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# Uptake and depuration of water-soluble fractions (WSF) of crude oil by the bivalve *Corbicula fluminea* (Müller) from Shatt Al-Arab river

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**Abstract -** This study includes laboratory uptake and depuration experiments. The bivalve *C. fluminea* is exposed to 5ml of WSF of Nahran–Umar crude oil for each liter of river water for 48 hours in the static toxicity test system. The exposure water and animals are periodically taken for analysis of total hydrocarbons concentrations by spectroflurometer. After the exposure period is over, the bivalve transferred to clean river water for depuration period of 48 hours. The experiments show that there are no mortalities due to WSF of Nahran–Umar crude oil. The bivalve is usually active during the 12–16 hours of the experiments. Hydrocarbons concentrations in the exposure water decreased are slowly in a linear fashion so that no measurable concentrations remained after 32 hours. Hydrocarbons accumulated by the bivalve are slowly during the first few hours of exposure. After 2 hours of exposure, the concentration of hydrocarbons is approximately 0.38 ppm in water, while in tissues it is 4.8 ppm. After 20 hours, hydrocarbons are present in water at a concentration of 0.09ppm, while the bivalve tissues contained 17.7 ppm hydrocarbons. After 25 hours of exposure, the hydrocarbons concentration decreased in the bivalve tissues. At the end of the exposure period, the bivalve contains approximately 7.4 ppm. After 48 hours in clean river water, tissues burden decreased to 0.22 ppm of hydrocarbons. The bivalve remained contaminated with small concentrations of hydrocarbons for 20 days. The complete depuration occurred at 26 days.

## Introduction

Many organisms accumulated petroleum hydrocarbons after exposed to various petroleum components. These organisms were of interest because of implications to the marine environment and human health (Zhou *et al*., 1996; Allen *et al*., 2002). The ability of bivalve molluscs to accumulate hydrocarbons had been demonstrated under both laboratory and field conditions. Menon and Menon (1999) had reported the occurrence of petroleum oils in bivalve molluscs sampled from the environment. These molluscs were sampled from area which had either been exposed to oil spills or from areas considered industrially contaminated. Laboratory studies of the accumulation of petroleum oils and oil fractions by bivalve molluscs indicated that various fractions of petroleum oils can be accumulated and retained by animals (Meador *et al*., 1995; Widdows and Donkin, 1992). Many studies with bivalve molluscs have also involved depuration experiments (Fossato and Cononier, 1976; Baussant *et al*., 2001).

The *Corbicula fluminea* (Müller) is a common bivalve in the water of Shatt Al–Arab river and is dominant member of its benthic macrofauna. As with many molluscs species their growth rates are slow and the animals are long lived. The *C. fluminea*, occur frequently in the areas receiving acute and chronic oil exposures and are often considered as an important part of food web in the stream. A few studies of oil accumulation and discharge have been performed on organisms of Shatt Al-Arab river in general and on molluscs in particular. Therefore, the present study performed to determine experimentally, a pattern of accumulation and discharge of WSF of crude oil in the bivalve *C. fluminea*.

The Crude oil was chosen for study because it is commonly shipped in water of Shatt Al-Arab river, used in industrial installations around the river and has already been involved in well documented spill. The crude oil was added as WSF form to simulate a potential naturally occurring condition. Wang and Fingas (2003) have reported that in the event of an oil spill, it is probable that much of the oil fractions would dissolve in the water column. These fractions are among the most toxic of all oil components. The mechanism for accumulation of water-soluble fractions of oils by bivalve molluscs has been reported by Widdows and Donkin (1992).

## Materials and Methods

*Collection and acclimation of animals:*

Specimens of adult and uniform size individuals of *C. fluminea* were collected from Shatt Al–Arab river (along the region extended from Abu–Al-Khasib to Garmat-Ali) during 2004 and 2005 (Figure 1). The animals were transferred to an aquarium for acclimation period of ten days prior to the experiments, under laboratory temperature of 20 ± 2°C with light/dark cycle (12:12) under aerated conditions.

