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Toxicity Effect of Oil Spill Dispersants on *Litopenaeus Vannamei*

Delshad, N.[1*], Emtyazjoo, M.[2] and Khezri, M.[3]

[1]Department of Environmental Science, faculty of environmental and energy, science and Research Branch, Islamic Azad University, Tehran, Iran

[2]Department of Marine Biology, Islamic Azad University, Marine Science and Technology, North Tehran Branch, Tehran, Iran

[3]Department of Environmental Engineering, Graduate Faculty of Environment and Energy, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT: One of the most chemical methods for controlling of oil spills is use of dispersants, which are widely being used worldwide. In this research, comparison of acute toxicity of crude oil of the Persian Gulf-Khark area (CO), Iranian Naftroob crude oil dispersant (IND), Radiagreen OSD dispersant crude oil (RD), the mixture of crude oil and Iranian Naftroob dispersant (CO-IND) and crude oil with Radiagreen OSD dispersant (CO-RD) was carried out. For this purpose, the concentrations of both dispersants were equal: 10.5, 21, 42, 84 and 168 mg/L. The concentrations of 8.4+16, 16.8+336, 33.6+672, 67.2+1344 and 134.4+2688 mg/L were used for dispersants-crude oil mixtures and the concentrations of 168, 336, 672, 1344 and 2688 mg/L were used for crude oil alone. *Litopenaeus vannamei* was used for bioassay toxicity test. 48-h LC50 was estimated by Probit value table. Relationship between efficiency and toxicity (RET) was also estimated. 48-h LC50 for CO, IND, RD, CO-IND and CO-RD were 1741, 17.21, 43.52, 631.456, 357.089 mg/L, respectively. The results were obtained by these tests were shown that CO alone is less toxic than the IND, RD and also CO-IND and CO-RD. Moreover, CO-IND was less toxic than CO-RD. With regard to the efficiency of IND had a lower (RET = 34.8) than RD (RET = 39) and so, it was more efficient.

Key words: Oil dispersant, Toxicity, Crude oil, RET, *Litopenaeus vannamei*

INTRODUCTION

Oil spills associated with marine transport and offshore production facilities often occur in the open sea and can cause dramatic damage (Khan & Payne, 2005). It is estimated that more than 2 million tons of oil enters marine environments from ships and other sea-based activities annually (GESAMP, 2007). After weathering of the surface oil layer by evaporation, dispersion, and dissolution by sedimentation of heavier slicks, dissolved hydrocarbons in the water may still be toxic for organisms (Tecon *et al*., 2010). Several clean-up options were usually considered to reduce the negative effects of oil spills, chemical dispersant and the mechanical containment and collection are two major methods widely used (Liu *et al*., 2006). Dispersants are a group of chemicals spray or apply to oil slicks to accelerate the dispersion of oil into the water column. They can't remove oil from the water, but are intended to limit the amount of oil forming a slick on the water surface or shoreline by driving that oil into a dissolved phase. It is, however important to note that most of the dispersants are very toxic on their own against organisms and in some cases, enhance the toxicity of spilled crude oil to exposed organism when deployed or utilized to control oil spills (Otitoloju, 2005). Since 1960s, the research on the aquatic toxicology of oil spill dispersants started to be considerable .past studies have evaluated the acute toxicity of dispersants (Perkins *et al*., 1973; Thompson & Wu, 1981; Law, 1995; Adams *et al*., 1999; Ares & Clark, 1999). More recent studies have taken into consideration the toxicity of the petroleum-dispersant interaction (Epstein *et al*., 2000; Long & Holdway, 2002; Lin *et al*., 2009). Some studies show that oil alone has less toxicity than dispersant and combination of oil and dispersant (Milinkovitch *et al*., 2011; Shafir *et al*., 2007; Gulec *et al*., 2000; Unsal, 1991) and some studies show that oil alone is more toxic than dispersant and mixture of oil and dispersant (Otitoloju, 2005; Yijun *et al*., 2010; Ndimela *et al*., 2010). The aim of this research was to determine acute toxicity on *Litopenaeus vannamei* against CO, IND, RD, CO-IND and CO-RD. This organism is a kind of shrimp that have benthic strategy in their life. L. vannamei is the

*Corresponding author E-mail:n_delshad@yahoo.com

most important penaeid shrimp species farmed worldwide.(Alcivar-Warren *et al.*, 2007) It is native in Eastern Pacific coast from the Gulf of California, Mexico to Tumbes, North of Peru (Perez-Farfante & Kensley, 1997). L. vannamei is culturing in south of Iran at Bushehr region.

