EFFECT OF WATER–SOLUBLE FRACTIONS (WSF) OF KEROSENE ON THE OSMOREGULATION OF THE PENAEID SHRIMP *METAPENAEUS AFFINIS* H. MILIN–EDWARDS (CRUSTACEA: DECAPODA: PENAEIDAE) [+]

تأثير الأجزاء الذائبة بالماء من الكيروسين على عملية التنظيم الازموزي للروبيان *METAPENAEUS AFFINIS* (H. MILIN-EDWARDS) (صنف: القشريات، رتبة: عشارية الأقدام، عائلة: البنايدي)

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Abstract:

In the present study, the effect of water soluble fractions (WSF) of kerosene on the osmoregulation of penaeid shrimp *M. affinis* was studied. The shrimp was exposed to concentration of 300 ppb of kerosene WSF for a period of 30 days. The osmotic concentration of the haemolymph of the shrimp was measured by freezing point determination once a week. The ability of shrimp to maintain haemolymph osmolality was significantly decreased ($p<0.05$) after one week exposure to the WSF of kerosene. The shrimp was also exposed to acute kerosene WSF pollution for 24 hours period. It was then placed in pure sea water for a recovery period of three weeks. The ability of shrimp to maintain haemolymph osmolality was reduced two weeks after exposed to acute oil pollution but it was retuned within three weeks.

المستخلص:

تم دراسة تأثير الأجزاء الذائبة بالماء من النفط الأبيض على التنظيم الازموزي للروبيان *M. affinis*، حيث عرض الروبيان إلى تركيز 300 جزء بالبليون من الأجزاء الذائبة بالماء من النفط الأبيض لمدة 30 يوم. تم قياس التركيز الازموزي لهيمولينف الروبيان بدلالة درجة الانجماد مرة بالأسبوع. أن قابلية الربيان على حفظ الازموزية انخفضت معنويا (عند مستوى احتمالية 0.05) بعد أسبوع واحد من التعرض إلى الأجزاء الذائبة بالماء من النفط الأبيض. كما تم تعريض الربيان إلى تلوث نفطي حاد بالأجزاء الذائبة بالماء من الكيروسين لمدة 24 ساعة، ثم تم وضعه في ماء بحر نظيف لمدة ثلاث أسابيع لغرض الاسترجاع. أن قابلية الروبيان على حفظ الازموزية انخفضت أسبوعين بعد التعرض إلى التلوث النفطي الحاد ولكن استعادها خلال الثلاث أسابيع.

Introduction:

The biological effects of marine pollution and problems of monitoring were the subject of many researchers, organizations and commissions [1, 2, 3, 4]. It was concluded that further efforts in this field should concentrate on finding procedures for biological monitoring and include them in routine programmes which were properly tested and evaluated in the field [3, 4].

Metapenaeus affinis (H. Milin–Edwards) is an Indo–West Pacific species which ranges from the Arabian Gulf to Malay Archipelago [5]. In the Arabian Gulf, *M. affinis* has been reported from Saudi Arabia [6], Kuwait [7] and Iraq [8]. *M. affinis* has a commercial importance. In Kuwait, the fishing grounds of this shrimp extend from the south and east Kuwait Bay northeastward beyond the mouth of Shatt Al-Arab river. Salinities in these areas are at least

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32‰. In Iraq, the species has long been fished traditionally from the marshes where salinities are around 0.4‰ [9].
In spit of this importance, little was known about the effects of pollutants on the shrimp *M. affinis* in general and on its osmoregulation in particular. Thus, in the present investigation, the shrimp was the test animal on the effects of water–soluble fractions (WSF) of kerosene on its osmoregulation.. [10] used the osmoregulation in monitoring pollution, who tested the effects of heavy metals on two species of estuarine crabs.

Materials and Methods:

Chemicals:

The oils (kerosene-specific gravity @15.6ºC maximum=0.8017, aromatic content volume% maximum=20 and sulpher% weight maximum=0.1 and Basrah regular crude oil-medium-API gravity between 28–34) used in this research were obtained from South Oil Company, Basrah, Iraq. The oils were transferred to the laboratory by blinding glass bottle closed tightly and kept in a cold and dark place till their used. Carbon tetrachloride (CCl_4), n-hexane and methylene chloride were supplied from Burdick and Jackson laboratories, Inc. Sodium sulphate was supplied by Supelco SA. It was extracted with methylene chloride for 36 hours in a soxhlet. Following clean up by extraction, It was dried in an oven at 130˚C for about 24 hours and deactivated with deionized water at the recommended percentage prior to use.

Collection and acclimation of test animals:

Specimens of penaeid shrimp *M. affinis* were caught during 2008 from north-west the Arabian Gulf (Figure 1). The animals were then transferred to the laboratory. Stock animals were maintained in a 150 liter aquarium contained sea water from the Arabian Gulf (Salinity, 28–33‰) under aerated conditions with light/dark cycle (12:12). The laboratory temperature was 20±5°C. The animals were fed every fourth day with algae.