*Preparation of WSF:*

The water–soluble fractions (WSF) were prepared freshly according to the procedure of Singer *et al.* (2001) by adding a known volume of the crude oil (20ml in the present study) to volumetric flask containing about 150 ml of water from the Shatt Al–Arab river which was filtered and boiled then cooled before use. The resulting mixture was then mixed for about three hours on magnetic stirrer at a temperature of 20±2°C. After stirring, the volume was brought up to one-liter of a filtered and boiled river water. The solution was to be poured into one liter separating funnel. It was allowed to separate for two hours at temperature 20 ± 2°C. Following separation, the lower 950ml of solution was removed to one liter flask and mixed for 30 minutes while the insoluble fraction was discarded.

*Uptake and Depuration Experiments:*

To study the uptake and depuration of petroleum hydrocarbons, the bivalve *C. fluminea* exposed to 40ml of WSF of Nahran–Umar crude oil for 48 hours in glass aquaria (40×22×15cm$^3$ in size) (static toxicity test system) containing 8 liters of the river water. The aquaria covered by a glass lids to reduce evaporation of hydrocarbons. After the addition of WSF, water and bivalve samples were periodically taken for analysis of total hydrocarbons

concentrations by Shimadzu RF-450 spectroflurometer. The apparatus equipped with a DR–3 data recorder. The basis quantitative measurements were made by measuring emission intensity at 360nm with excitation set at 310nm and monochrometer slits of 10nm. The recorded values were converted to ppm total hydrocarbons. Approximately 140 bivalves were used, 20 were freeze–dried, grounded and sieved as a control tissue and the remainder placed in the exposure aquaria. The exposure water was aerated. The animals were observed closely during the exposure period. The water and bivalve samples that consisted of the pooled tissues of 10-15 animals were sufficient to the three replicate samples. As the exposure period was over, more river water was added to the aquaria. Water and tissue samples were continuously taken for analysis.

