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TOXICITY, UPTAKE, AND DEPURATION OF CRUDE OIL-WATER MIXTURES BY SHRIMPS FROM SHATT AL-ARAB RIVER

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ABSTRACT

The intent of present study was to examine the toxicity, uptake, and depuration of oil–water mixtures from Nahran–Umar crude oil of two types , water soluble fractions (WSF) and oil– in – water dispersions (OWD). The test organisms used in these experiments were the shrimps, *Caridina babaulti* basrensis and *Atyaephyra desmaresti* mesopotamica, presence in Shatt Al–Arab river. In the toxicity test experiments, the two species of shrimps were subjected in river water to different concentrations of WSF and OWD using renewal toxicity system for 24 and 48 hours under laboratory conditions. In the median lethal concentration (LC_{50}) parameter, The shrimp *C. babaulti* basrensis was more sensitive to oil – water mixtures than the shrimp *A. desmaresti* mesopotamica. The WSF was slightly more toxic to shrimps than the OWD . In the uptake and depuration experiments, we exposed the shrimp, *C. babaulti* basrensis to 2 ml of WSF / l of river water for 24 hours. Exposure water and organisms were sampled periodically for the total hydrocarbons concentration. Hydrocarbons levels in the exposure solution decreased rapidly while concentration in the shrimp tissue increased dramatically. After 6 hours, tissue levels of hydrocarbons were 150 times grater than water levels. Depuration of hydrocarbons was rapid and began during the exposure period, complete depuration did not occur.

INTRODUCTION

Aquatic organisms are often contaminated by petroleum hydrocarbons during oil spills. Numerous studies have been conducted on the acute toxicity of oil – water mixtures to aquatic organisms. Rice *et al.* (1976 a) tested five species with dispersions and water – soluble fractions (WSF) of two crude oils . Rice *et al.* (1976 a) compared toxicities of dispersions and water–soluble fractions (WSF) of two crude oil to each of six test species.

Widdows (1994) studied the characteristic of dispersions and water–soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. These studies provide useful information on both the relative sensitivities of aquatic organisms and the relative toxicities of oil–water mixtures.

Many studies have been also made to measure uptake and loss of petroleum hydrocarbons from tissues of aquatic organisms. Mckim and Schmieder (1990) reported that the various species of molluscs and crustaceans rapidly uptake petroleum hydrocarbons from either the water or their food. Cox *et al.* (1972) add No.2 fuel oil to a large pond in south Texas which contained shrimps, clams and oysters. After 38 days, the animals were transferred to clean water and 10 days of depuration were sufficient to discharge all naphthalene from the shrimps. Clams and oysters still retained 4.0 and 2.0 µg/g of naphthalene, respectively. Fayad *et al.* (1996) has observed that brown shrimp *Crangon crangon* consume sunken crude oil which remains in the foregut until the next molt .

Shatt Al–Arab river are polluted with oils from a variety of sources including routine tanker and shipping operations, terrestrial runoff, atmospheric fallout, natural seepage, and shipping and offshore well disasters (Al–Saad, 1995). Very little information are available on toxicity, uptake, and depuration of oils pollutants to the organisms that habited in the river. Thus, the objective of this study is to examine the toxicity, uptake, and depuration of oil–water mixtures from crude oil on important species in the food web of Shatt Al–Arab river. These are the shrimps, *Caridina babaulti* basrensis and *Atyaephyra desmaresti* mesopotamica.

MATERIALS AND METHODES

Specimens of uniform size of adult shrimps, *C. babaulti* basrensis and *A. desmaresti* mesopotamica (25–30 mm length) were collected during 2004 from Shatt Al-Arab river, (Figure- 1). Then the animals were transferred to keep in an aquarium for an acclimation period of one week prior to start of the experiments, under laboratory temperature 20 ± 2 °C with photo regimen consist of 12 hours light and 12 hours dark under aerated conditions.

The test crude oil, Nahran– Umar used in this research was obtained from South Oil Company, Basrah, Iraq. The oil was transferred to the laboratory by blinding glass bottle closed tightly and kept in a cold and dark place till its used.