MATERIALS & METHODS

L.vannamei with an average weight of 14±1 g and 128 days old were selected from Delvar shrimp farm in Bushehr-Iran. They were carefully placed in ice chests with water from the farm and transported to the laboratory near here. Shrimps were acclimated slowly in 25°C for 24-h period in washed aquaria with flow-through air (Bordbar *et al.*, 2008; APHA, 1995). About 80 aquariums of 50L of useful volumes were provided and all aquariums were cleaned and dried carefully. Then 30 liters of seawater were added to each aquarium. Aeration pumps were put in each aquarium. Used water in the experiment had been Filtered and decolorized in Pars abzistan reproduction shrimp center in Bushehr-Iran. 20 shrimps were placed in each aquarium in 14±1 gr. The shrimp health was closely monitored. 24- h before starting the test, due to the shrimp adoption, they were introduced to the aquariums. They were neither fed during the experiment (48-h) nor 24-h before starting the test. The majority of studies had three replicates. The water was not changed either. To prevent the shrimps from jumping out of aquariums, they were all covered by cloth net.

The 48-h acute toxicity of Iranian oil dispersant naftroob (IND), dispersant Radiagreen OSD (RD) and Persian Gulf crude oil (CO) were tested. The experiment conducted five treatments, Crude oil alone, Dispersant Naftroob alone, Dispersant Radiagreen alone and combinations of crude oil with each of the two dispersants (CO-IND, CO-RD). Each treatment conducted five concentrations.

The following concentrations were used:

CO: 168, 336, 672, 1344, 2688 mg/L

RD: 10.5, 21, 42, 84, 168 mg/L

IND: 10.5, 21, 42, 84, 168 mg/L

CO-RD: 8.4+168, 16.8+336, 33.6+ 672, 67.2+ 1344, 134.4+ 2688 mg/L

CO-IND: 8.4+168, 16.8+336, 33.6+ 672, 67.2+ 1344, 134.4+ 2688 mg/L

Three replicates and one control for each treatment were considered. Shrimps mortality were recorded daily and dead shrimps were removed. Water temperature, dissolved oxygen and pH were measured for each test twice, before and after the 48-h bioassay .The test parameters before the test were as follows: temperature 32±1 °C, dissolved oxygen 6.9 mg/L, pH 8.1 and salinity 40±1 ppt. Tables 1, 2, 3, 4 and 5 indicate Water temperature, dissolved oxygen and pH after the test. RET is measured to compare the efficacy of oil dispersants. RET shows the relationship between toxicity and effectiveness. DOR (the rate of dispersant of oil to oil required to disperse 90% of oil) has to be measured in order to measure RET (Anderson *et al.*, 1985).

Table 1. Dissolved oxygen, temperature and pH after the experiment finished in CO
CO = Crude oil of the Persian Gulf-Khark area

CO Concentration(ppm)	CO		
	D.O (mg/L)	Temp(°C)	pH
168	6.3	30	7.9
336	6.2	31	7.9
672	6.2	30	7.9
1344	5.9	30	7.8
2688	5.7	31	7.7

Table 2. Dissolved oxygen, temperature and pH after the experiment finished in IND
IND = Iranian Naftroob crude oil dispersant

IND Concentration (ppm)	IND		
	D.O (mg/L)	Temp (°C)	pH
10.5	6.4	30	7.9
21	6.4	30	7.9
42	6.4	30	8
84	6.2	30	8
168	5.9	30	8.1

Table 3. Dissolved oxygen, temperature and pH after the experiment finished in RD RD = Radiagreen OSD dispersant crude oil

RD Concentration (ppm)	RD		
	D.O (mg/L)	Temp (°C)	pH
10.5	6.4	30	7.9
21	6.4	30	7.9
42	5.9	30	7.9
84	5.9	30	8
168	5.7	30	8

Table 4. Dissolved oxygen, temperature and pH after the experiment finished in CO-IND CO-IND = The mixture of crude oil and Iranian Naftroob dispersant

CO-IND Concentration (ppm)	CO-IND		
	D.O (mg/L)	Temp (°C)	pH
8.4+168	5.8	30	7.7
16.8+336	4.8	30	7.7
33.6+672	4.8	31	7.8
67.2+1344	4.5	30	7.8
134.4+2688	4.2	30	7.9

The 48-h median lethal concentrations (LC50) of the toxicants for *litopenaeus vannamei* are shown in Table 6.