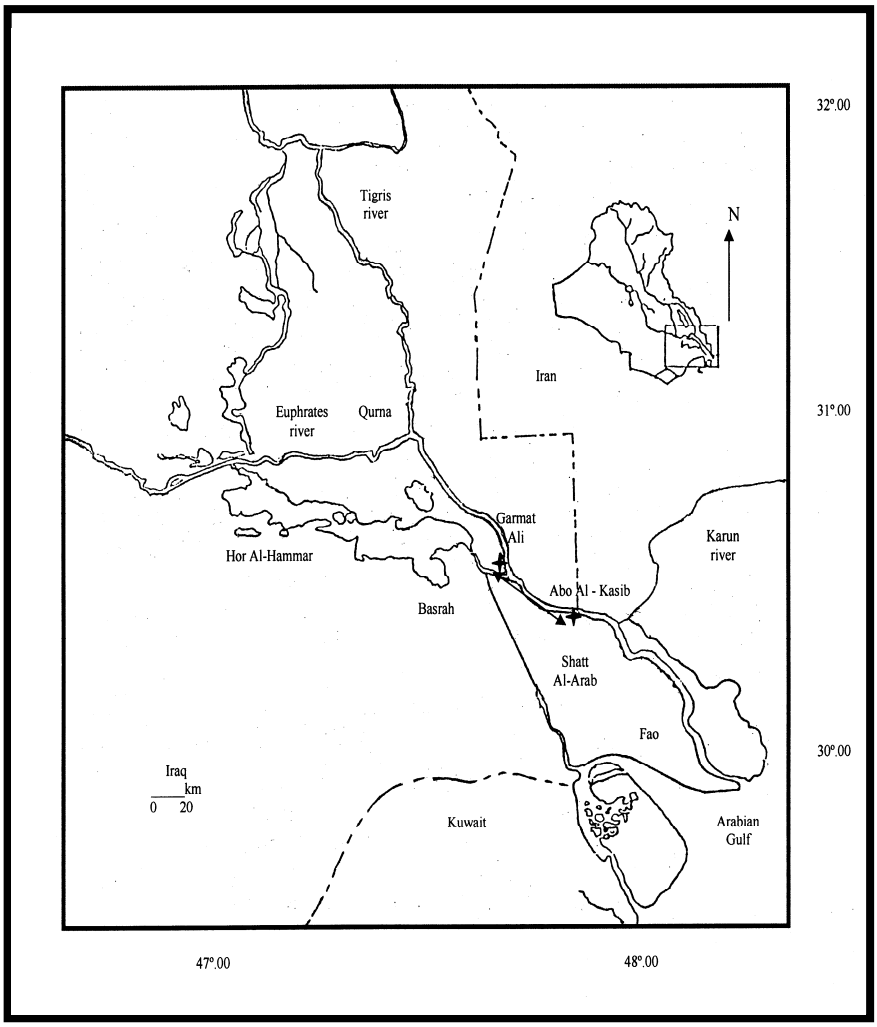


Figure 1. Map of sampling locations.

Extraction of hydrocarbons from exposure water:

The hydrocarbons were extracted from water following the procedure of UNEP (1989). According to which, 100ml of nangrade 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 $CCl_4$. A small amount of anhydrous sodium sulphate was added to these extracts to remove excess water. The $CCl_4$ extracts were reduced to volume less than 5 ml by using a rotary evaporator. The reduced extract was carefully pipette into a precleaned 10ml volumetric flask, making sure that any residual particles of sodium sulphate were excluded and evaporated to dryness by a stream of pure nitrogen. The flask was then rinsed with a fresh hexane. The rinsing was used to make the sample volume up to exactly 5ml prior to analysis by spectroflurometer.

*Extraction of hydrocarbons from animal tissues:*

The procedure of Grimalt and Oliver (1993) was used in the extraction of hydrocarbons from bivalve tissues. Ten grams of freeze-dried, grounded and sieved tissues of bivalve were placed in a pre-extracted cellulose thimble and soxhlet extracted with 150ml 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 combined extracts were reduced in volume to ca 10 ml in a rotary vacuum evaporator. They were then saponified for 2 hours with a solution of 4N KOH in 1:1 methanol:benzene. After extraction of the unsaponified matter with hexane, the extract was dried over anhydrous $Na_2 SO_4$ and concentrated by a stream of $N_2$ for analysis by spectroflurometer.

*Test solutions:*

The tests solutions of experiments were monitored for temperature, dissolved oxygen, pH and salinity at regular intervals. The temperature was at 20 ± 2°C. The dissolved oxygen ranged was 8.5 to 10.1 mg/l as to the other characteristics of tests solutions, pH was 7.1 to 7.8 and salinity was 1.6 to 1.8 ppt.

*Blank:*

Strenuous efforts are made to minimize the contamination of the samples; for such contamination would otherwise yield in erroneous results. Throughout the procedure, a great care is taken to ensure that samples are not contaminated; it is very important to avoid an unnecessary exposure of the samples (whether the solvent or the final extract) to the atmosphere or other potential contamination sources. However, procedural blanks of all reagents and glassware that were used during the analysis are periodically determined. It is preferred to eliminate contamination sources rather than adjusting or correcting the data that were actually obtained according to the blank value.

*Calibration:*

The fluorescence intensity of the sample analyzed is compared with the fluorescence of a reference solution (having almost the same concentration as the unknown extract) or to a series of reference solution (wherever, the measurement of fluorescence of the sample took more than one day). The fluorescence of reference solution was measured at least once a day under identical instrumental conditions. The reference oil was used in the spectroflurometer obtained from Iraqi South Oil Company (Basrah regular crude oil) (Figure 2).