The oil – in- water dispersions (OWD) were prepared depending on procedure of Bragin *et al.* (1994). A known volume of crude oil was adding to 500 or 750 ml of Shatt Al-Arab river freshwater (which was filtered and

boiled then cooled before use) in one liter volumetric flask and shaking the mixture for 5 minute at approximately 200 cycles per minute on a magnetic stirrer. After shaking the volume was brought up to one liter with river water. The dispersions were allowed to equilibrate for one hour before the animals were added for exposure.

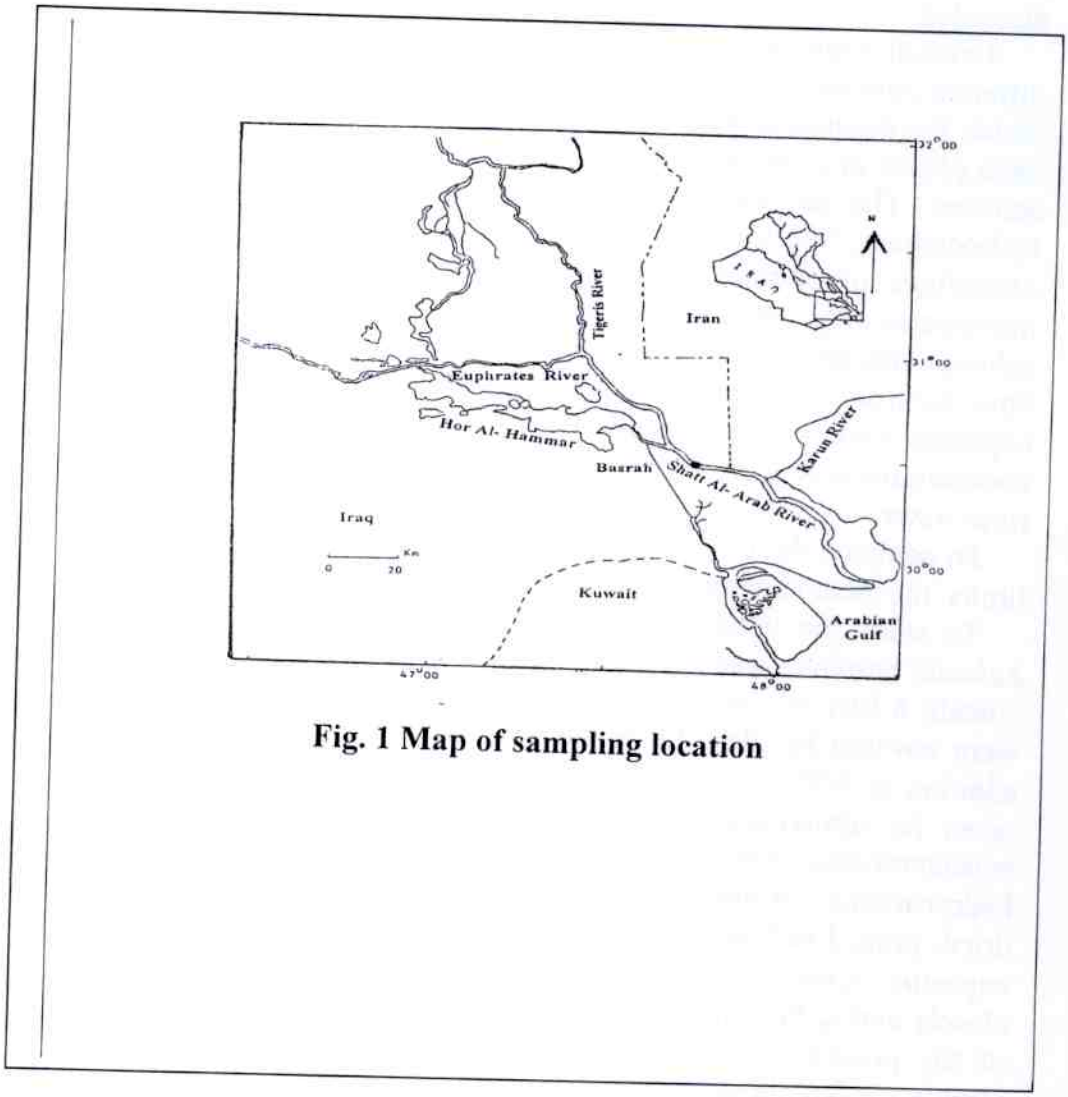

Fig. 1 Map of sampling location

The water–soluble fractions (WSF) were prepared freshly according procedure of Singer *et al.* (2001) by adding a known dose of the crude oil to volumetric flask containing about 150 ml of freshwater collected from Shatt Al-Arab river, which was filtered and boiled before use. The resulting mixture was then mixed for about three hours on magnetic stirrer at

