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Effect of Sublethal Concentrations of Fuel Oil on the Behavior and Survival of Larvae and Adults of the Barnacle *Balanus amphitrite amphitrite* (Darwin)

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Abstract

The sublethal effect of petroleum hydrocarbons (fuel oil) was investigated using different developmental stages of *Balanus amphitrite amphitrite*. 5, 10, 15 and 20 ppm concentrations of the water soluble fraction of the fuel oil were applied and found to have significant effect on the behavior and survival of the organism. Larval stage II was more sensitive than the other stages and was affected even with the lowest concentration of hydrocarbons (5 ppm). Nutritional activity of the adults was affected by fuel oils due to effects on the movements of the cirri as indicated by altering the number of cirri beats. At 15 and 20 ppm concentration, cirri stopped moving and the organisms died. Accumulation of the hydrocarbons in the tissues of organisms' was determined and was the highest at 15 ppm concentration (after 48 hours exposure time) where it reached 240 µg/g dry weight. After 96 hours of exposure, only adults exposed to 5 ppm could be recovered. Due to continuous pollution in the study area, there have always been petroleum hydrocarbons in the organisms tissue which reach 55 µg/g, but the barnacles can tolerate up to 100 µg/g.

Keywords: Fuel oil, Barnacle, sublethal concentration, South Iraq

Ölümcül olmayan Akaryakıt Konsantrasyonlarının Kaya Midyesinin *Balanus Amphitrite Amphitrite* (Darwin) Larvası ve Erginlerinin Davranışları ve Yaşama Oranı Üzerindeki Etkisi

Özet

Balanus amphitrite amphitrite'in farklı gelişim evreleri kullanılarak petrol hidrokarbonların (akaryakıt) ölümcül olmayan etkisi araştırılmıştır. Suda çözünen akaryakıtların 5, 10, 15 ve 20 ppm'lik konsantrasyonları uygulanan organizmanın davranışı ve yaşama oranı üzerinde anlamlı etkisi olduğu görülmüştür. II. larva evresi, diğer evrelerden daha hassas olmuş ve en düşük hidrokarbon (5 ppm) konsantrasyonundan dahi etkilenmiştir. Cirri beat sayısında değişiklik yapılarak da belirtildiği gibi, cirri hareketleri üzerindeki etkileri sebebiyle erginlerin beslenme faaliyeti akaryakıttan etkilenmiştir. 15 ve 20 ppm'lik konsantrasyonlarda cirri hareket etmeyi bırakmış ve organizmalar ölmüştür. Organizmaların dokusu üzerinde hidrokarbonların biriktiği tespit edilmiş ve en fazla akümülasyon, 240 µg/g kuru ağırlığa eriştiği 15 ppm'lik konsantrasyonda (48 saatlik maruziyet süresinden sonra) olmuştur. 96 saatlik uygulama sonrasında yalnızca 5 ppm'e maruz kalmış olan erginler kurtarılmıştır. Çalışma yapılan alanda sürekli kirliliğin olması sebebiyle organizma dokusu üzerinde her zaman 55 µg/g'a ulaşan petrol hidrokarbonlar bulunmaktadır. Fakat kaya midyesi 100 µg/g'ye kadar tolere edebilmektedir.

Anahtar Kelimeler: Akaryakıt, Kayamidyesi, yaşamsal konsantrasyon, Güney Irak.

Introduction

The Arabian Gulf is one of the most oil loading and transporting places in the world where many oil ports are functioning. It has been estimated that 49% of the world's oil production comes from the Gulf and its liability to pollution is 48 times more than any other place on earth (Al-Awadhi, 1999). Even before the Gulf war, environmental impacts associated with oil exploitation, refining, handling and discharge of slop oil as well as natural seepage were rather high (Zarba *et al*., 1985). Although the Iraqi coastal line is rather short, two busy oil platforms exporting oil the whole year round, these are the Deep and Basrah oil ports. Iraqi shores were exposed to ecological disasters during the Gulf wars where tremendous amounts of crude oil were spilled. Oil seepage during tankers loading is likely to occur resulting in continuous sea water pollution.

Oil discharged to the marine environment can result in both acute and chronic effects to marine organisms. In coastal waters, polycyclic aromatic hydrocarbons can cause variety of effects to all organisms. Fishes may seem more tolerant to hydrocarbons owing to the mucous membrane surrounding their bodies which prevent absorption of these chemicals, but their early life stages can be severely affected (Baron *et al*., 2004). Adult fish, however, will die when exposed for long to hydrocarbons (Fadaj, 2000).