Preparation of WSF of kerosene test solution:

The stock solution of water–soluble fractions (WSF) of kerosene were prepared freshly according to . [11]. A known volume of kerosene (6ml) was added to volumetric flask containing about 250ml of sea water. The mixture was stirred slowly for about 24 hours (avoiding emulsification) on magnetic flea. After stirring, the volume was brought to one liter with sea water. The solution was then poured into one liter separating funnel and left for 2 hours to separate. After separation, the lower 950ml of solution was removed to one liter flask and mixed for 30 minute resulting in a maximum concentration of kerosene WSF of 2ppm. The insoluble fraction was discarded.

To determine the concentration of WSF of kerosene, the procedure of UNEP [12] was used. 100ml of nanograde carbon tetrachloride (CCl_4) was used in two successive 50ml extractions and the extracts were combined. The mixture was vigorously shaken to disperse the CCl_4 thoroughly throughout the water sample. The shaking is repeated several times before decanting the CCl_4. To these extracts a small amount of anhydrous sodium sulphate was added to remove excess water. The CCl_4 extracts were reduced in volume to less than 5ml by using a rotary evaporator. The reduced extract was carefully pipetted into a precleaned 10ml volumetric flask, making sure any residual particles of sodium sulphate were excluded and evaporated to dryness by a stream of pure nitrogen. The flask was then rinsed with fresh hexane and the rinsing used to make the sample volume up to exactly 5ml prior to UVF analysis (spectrofluorometer).

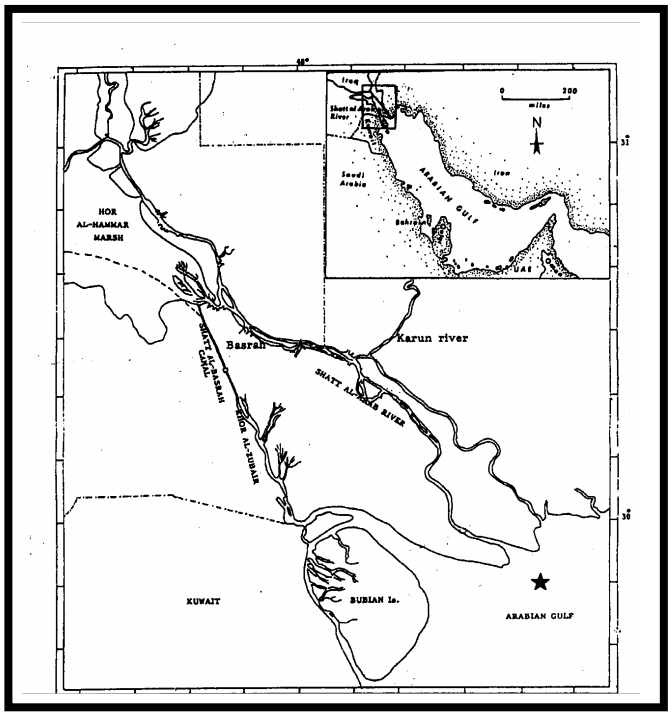

Figure (1): Map of Shatt Al–Arab river and north-west Arabian Gulf showing the position of samples.

Exposure of test animals to WSF of kerosene:

Five individuals of uniform size of adult female shrimps (60–70mm length) were transferred to each 10 liter aquarium contained sea water. Sand, stones and pieces of fine–meshed fishing net formed the substratum. Dead animals were replaced by living ones (the accidental stoppage of aeration and cannibalism of molting specimens were the most common causes of death). In the aquaria, the shrimps were subjected to concentration of 300ppb of kerosene WSF for a month period. This concentration was maintained by continuous renewal of the mixture of sea water and WSF of kerosene solution every day. Pure sea water was supplied in the same way in the zero ppb aquarium (control). The test solutions of the experiment were monitored for temperature, dissolved oxygen, pH and salinity at regular intervals. The temperature was at 20±5°C. The dissolved oxygen ranged was 8.5 to 10.1mg/l. As to the other characteristics of test solutions, pH was 7.1 to 8.2 and salinity was 30±2ppt.

Determine the osmoregulation:

The osmolality of haemolymph and sea water was determined by freezing point determination [13]. Two haemolymph samples from each animal were taken once a week by inserting a capillary tube between two abdominal segments. The animals were kept in pure sea water for a couple of hours after sampling to prevent infection. The maximum exposure time of shrimps to WSF of kerosene was 30 days.

Exposure of test animals to acute kerosene WSF pollution:

In this experiment, the kerosene WSF was accidentally supplied into the 10 liter aquaria contained shrimps. The water surface of aquaria was covered with oil. The animals were exposed to this pollution for 24 hours period. The animals then kept in unpolluted sea water

for three weeks for recovery. Samples of haemolymph and sea water were taken every week for freezing point determination.

Spectrofluorometer:

The Shimadzu RF–540 spectrofluorometer equipped with a DR–3 data recorder was used to determine the concentration of WSF of kerosene. The basic quantitative measurements were made by measuring emission intensity at 360nm with excitation set at 310nm and monochrometer slits of 10nm. The reference oil used for calibration was Basrah regular crude oil obtained from South Oil Company.