temperature of 20 ± 2 °C. After stirring, the volume was brought to one liter with filtered and boiled river water. The solution was then poured into one liter separating funnel and allowed to separate for two hours at temperature 20 ± 2 °C. Following separation, the lower 950 ml of solution was removed to one liter flask and mixed for 30 minute, while the insoluble fraction was discarded.

Renewal toxicity tests were conducted by exposing the shrimps to different concentrations for definite exposure time (24 and 48 hours) after which the organisms were transferred to clean river water, 10 individuals were placed in glass jar, (14 ×10 × 20 cm^3 in size) containing of the test solution. The jar was covered by glass lid to reduce evaporation of hydrocarbons. The shrimps were not fed during the exposure experiments. Mortalities of animals were recorded as being dead if no dissection movements after 2 hours recovery in clean river water. In all cases, the test solution changed daily. The test was set up in three replicates together with three control. Thirty individuals were used for each replicated and for an exposure time of 24 and 48 hours. The tests were carried out using concentrations of 2, 5, 10, 20, 30, 35, 40 and 45 ml of WSF or OWD / l of river water.

To compute the LC_{50} values and their upper and lower 95% confidence limits, the method described by UNEP (1989) was used.

To study the uptake and depuration of hydrocarbons, the shrimp, *C. babaulti* basrensis was exposed in glass aquaria (40 × 22 × 15 cm^3 in size) contain 8 liter of river water to 16 ml of WSF for 24 hours. The aquaria were covered by glass lid to reduce evaporation of hydrocarbons. After addition of WSF to test water, water and shrimp samples were periodically taken for ultraviolet fluorescence (UVF) analysis of total hydrocarbons concentrations. The recorded values were converted to ppm total hydrocarbons. Approximately 140 shrimps were used; 20 were freeze – dried, ground and sieved as control tissue and the remainder placed in the exposure aquaria. Exposure water was aerated. Organisms were observed closely during the exposure period and water and shrimp samples consisted of the pooled tissues of 10-15 organisms sufficient for three replicate samples. After the exposure period was over, additional river water was added to the aquaria. water and tissue samples continued to be taken for analysis.

The hydrocarbons were extracted from water following the procedure of UNEP (1989). In this, 100 ml of nanograde carbon tetrachloride (CCl_4) was used in two successive 50 ml 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 5 ml by using a rotary evaporator. The reduced extract was carefully pipetted into a precleaned 10 ml 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 5 ml prior to UVF analysis.

The procedure of Grimalt and Oliver (1993) was used in the extraction of hydrocarbons from shrimp tissues. The tissues of animals were pooled and macerated in a food liquidizer. The tissues were then freeze-dried, ground and sieved through a 63 μ metal sieve. The dried shrimp tissues were placed in a per–extracted cellulose thimble and soxhlet extracted with 150 ml methanol : benzene (1:1 ratio) for 24 hours. The extract was then transferred to a storage flask and the sample was further extracted with fresh solvent. The combined extracts were reduced in volume to ca 10 ml in a rotary vacuum evaporator and was then saponified for 2 hours with a solution of 4 N KOH in 1:1 methanol: benzene. After extraction the unsaponified matter with hexane, the extract was dried over anhydrous Na_2SO_4 and concentrated by stream of N_2 for UVF analysis.

The Shimadzu RF–540 spectrofluorometer equipped with a DR – 3 data recorder was used for analysis of total hydrocarbons. The basis quantitative measurements were made by measuring emission intensity at 360 nm with excitation set at 310 nm and monochrometer slits of 10 nm.

The procedural blanks consisting of all reagents and glassware used during the analysis, ware periodically determined.

The reference oil used for calibration was Basrah regular crude oil obtained from South Oil Company.