Barnacles are abundant in the Arabian Gulf where four species were recorded. *Balanus amphitrite amphitrite* is the only species found in the Iraqi coastal waters up to Shatt Al-Arab estuary (Arif, 1977). This species is widely distributed through the whole gulf and can quickly encrust any submerged object down to 2-3 meters. Despite of the adverse effect of barnacles on ships, cooling pipes of power plants and other submerged objects, its larvae play an important role in the trophic levels of coastal waters where it forms an important part of the diet of many fishes. In there Iraqi coastal water, two fishes, *Acanthopagrus latus* and *Pathygablus fussus* feed mainly on barnacle's larvae as well as young adults by breaking their shells (Abdul Sahib, 1997). Removal of this organism due to oil pollution affects the stock of such fishes.

The present study aims at investigating the effect of fuel oil on the survival and activity of larvae and adult barnacles. A locality in the Shatt Al-Arab estuary was selected as it is exposed to high pollution by oil due to heavy boats traffic, oil transport and several cleaning processes.

Material and Methods

Sampling was made in a locality opposite to Fao city, 100 km south of Basrah (Figure 1). Adult barnacles were scrapped from an old, sunken ship where its body is heavily covered with barnacle shells. Samples were kept in large bowls filled with water from the same locality. Larvae were collected from the water column by means of plankton net. These were kept in 1 liter jars filled with water and kept in isolated boxed to prosses the same water temperature until returning to laboratory.

In the laboratory, adult barnacles (shells) were kept in the experiment containers for four days for acclimatization. They were then transferred into glass jars, 8-10 shells in each jar for pollution experiments. Larvae were separated under a dissecting microscope into two groups, stage II and stage III groups. 10 larvae of each stage were transferred into glass dishes containing water from the site of collection. Three sets of each larval stage were prepared for the experiment as well as an additional set treated as a control. The effect of the fuel oil on larval activity was determined according to the score index presented in Table 1.

The water soluble fraction (WSF) of the fuel oil was prepared according to Anderson *et al*. (1974). One volume of the fuel oil used locally was shaken with nine volumes of water collected at time of sampling (salinity 17 ppt) for 12 hours. After the mixture was well homogenized, concentrations of 5, 10, 15 and 20 ppm were prepared for the experiment. The control dishes contained only water. All larvae and adults were placed in these concentrations and observations for mortality and behavior were made every two, four, six, 12, 48, and 96 hours. To eliminate the effect of temperature variation, all experiments were made in the same temperature which is similar to that of the natural habitat. Observations on larval behavior were made as shown in Table 1 a score is given to larvae condition in each WSF concentration. For the adults, the number of cirri movements (beats) per five seconds was calculated as an indicator of its activity.

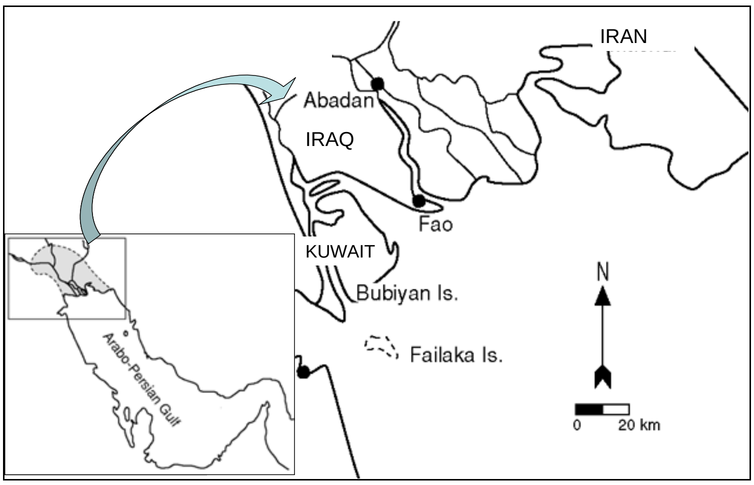

Figure 1. Map showing lower reaches of the Shatt Al-Arab estuary and the sampling location.

Table 1. Index of barnacle larval behavior exposed to fuel oil

Behavior	score
Healthy, swimming well	4
Slow movement in one direction	3
No movement except for appendages	2
Narcoted, with sporadic movement	1
Dead after two hours	0

Table 2. Larval activity in different concentrations of fuel oil during various times of exposure

	Stage II larvae							Stage III larvae						
	Time of exposure (hours)													
Concentration	2	4	6	12	24	48	96	2	4	6	12	24	48	96
(ppm)	Score													
5	3	3	3	3	3	3	3	3	3	4	4	4	4	4
10	2	2	2	3	3	3	3	3	3	3	4	4	4	4
15	2	1	1	1	1	1	1	2	2	3	3	3	3	2
20	0	0	0	0	0	0	0	1	1	2	2	2	2	1

Score 4: healthy, 3: slow movement, 2: no movement, 1: narcotid, 0: dead.