Table 5. Dissolved oxygen, temperature and pH after the experiment finished in CO-RD
CO-RD = Crude oil with Radiagreen OSD dispersant

CO-RD Concentration (ppm)	CO-RD		
	D.O (mg/L)	Temp (°C)	pH
8.4+168	5.2	30	7.5
16.8+336	5.1	30	7.5
33.6+672	4.8	30	7.4
67.2+1344	4.2	30	7.4
134.4+2688	4.2	30	7.4

Table 6. LC50 48-h for *litopenaeus vannamei*

Materials tested	48-h LC50 (ppm)
IND	17.21
RD	43.52
CO-IND	631.456
CO-RD	357.089
CO	1741

It should be bore in mind that the quality of a dispersant cannot be measured by LC50 value. RET is measured to compare the efficiency of oil dispersants. RET shows the relationship between the toxicity and efficiency and it is measured by the following formula: (Anderson *et al.*,1985)

$RET = DOR\ 90.\ 10^4/LC50$

DOR shows the amount of the dispersant that can disperse 90% of the oil spill. DOR gives us the size of detergent that is needed for cleaning the oil spill and it also analyzes its toxicity and cost. DOR of IND and RD is measured by a 20:1 ratio for oil-dispersant mixture by the Research Institute of Oil Company in Tehran. The table shows RET, DOR and LC50 for IND and RD. The smaller the RET, the less toxic the product, that is to say the smaller size of the material is more efficient (Liden, 1975). Although LC50 for IND is less than for RD but RET in IND is smaller, therefor it is more efficient than RD.

Table 7. Relative effectiveness and toxicity of oil dispersants

Oil Dispersant	Dispersants to oil ratio (DOR_{90})	RET	48- h LC50 (ppm)
RD	0.17	39	43.52
IND	0.06	34.8	17.21

The toxicity of RD and IND was higher than the combination of dispersants- crude oil and the toxicity of the combination of dispersants- crude oil was higher than the crude oil alone on *Litopenaeus vannamei*. The result of this study support those reported by Unsal in 1991 for *Palaemon elegans*. He reported that the dispersants are more toxic than the oil spill and the oil alone (Unsal,1991). As Gulec reports in 2000 by measuring 96-h LC50 for crude oil, dispersant and oil spilled on *Palaemon serenus*, the mixture of oil spill are remarkably more toxic than the crude oil alone (Gulec *et al.*, 2000). In this study, after the addition of oil to sea water, while the water surface was covered by oil, the shrimps gathered at the bottom of the aquarium; while, the addition of dispersant to the oil caused more oil particles to be at the disposal of the shrimps and the shrimps reacted more intensely to the mixture.

As Trazwell, Verriopoulos and Bobra have reported in their studies that the spilled oil is more toxic for the marine organisms than the oil alone (Tarzwell, 1971; Verriopoulos & Moraitiou-Apostolopoulou, 1982; Bobra *et al.*, 1989). Although the results of the researches made by Hemmer in 2011 to compare the toxicity effect of eight oil spill dispersants on mysid shrimp *(Americamysis Bahia)* and the inland silversides *(Menidia Berylina)* revealed that for all eight dispersants in both test species, the dispersant alone were less toxic than the dispersant--crude oil mixtures. Crude oil alone had generally similar toxicity for A. Bahia and M. Berylina as the dispersant-crude oil mixtures (Hemmer *et al.*, 2011). The species tested, the age of the organism, length of the organism, formulation of the dispersants, dissolved oxygen, temperature of sea water, salinity of sea water and etc., are among the factors affecting the results of the experiments that have to be taken into consideration (Yijun et al.,2010; Oyewo,1986; Swedmark *et al.*,1973).

CONCLUSION

The results of this study indicate that the toxicity of CO is remarkably less than the toxicity of the dispersants and the mixture of dispersants with crude oil; moreover, the toxicity of IND is higher than RD, but in comparison to the efficiency of RD and IND, the RET of IND is less than RD and so it is more efficient than RD. Based on the results of this study, the dispersants, should only be used when it is essential, that is when the application of mechanical solutions is not sufficient. The dispersants should not be used as the only and the first choice for responding to oil pollution.

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