RESULTS AND DISCUSSION

Toxicity data and the results of analysis of these data for the shrimps *C. babouliti* basrensis and *A. desmaresti* mesopotamica exposed to different concentrations of oil–water mixtures (WSF and OWD) at two time intervals are presented in (Table - 1). It has been noted that values of 2 ml / l and 5 ml/l of WSF from crude oil are the upper nonlethal concentration values after exposure of 24 hours for the shrimp *C. babouliti* basrensis and only 2 ml/l concentration for the shrimp *A. desmaresti* mesopotamica. While values of 2 ml / l and 5 ml / l of OWD of crude oil are the upper nonlethal concentration values after exposure of 24 hours for the shrimp *C. babouliti* basrensis and *A. desmaresti* mesopotamica and only concentration 2 ml / l

Table 1 . Percentage mortality of two species of shrimps exposed to different concentrations of oil - water mixtures from Nahran – Umar crude oil and LC_{50} values and their upper and lower 95 % confidence limits at two time intervals .

Concentration of Nahran – Umar crude oil ml / l	*C . baboulti* basrensis				*A . desmaresti* mesopotamica			
	WSF		OWD		WSF		OWD	
	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours	24 hours	48 hours
2	0	10.0	0	0	0	10.0	0	0
5	0	23.3	0	16.6	10.0	26.6	0	6.6
10	26.6	50.0	20.0	36.6	30.0	40.0	6.6	23.3
20	53.3	76.6	35.3	60.0	40.0	63.3	20.0	53.3
30	80.0	86.6	76.6	73.3	66.6	76.6	50.0	76.6
35	100	100	90.0	90.0	83.3	69.6	63.3	90.0
40	100	100	100	100	96.6	100	76.6	100
45	100	100	100	100	100	100	100	100
Control	0	0	0	0	0	0	0	0
LC_{50}	18.9	10.1	19.1	15.8	22.2	14.4	25.0	19.2
Upper 95 % confidence limit of LC_{50}	28.8	16.5	26.4	24.8	45.9	37.0	73.3	33.9
Lower 95 % confidence limit of LC_{50}	12.3	6.1	6.1	10.0	10.7	5.5	16.7	10.8

after exposure of 48 hours for both species. The concentration values of 35, 40, and 45 ml / l showed approximately 100% mortality in both species of shrimps after 24 and 48 hours exposure to oil–water mixture when the animals were transferred to clean river water. Generally, the survivals of the shrimps are decreased with increasing the concentration and exposure time to WSF and OWD. Calculated 24 and 48 hours LC_{50} values were (18.9 ml/ l) and (10.1 ml / l) Nahran-Umar crude oil WSF for shrimp *C. babouliti basrensis*, and (22.2 ml / l) and (14.4 ml / l) crude oil WSF for shrimp *A. desmaresti mesopotamica*. While, the 24 and 48 hours LC_{50} values were (19.1 ml /l) and (15.8 ml/l) crude oil OWD for shrimp *C. babouliti basrensis*, and (25.0 ml / l) and (19.2 ml / l) crude oil OWD for shrimp *A. desmaresti mesopotamica*. It is appeared that the 24 and 48 hours LC_{50} values of shrimp *C. babouliti* basrensis at two different exposure times of oil–water mixtures are lower than those values of the shrimp *A. desmaresti mesopotamica* in comparison with the same exposure time and oil–water mixing type. This is indicated that the shrimp *C. babouliti basrensis* is more sensitive to oil–water mixtures than the shrimp *A. desmaresti mesopotamica*. It is possible that the different in sensitivity to hydrocarbons between the shrimps due to the difference in the wall structure of their bodies, their ability on metabolism, excretion and storage of hydrocarbons and/ or the difference in permeability of hydrocarbons into the sit of action and their effecting in the animals. The difference in sensitivity to petroleum hydrocarbons among different species is well known (Widdows, 1994). The study by Rice *et al.* (1976b) tested 27 species of marine fish and invertebrates, and permits the best comparisons of species sensitivities since methods, temperature, etc. were all similar. Fish and shrimp were usually among the more sensitive species tested, while intertidal animals were generally more tolerant. Animals are probably more tolerant to static exposures because they can temporarily insulate themselves from the exposures, at least until the concentrations in the static exposures have declined to sublethal levels. The intertidal limpets and chitons were more sensitive than the other intertidal animals, but this may have been due to damage occurring when they were collected (pried off the substrate). The sensitivities of cold–water shrimps appear greater than sensitivities of similar species from warmer climatic (Rice *et al.*, 1976b). Laboratory studies on the snails (*Melanoides nodosa*, *Theodaxus jordan*, *Lymna aurcularia* and *Melanoides tuberculata*) and the bivalves (*Corbicula fluminalis* and *Corbicula fluminea*), a common inhabitant in Shatt Al–Arab estuary area, were made with crude oils, refined products and oil fractions (Farid, 2002; Farid *et al.*, 2002; Farid, 2003). These studies showed that the