For the determination of accumulated hydrocarbons in the tissues of barnacles, the procedure of Grimalt and Oliver (1993) was followed with slight modification. Few randomly selected shells from each experiment were broken and their tissues were taken. Two grams of the freeze-dried and grounded tissues were placed in pre-extracted cellulose thimble and soxhlet extracted 150 ml methanol:benzene (1:1 ratio) for 24 hours. The extract was then transferred into a storage flask. The sample was further extracted with a fresh solvent. The extracts were reduced in volume to ca 10 ml in a rotary vacuum evaporator and then saponified for two hours with a solution of 4N KOH in 1:1 methanol benzene solution. After extraction of the unsaponicated matter with hexane, the extract was dried over unhydrous Na_2SO_4 and concentrated by a stream of N_2 for UVF analysis. A Schimadzu RF-540 spectrophotometer was used to determine total petroleum hydrocarbons. Quantitative measurements were made by measuring emission intensity at 360 nm with excitation set at 310 nm and monochrommeter slits of 10 nm.

Results

The effect of different concentrations of the WSF of the fuel oil on the larvae of *Balanus amphitrite amphitrite* is shown in Table 2. It seems that larvae of stage II is more sensitive to hydrocarbons and get affected faster than those of stage III. At the lowest concentration (5 ppm) of WSF, the activity of stage II larvae decreased after two hours of exposure and remained in the same condition for the rest of the experiment time (96 hours), whereas stage III larvae were able to retain its activity and recover after four hours of exposure at this concentration. The larvae of the latter stage have also recovered after 12 hours when exposed to 10 ppm concentration of the WSF of fuel oil. At 20 ppm concentration, none of the larvae were recovered and all of stage II larvae were dead after two hours of exposure. Accordingly, the early larval stages have no opportunity of surviving if exposed for long time to even low concentrations of crude oil.

The effect of WSF of fuel oil on the adults was studied by observing the number of cirri movements by calculating the cirri beat numbers per second (five seconds for easy counting). The organism uses its cirri to generate inward current for nutrition and any interference with this behavior may lead to death.

Normal number of cirri beats ranges between 9 to 11 beats per five seconds depending on feeding activity. When exposed to 5 ppm of WSF of fuel oil, cirri beats became considerably slow after two hours, but then after 12 hours the organism seemed to recover and cirri movement returned to normal (Figure 2). The same was also observed when adults were exposed to 10 ppm concentration. In the higher concentrations (15 and 20 ppm), the organism could not retain its ability of feeding and its cirri were stopped after 24 hours of exposure, leading to death (Figure 2).

Samples of *Balanus* shells exposed to different concentrations of WSF of fuel oil were taken for examining hydrocarbons accumulation in their tissues. As shown in Figure 3, the organism can tolerate up to 100 µg of hydrocarbons per gram of live tissue. Shells exposed to 10 ppm of WSF of fuel oil could survive, while those exposed to 15 ppm were dead and the concentration of hydrocarbons in their tissues reached 240 µg/g after only 24 hours of exposure. Since the water of the studied locality is polluted with fuel oil, a concentration of 55 µg/g is always found in the living organisms as seen in the shells of the control treatment as well as those

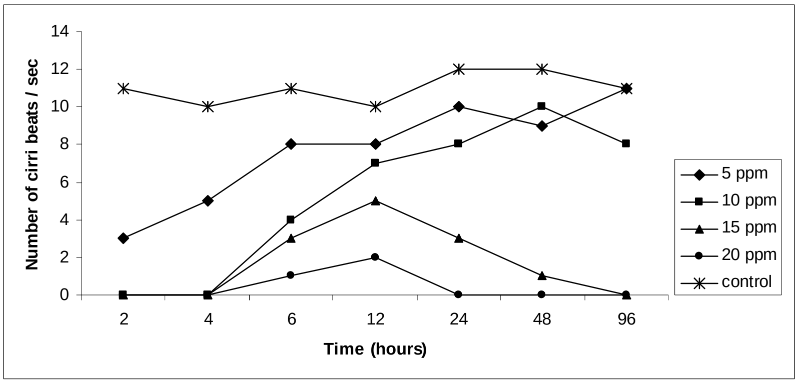

Figure 2. Number of cirri beats (in 5 seconds) with different concentrations of WSF and exposure time.

