit 9500 to green hydra was found to be 230 and 160 ppm (LC_{50}, 96 hour). Bhattacharyya et al (2003) in their study on the toxicity of South Louisiana crude oil or diesel fuel oil and a cleaner Corexit 9580 or dispersant Corexit 9500 to *Chironomus tentans*, *Daphnia pulex*, and *Oryzias latipes* found that the crude oil was less toxic than diesel fuel oil, chemical additives enhanced oil toxicity, the Corexit 9500 was more toxic than the Corexit 9580. Scarlett et al (2005) compared the toxicity of the two dispersants, Corexit 9527 and Superdispersant-25 (SD-25) to the marine sediment-dwelling amphipod *Corophium volutator* (Pallas), the common mussel, *Mytilus edulis*, the symbiotic snakelocks anemone, *Anemonia viridis*, and the seagrass *Zostera marina*. Superdispersant-25 was found overall to be less toxic than Corexit 9527.

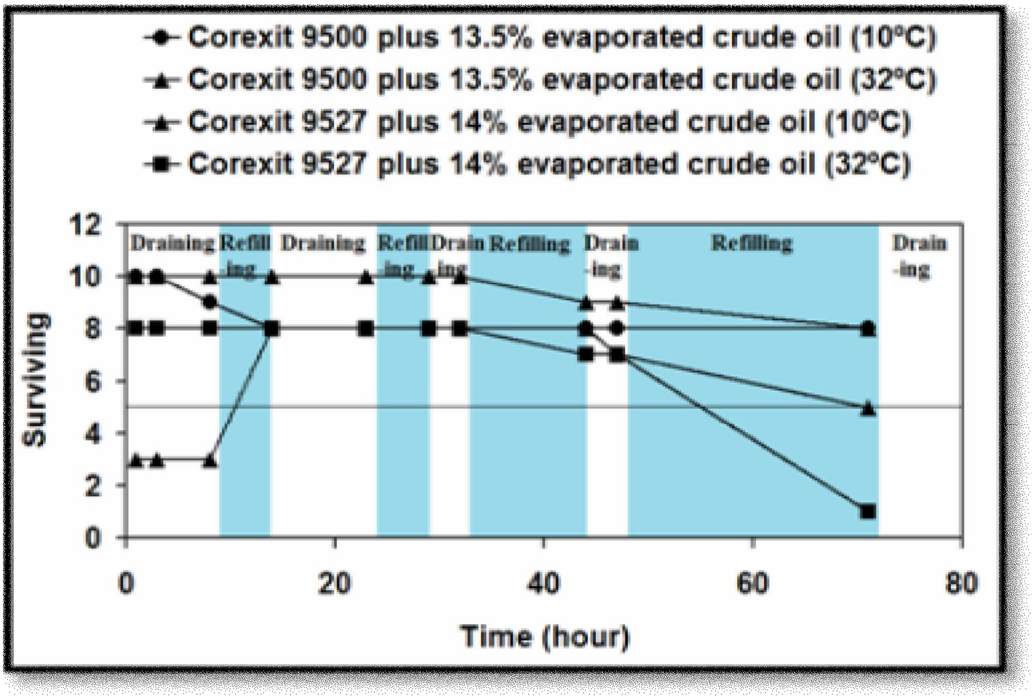

Figure 2. Tidal regime of *Corbicula fluminalis* subjected to crude oil plus dispersants.

When the *M. tuberculata* were transferred to clean river water, the effects that had been seen during the tidal regime become immediately sublethal. Both snails which had been subjected to crude oil alone and those subjected to crude oil plus dispersants were able to recovery, but those snails subjected to crude oil plus dispersants recovered at longer time. The same conclusion was obtained by Bhattacharyya et al (2003). Recovery of snails subjected to crude oil plus dispersants was more rapid at 10ºC, where the snails RT_{50} value was 24 hours for 12% evaporated crude oil plus Corexit 9500 and 39 hours for 13% evaporated crude oil plus Corexit 9527, than at 32ºC, where the snails RT_{50} value was 85 hours for 12% evaporated crude oil plus corexit 9500 and 84 hours for 13% evaporated crude oil plus Corexit 9527 (Table 4; Figure 3 & 4). The temperature and the volume of crude oil lost by evaporation appeared to not affect significantly the snails RT_{50} values for crude oil alone. The snails RT_{50} value at 10ºC was 12 hours for both 12% and 13% evaporated crude oil, while at 32ºC the snails RT_{50} values were 16 and 12 hours respectively. The difference of 61 hours of snails exposed to 12% evaporated crude oil plus Corexit 9500 and 45 hours for those subjected to 13% evaporated crude oil plus Corexit 9527 in the TR_{50} values between 10ºC and 32ºC is interesting because although a distinct temperature affect was observed in the LT_{50} values of snails subjected to crude oil alone it was not seen in the LT_{50} values of snails exposed to crude oil plus dispersants (Table 5). Thus, the toxicity of the crude oil plus dispersants was also acute to snails at temperatures of 10°C and 32°C. The difference between the 10ºC TR_{50} values of snails subjected to 12% evaporated crude oil plus Corexit 9500 (24 hours) and those subjected to 13% evaporated crude oil plus Corexit 9527 (39 hours) is not significant.