sensitivity of molluscs to oil pollutants were very different as following trends; *L. aurcularia* > *M. nodosa* > *T. jordani* > *M. tuberculata* > *C. fluminalis* > *C. fluminea* . Jaweir and Habash (1987) in their experiments on the toxicity of water–soluble hydrocarbons (WSF) of kerosene to two species of polychaetes from Shatt Al–Arab river (*Namalycastic indica* and *Dendronereides heteropoda*), found that *D. heteropoda* was slightly more sensitive to WSF of kerosene than *N. indica*. The 96 hours LC_{50} values recorded were 1.5 ml / l for *D.* heteropoda and 4 ml / l for *N. indica*.

It is clear from the data that the LC_{50} values of shrimps exposed to WSF of crude oil are slightly lower than the LC_{50} values of the same species subjected to OWD of crude oil in comparison with the same exposure time. This is indicated that the OWD of crude oil is less toxic to shrimps than WSF of crude oil. The toxicity of oil–water mixtures depends in part upon the way the oil is associated with the water. Experiments comparing the toxicity of water–soluble fractions and dispersions of oil suggest that the toxicity of oil is due to the soluble compounds contained in that oil, and not due to compounds in dispersed droplets (Singer *et al.*, 2001). The chemical composition of the droplets is probably very similar to that of the parent oil, except that droplets probably contain slightly lower concentrations of soluble compounds because of losses of compounds due to equilibration with the water. If oil dispersions are less than or equally as toxic as WSF (which contain for fewer dispersed droplets of oil), the toxicity must be due to the soluble fractions of oil. Rice *et al.*, (1976 a) tested five species with dispersions and WSF of two crude oils and found that toxicities of dispersions were always less than, but within an order of magnitude of the toxicities for corresponding WSF. Widdows (1994) did not analytically determine oil concentrations in the doses they used to determine the toxicity of dispersions. However, they did provide data relating the amount of oil used to prepare dispersion to the amount of oil found in a typical dispersion (as determined by IR). After calculating the amount of oil found in toxic dispersions , Peterson (1994) estimate that the toxicity difference between the dispersions and WSF studied by Widdows (1994) ranged from nil to about an order of magnitude, with the dispersions being consistently less toxic. Rice *et al.* (1976 a) compared toxicities of dispersions and WSF of two crude oil to each of 6 test species. They found significant differences in only 4 of 12 cases when oil concentrations were determined by UV. In 3 of these 4 cases, the dispersions were slightly more toxic than the corresponding WSF. The lack of significant differences in toxicity suggests that the toxicity of oil is due to water–soluble compounds.

Zhou *et al.* (1997) suggested that toxicity of oil is due to chemical toxicity of soluble aromatic, rather than physical toxicity of dispersed droplets

In present study, the total acute effects of hydrocarbons on the shrimps are restrict their normal activities, bring narcosis and anesthesia, loss their ability to react to the external cure, rapture their tissues, and finally lead them to die. Those above causes are due to physical and chemical toxic effects of hydrocarbons which produce the abnormal activities in animals and die. Widdows (1994) reported the toxicity significance of the various fractions of hydrocarbons; the low boiling saturated hydrocarbons have been showed to produce anesthesia and narcosis at low concentrations, and at higher concentrations cell damage to a wide variety of animals. Higher–boiling saturated hydrocarbons may not be directly toxic, although it is suggested that they may interfere with nutrition. The aromatic hydrocarbons are the most dangerous fractions. The low–boiling aromatics represent the acute toxic hazard, while the higher molecular weight polynuclear species may well be of significance in their long-term effects. Asimilar toxic effects of hydrocarbons on other species of aquatic organisms were also reported by (Rice *et al.*, 1976a; Jaweir and Habash, 1987; Farid *et al.*, 2002).