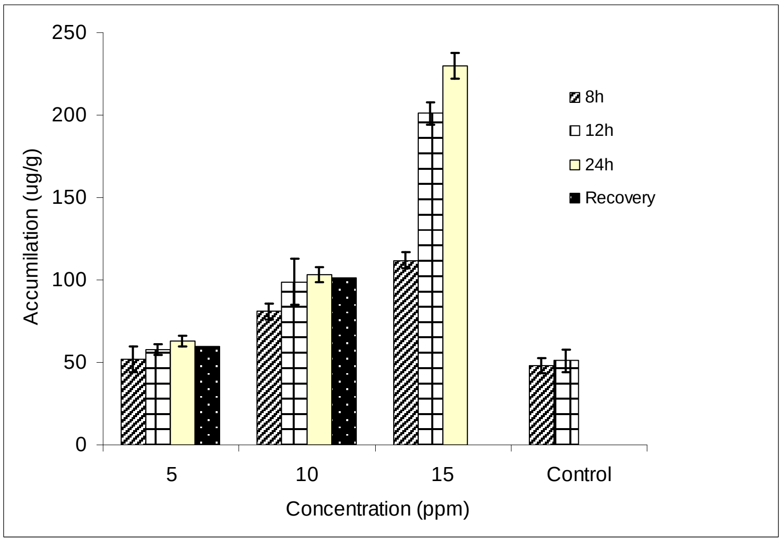

Figure 3. Concentration of accumulated hydrocarbons in the tissues of *Balanus amphitrite amphitrite* exposed to different concentrations of fuel oil.

examined directly after collection from the field (Figure 3). It was also noticed that the gills of the organisms exposed for longer time (over 24 hours) were covered by a mucoid film, probably excreted in response to exposure to hydrocarbons.

Discussion

It has been found that the aromatic hydrocarbons of the crude oil have direct effect on the central nervous system which leads to imbalance of movement and decrease of oxygen consumption (Hashim and Ahmed, 1998; Ahmed *et al*., 2001). This effect is quite evident when stage II and stage III larvae were narcoted with uncontrolled movement when exposed to 15 and 20 ppm of WSF respectively. Larval stages are very sensitive and intolerable to even low concentrations of petroleum hydrocarbons.

The adults although are more tolerable to hydrocarbons, yet some physiological reaction can occur which in turn interfere with normal activity as a result of its interference with the central nervous system. This also leads to the secretion of a mucoid film on the gills. Such film would clearly interfere with oxygen consumption and lead to death if exposure to hydrocarbons extends for longer periods. Such effect has also been observed in the crab *Sesarma bolungeri* in the Shatt Al-Arab River, south Iraq (Ahmed, 2007).

From the results of the present study as well as other works made elsewhere, it can be concluded that early stages of larval development of *B. amphitrites amphitrite* as well as other meroplanktonic organisms are more sensitive to WSF of oils than adults. Even the survived larvae exposed to low concentrations of fuel oil may develop into abnormal adults (Mironov, 1970; Moore and Dwyer, 1974; Holland *et al*., 1984; Blanchard *et al*., 2003). In the lower reaches of the Shatt Al-Arab estuary, water is contaminated with different hydrocarbons coming from oil loading, fuel loading and fuel wastes discharged into the river. Weathering may play an important role in releasing WSF of different hydrocarbons (Neff *et al*., 2000) to make these compounds widely available to be ingested by planktonic and benthic organisms as in the case of *Balanus amphitrite amphitrite*.

Concentrations of hydrocarbons in both water and sediments, however, can be very toxic to both planktonic and benthic organisms (Wetzel and Van Vleet, 2004). Although the sediment may contain higher concentrations of toxic hydrocarbons due adsorption by clay and other particles, yet benthic organisms can absorb hydrocarbons from the water rather faster than that from their substrata (Ho and Karim, 1978). This has been demonstrated in the present study where adult *Balanus* absorbed accumulated the WSF of fuel oil rapidly.

The lower reaches of the Shatt Al-Arab River are rather a confined area with heavy traffic of boats and ships where fuel oil with its soluble fractions of different hydrocarbons can easily find its way to the water. Dispersion of these pollutants is unlikely to occur in comparison with the open parts of the Gulf and therefore organisms are more likely to be affected. Further studies on different marine organisms are needed to elucidate the effect of fuel oil on the biodiversity in the Shatt Al-Arab estuary and North parts of the Gulf which is polluted with oils.

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