Table 4

Numbers of *Melanoides tuberculata* recovered from crude oil and crude oil plus dispersants during the recovery period and RT_{50} values

Toxicant	*Temperature (ºC)*	*After 12 hrs*	*After 16 hrs*	*After 24 hrs*	*After 84 hrs*	*RT_{50} (hour)*
12% evaporated crude oil + Corexit 9500	10	3	4	5	6	24
	32	1	2	2	6	85
12% evaporated crude oil	10	5	6	7	9	12
	32	2	5	6	8	16
13% evaporated crude oil + Corexit 9527	10	4	4	4	8	39
	32	0	1	1	5	84
13% evaporated crude oil	10	5	7	7	8	12
	32	5	6	6	8	12
Control	10	10	10	10	10	---
	32	29	10	10	10	---
Total time (hour)		12	26	24	84	

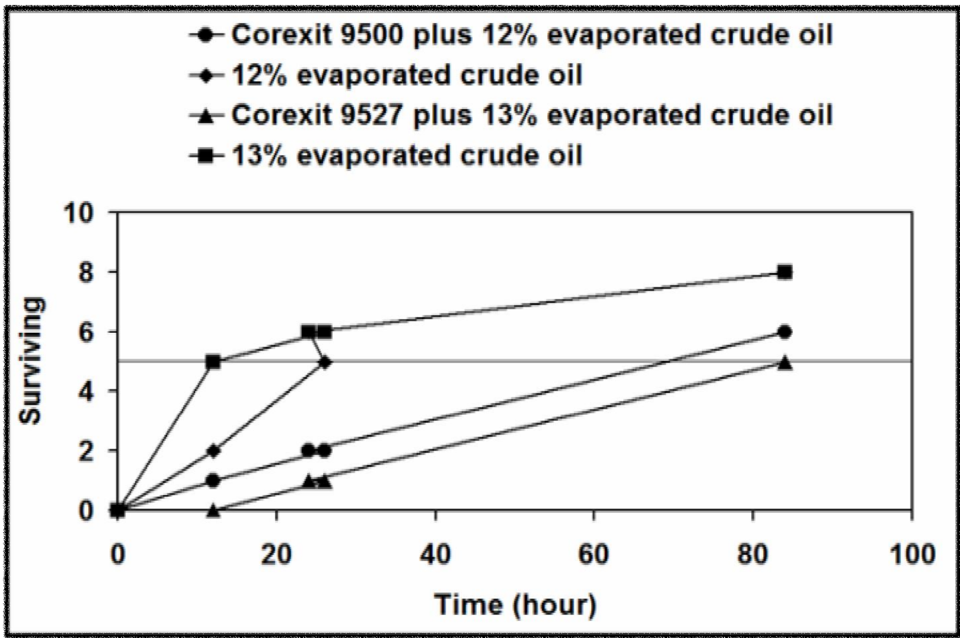

Figure 3. Recovery of *Melanoides tuberculata* subjected to crude oil and crude oil plus dispersants at 10 ºC.