***Statistical analysis*:**

The data of the present study was analyzed by using Randomized Complete Block Design (RCBD). Significant results were then analyzed by RLSD test.

Results and Discussion:

The osmoregulation in shrimp was achieved by active uptake or excretion of water and ions through the surface, gills or gut and excretion via the antennal glands [14]. Exposure of the shrimp *M. affinis* to WSF of kerosene in the concentration of 300ppb for 30 days reduced its ability to maintain the haemolymph osmolality in the sea water with the time during the exposure. The difference between haemolymph osmolality of control shrimp and kerosene WSF exposed shrimp was significant ($p<0.05$) when exposure exceeded 8 days. The maximum reduction value (62.068%) of the haemolymph osmolality of exposed shrimp was obtained after two weeks period of exposure. This value was almost unchanged during the three weeks. The haemolymph osmolality of the exposed shrimp was about 21% less than of the control shrimp after 30 days of exposure. At 300ppb concentration of kerosene WSF, however, the shrimp was seemed to be able to maintain some of the haemolymph osmolality in spite of continued exposure to the pollutant (Table 1 and 2). The decreased ability of the shrimp *M. affinis* to maintain the haemolymph osmolality was also apparent after 24 hours of exposure to acute kerosene WSF pollution followed by the 21 days recovery period in the sea water. After three weeks in unpolluted sea water, the haemolymph osmolality of the shrimp had retuned to normal value (Table 3 and 4). Thurberg *etal*. [10] reported that the haemolymph of the crab *Carcinus maenas* was became more osmotic than that of the control crab in salinities up to 32‰ at 20ºC, when the carb was exposed to 2ppm concentration of copper and cadmium for 48 hours. This indicated that different pollutants may change the membrane osmoregulation in different ways. The water–soluble fractions of kerosene were known to contain aromatic compounds. It was suggested that these chemicals expanded cell membranes, primarily due to distortion of proteins, obstruction of sodium channels and liquefaction of lipid regions. A possible effect of membrane expansion was inhibition of active uptake of ions from the medium. A contributing factor to those mentioned above may be increased passive uptake of water [15]. The results of the present study showed the delayer effects of WSF on the osmoregutation of shrimp (2–9 days) (Table 1and 2) with

faster recovery taking one to two weeks (Table 3 and 4). Baden and Hagerrman [16] demonstrated the earlier effects of WSF on the ventilatory behavior of the shrimp *Palaemon adspersus* with slower recovery taking more than six weeks. The difference may depend on the kind of the tissue effected. The osmoregulation took place mainly in the gills whereas the ventilatory behavior was controlled by the central nervous system [17]. Accordingly, It can be expected that the cells in the gill membrane may be lower affected and faster recovered than the nerve cells. The use of osmoregulation in the monitoring of pollutants in the marine environment had several characteristics. The sampling of haemolymph was easy, simple and fast. The method was suitable for all animals sizes (small or big) without killing the animal. Determination of osmoregulation by freezing point technique required small samples than osmometer demanding bigger samples. The method can be adopted and applied to field work.

Table (1): The freezing point (ºC) of the haemolymph of control and kerosene WSF exposed shrimp *M affinis*, and the sea water.

Exposure time (day)	Freezing point (ºC)		
	(a) Haemolymph of control shrimps (n=5)	(b) Haemolymph of kerosene WSF exposed shrimps (n=5)	(c) Sea water (n=5)
2	1.344	1.319	1.081
9	1.360	1.290	1.100
16	1.601	1.421	1.311
23	1.493	1.272	1.010
30	1.414	1.201	0.967

Table (2): The percentage loss of osmoregulation of the shrimp *M. affinis* exposed to WSF of kerosene for 30 days.

Exposure time (day)	(d) %loss of osmoregulation
2	9.505
9	26.923*
16	62.068*
23	45.755*
30	47.651*

(d)=[(a)–(b)/(a)–(c)]x100; * Significant at 0.05 levels.

Table (3): The freezing point (ºC) of the haemolymph of control and acute kerosene WSF pollution exposed shrimp *M. affinis* after 21 days recovery in sea water, and the sea water.

Recovery time (day)	Freezing point (ºC)		
	(a) Haemolymph of control shrimps (n=5)	(b) Haemolymph of acute oil pollution exposed shrimps (n=5)	(c) Sea water (n=5)
1	1.412	1.356	1.202
7	1.464	1.322	1.231
14	1.425	1.372	1.210
21	1.373	1.352	1.201

Table (4): The percentage loss of osmoregulation of the shrimp *M. affinis* exposed to acute Kerosene WSF pollution for 24 hours after 21 days recovery in sea water.

Recovery time (day)	(d) %loss of osmoregulation
1	26.666*
7	60.944*
14	24.186*
21	12.209

(d)=[(a)–(b)/(a)–(c)]x100; * Significant at 0.05 levels.

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