The data showing the accumulation and depuration of hydrocarbons by the shrimp *C. baboulti basrensis* are presented in (Table 2) and (Figs.2, 3, and 4). Shrimp is exposed for 24 hours to WSF of Nahran - Umar crude oil. There were no mortalities due to the WSF. Shrimp is unusually active during the first 6–8 hours of the experiment. Hydrocarbons concentration in the exposure water decreased rapidly in a linear fashion such that no measurable concentration remained after 12 hours. Hydrocarbons accumulated by the shrimp are very rapid during the first few hours of exposure, yet depuration began as the water concentration decreased to approximately 0.1 ppm of hydrocarbons. After 2 hours of exposure, water levels of hydrocarbons compounds are approximately 0.22 ppm, while in tissue concentrations are 7.3 ppm. At 6 hours, hydrocarbons are present in the water at a concentration of 0.1ppm, while shrimp tissue contained over 15.1 ppm hydrocarbons. After 6 hours of exposure, the hydrocarbons concentration decreased in the tissues rapidly. At the end of the exposure period, shrimp contained approximately 2.1 ppm. After 24 hours in clean river water, tissues burden had decreased to 0.2 ppm of hydrocarbons. The shrimp remained contaminated at these low levels for 18 days. Shrimps available for tissue samples are exhausted at this point. Additional experiments of a similar design have revealed essentially complete depuration of hydrocarbon by shrimp in 1 – 4 days (Rice *et al.*, 1976 b; Fayad *et al.*, 1996).

Table 2. Accumulation and depuration of hydrocarbons by the shrimp *C. baboulti bassrensis* exposed for 24 hours to WSF from Nahran–Umar crude oil.

Status	Sampling time (hours)	Concentrations of hydrocarbons (ppm)
Accumulation of hydrocarbons by the shrimp *C. baboulti basrensis*	1	2.8 ± 0.1
	2	7.3 ± 0.4
	6	15.1 ± 0.4
	10	4.8 ± 0.5
	20	3.0 ± 0.3
	24	2.1 ± 0.1
Concentration of hydrocarbons in the exposure water	1	0.28 ± 0.04
	2	0.22 ± 0.07
	4	0.14 ± 0.04
	6	0.1 ± 0.05
	10	0.03 ± 0.01
	12	0
Depuration of hydrocarbons by the shrimp *C . baboulti basrensis* (After 24 hours in clean river water)	0	0.2 ± 0.06
	4	0.19 ± 0.03
	24	0.19 ± 0.01
	90	0.17 ± 0.01
	280	0.16 ± 0.01
	432	0.14 ± 0.02

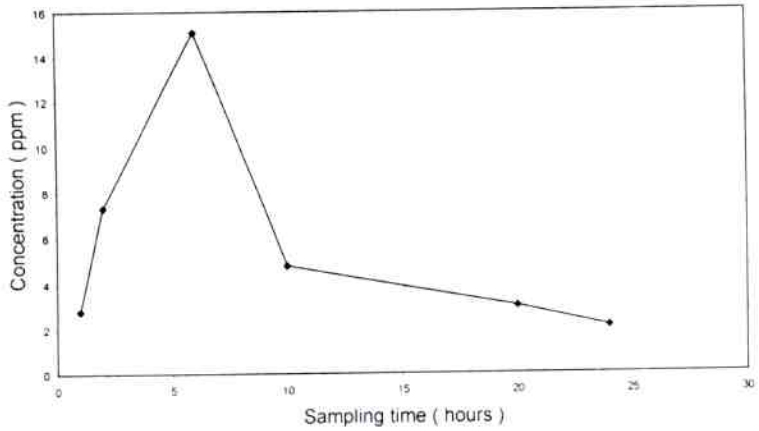

Figure 2 . Accumulation of hydrocarbons by the shrimp *C . baboliti* basrensis exposed for 24 hours to WSF from Nahran - Umar crude oil