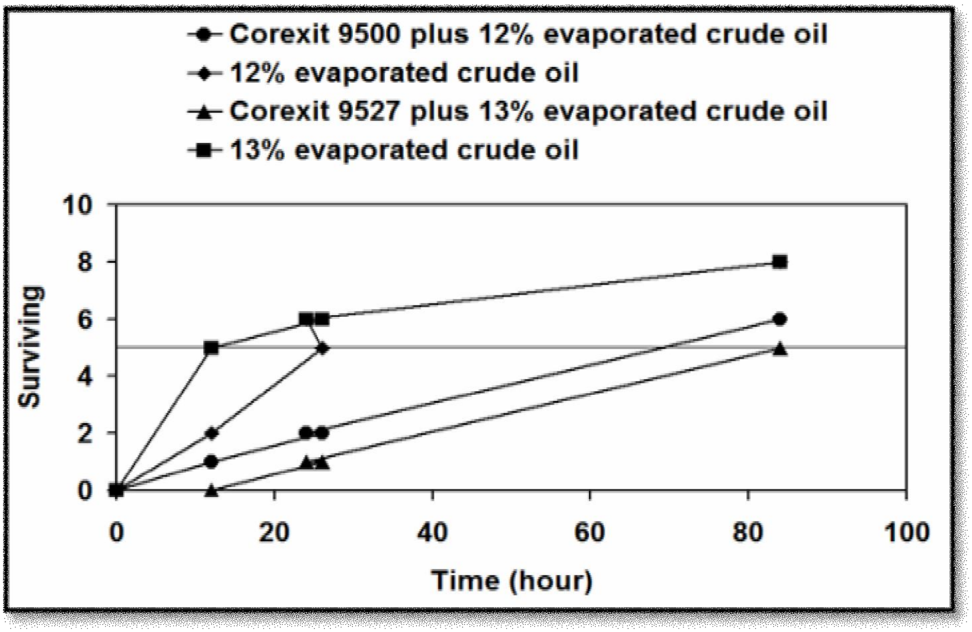

Figure 4. Recovery of *Melanoides tuberculata* subjected to crude oil and crude oil plus dispersants at 32 ºC.

No recovery took place among *C. fluminalis* which had been subjected to crude oil plus dispersants. The bivalves that subjected to crude oil alone showed progressive recovery with a TR_{50} value of about 17 to 20 hours. The bivalves exposed to 13.5% evaporated crude oil becoming recovered somewhat more slowly, at least in the earlier part of the recovery period than those subjected to 14% evaporated crude oil (Table 4 & Figure 5).

Table 5

Number of *Corbicula fluminalis* recovered from crude oil and crude oil plus dispersants during recovery period and RT_{50} values

Toxicant	*Temperature (ºC)*	*After 10 hrs*	*After 20 hrs*	*After 46 hrs*	*RT_{50} (hour)*
13.5% evaporated crude oil + Corexit 9500	10	0	1	1	-
	32	0	1	1	-
13.5% evaporated crude oil	10	1	5	5	20
	32	1	7	7	17
14% evaporated crude oil + Corexit 9527	10	0	0	0	-
	32	0	0	0	-
14% evaporated crude oil	10	3	6	7	17
	32	3	6	7	17
Control	10	10	10	10	-
	32	10	10	10	-
Total time (hour)		10	20	46	-

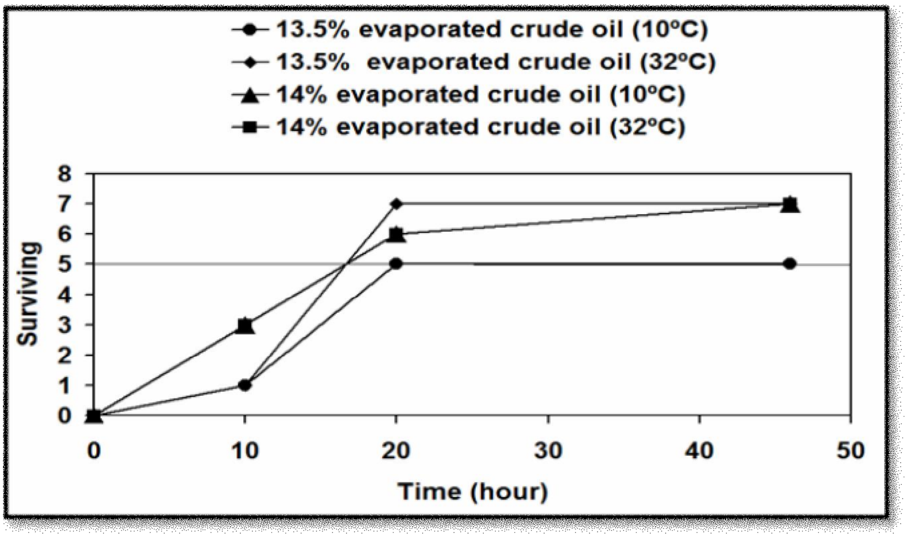

Figure 5. Recovery of *Corbicula fluminalis* subjected to crude oil.

Conclusions. The effects on intertidal organisms of chemically treated crude oil with a dispersants are more severe than the effects of non-chemically dispersed crude oil. The results of this study can be considered relevant when the oil dispersants are used in the field. Even if an effect is only sublethal under laboratory experiments, a narcotized organism in an intertidal habitat is at an obvious disadvantage with regard to predators and other negative factors.

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