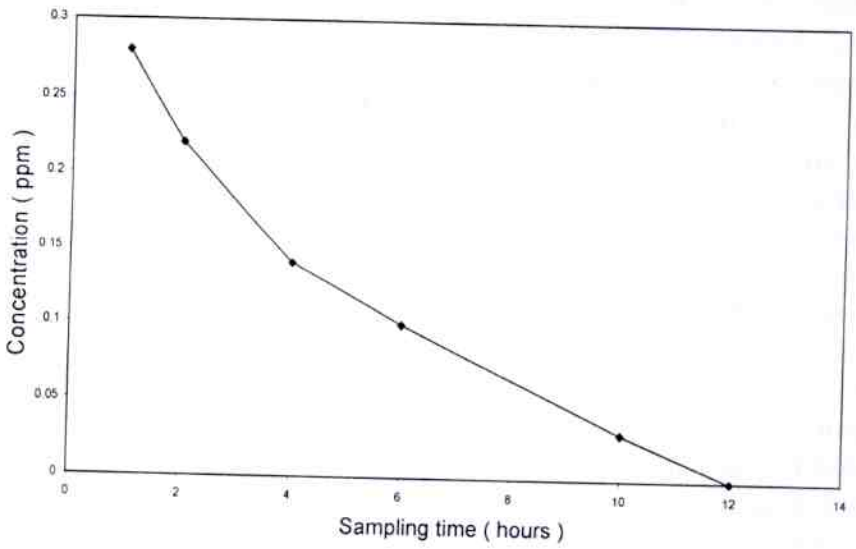

Figure 3 . Hydrocarbons concentration in the exposure water

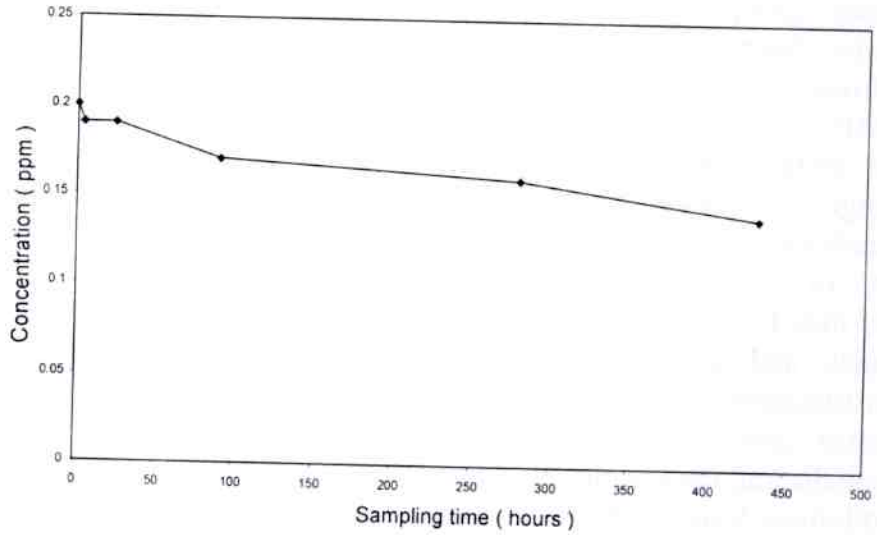

Figure 4 . Depuration of hydrocarbons by the shrimp *C . baboulti* basrensis exposed for 24 hours to WSF from Nahran - Umar crude oil

The present investigation has demonstrated the rapid accumulation and depuration of hydrocarbons compounds from an oil–water mixture by shrimp *C. baboulti basrensis*. The shrimp *C. baboulti basrensis* is similar in this respect to the *Penaeid aztecus* (Cox, 1974). It has been noted that, in general, estuarine fish and crustaceans accumulate hydrocarbons very rapidly and begin depuration immediately, whereas molluscs accumulate the hydrocarbons more slowly with depuration taking many days (Widdows and Donkin, 1992) Accumulation with the shrimp *C. baboulti* basrensis was most rapid during the first 2 hours of exposure. Depuration began during the exposure period as the hydrocarbons in the water decreased to levels near 0.1 ppm.

The most likely site for the transfer of hydrocarbons from water to organism's tissues and vice versa is the respiratory membrane. Data discussed by Rice *et al.* (1976a) have shown that accumulation is associated with the digestive gland and gill regions of shrimp until their complete release. Since hydrocarbons are volatile and are released from the surface organisms which spend more time in the water column might be exposed to accumulate greater amounts of hydrocarbons in shorter time periods compared to more sessile benthic animals. It is possible that the release of hydrocarbons due in part to metabolism of these compounds by the shrimp *C. baboulti basrensis*. Porte and Albaiges (1993) have concluded that many crustacean group including copepods, amphibods, crab zoea, and euphausiids have the ability to metabolism hydrocarbons. Further evidence of hydrocarbons metabolism by crustaceans has been published by GESAMP (1995). In the majority of these various accumulation studies, whether metabolism occurred or not, exposed organisms retained some percentage of the accumulated hydrocarbons for an indefinite time period. Hydrocarbons were retained in tissues or organs containing significant amounts of lipids. Molluscs may contain greater percentages of lipid material than fish or crustaceans. This could partially explain why molluscs accumulate and depurate petroleum hydrocarbons at a much slower rate than crustaceans. Also, molluscs have not been found to be able to metabolize these compounds.

In conclusion, the shrimps species very varied in their sensitivity to oil – water mixtures from crude oil. The shrimp *C. baboulti basrensis* was more sensitive to hydrocarbons from oil – water mixtures than the shrimp *A. desmareasti mesopotamica*. The WSF of crude oil was slightly more toxic to shrimps than OWD. The total acute effects of hydrocarbons on shrimps were abnormal activities, narcosis and anesthesia, loss the ability to react to the external cure, rapture tissues and die. The shrimp accumulate the

hydrocarbons from oil–water mixtures very rapidly and begin depuration immediately.

Finally, we recommend further studies to (1) test the acute and chronic toxicity of oils and that oils products and oil fractions in Shatt Al – Arab river environment. This will help to predict effects of oil pollution on living organisms and ecology, to determine permissible effluent discharge rates into the aquatic environment of the Shatt Al–Arab river, and to monitoring levels of oil pollution in stream with respect to water quality standards, and (2) to study accumulation and turnover of petroleum hydrocarbons in aquatic organisms. Since organisms which accumulate petroleum hydrocarbons after exposure to various petroleum components are interest because of implications to the aquatic environment and to human health.

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السمية والأخذ والاسترجاع لخلائط النفط الخام – الماء لنوعين من الروبيان المتواجدة في نهر شط العرب

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الخلاصة

تضمنت الدراسة الحالية تجارب لفحص السمية و الاخذ و الاسترجاع لخلائط نفط خام نهران عمر مع الماء والتي كانت على نوعين، الاجزاء الذائبة بالماء و الاجزاء المشتتة بالماء. استخدم في هذه التجارب نوعين من الروبيان *Caridina babaulti* basrensis و *Atyaephyra desmaresti* mesopotamica المنتشرة في نهر شط العرب ككائنات اختبار. عرض كلا النوعين من الروبيان في تجارب فحص السمية بماء النهر الى تراكيز مختلفة من الاجزاء الذائبة بالماء و الاجزاء المشتتة بالماء باستخدام اختبارات السمية المتجددة لمدة 24 و 48 ساعة تحت الظروف المختبرية . اشارت قيم متوسط التركيز المميت الى ان الروبيان *C. babaulti basrensis* كان اكثر حساسية الى خلائط النفط الخام – الماء من الروبيان *A. desmaresti mesopotamica* . وكانت الاجزاء الذائبة بالماء اكثر سمية اتجاه الربيان من الاجزاء المشتتة بالماء. في تجارب الاخذ و الاسترجاع عرض الروبيان *C. babaulti basrensis* الى 2 مل من الاجزاء الذائبة بالماء لكل لتر من ماء النهر لمدة 24 ساعة. اخذت عينات من ماء التعريض و الروبيان على فترات زمنية مختلفة لقياس تركيز الهيدروكاربونات الكلية. انخفض مستوى الهيدروكاربونات سريعا في محلول التعريض بينما ازداد مستواها دراماتكيا في انسجة الروبيان. وبعد مرور 6 ساعات من التعرض اصبح مستوى انسجة الروبيان من الهيدروكاربونات 150 مرة اكثر من مستواها في الماء. كان استرجاع الهيدروكاربونات من الروبيان سريعا فقد ابتدأ خلال فترة التعريض الا انه لم يحصل بشكل كامل.