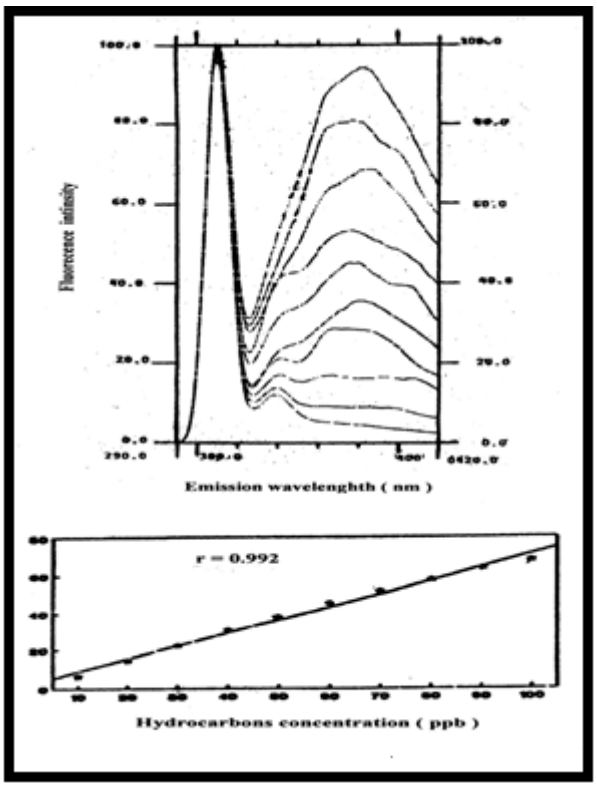


Figure 2. Fluorescence spectra with calibration curve of standard crude oil (Basrah Regular).

## Results and Discussion

The concentration of petroleum hydrocarbons in animals exposed to oil reflects the relative importance of the processes of uptake, storage and discharge. (Baussant *et al*., 2001). The present study showed there were no mortalities in the bivalve *C. fluminea* due to WSF. The bivalve was usually active during the 12 to 16 hours of the experiment (Tables 1) and (Figures 3 and 4). Hydrocarbons concentrations in the exposure water decreased slowly in a linear fashion so that no measurable concentrations remained after 32 hours (Table 2) and (Figure 5). The study has showed the slowly

accumulation of hydrocarbons compounds from a WSF of crude oil by the bivalve *C. fluminea* specially during the first hours of exposure (Tables 1) and (Figures 3 and 4). The *C. fluminea* was similar in this respect to the *Mytilus edulis* (Widdows and Donkin, 1992). It has been noted that, in general, estuarine molluscs accumulated hydrocarbons very slowly, whereas fish and crustaceans accumulated the hydrocarbons more rapidly (GESAMP, 1995). After 2 hours of exposure, the concentration of hydrocarbons was approximately 0.38 ppm in water (Table 2) and (Figure 5), while in tissues concentration was 4.8 ppm (Tables 1) and (Figures 3 and 4). After 20 hours, hydrocarbons were present in the water at a concentration of 0.09 ppm (Table 2) and (Figure 5), while the bivalve tissues contained 17.7 ppm hydrocarbons. After 25 hours of exposure, the hydrocarbons concentration deceased in the bivalve tissues to 15.6 ppm. At the end of the exposure period, the bivalve contained approximately 7.4 ppm (Tables 1) and (Figures 3 and 4). Bivalves, which filter large volumes of water while feeding, can take up and concentrate hydrocarbons from the water, either from solution or adsorbed to suspended particles (UNEP, 1993). Gill tissues of bivalves have a micellar layer on their surfaces which were responsible for the absorption of hydrophobic compounds, such as hydrocarbons (Aberkali and Trueman, 1985). Several studies have demonstrated that bivalves can concentrate hydrocarbons several orders of magnitude over their concentration in the water. Widdows and Dankin (1992) reported that the mussels exposed to fuel oil at 200–400 µg/g resulted in animals with concentrations of 335 µg/g. NRC (2003) reported that the maximum concentration of petroleum hydrocarbons in bivalves exposed to oil, under laboratory and field conditions was between 300 and 400 µg/g. The bivalves differ in their rates of hydrocarbon uptake, possibly due to differences in filtering rates and amounts of lipids (Zhou *et al*., 1996). Gold–Bouchot *et al*. (1995) found that oysters with high lipid content took up more fuel oil from the water than low lipid oysters.

In the present study, the depuration began during the exposure period (Table 1) and (Figures 3 and 4). The study demonstrated an initial rapid depuration of hydrocarbons by the bivalves *C. fluminea*. After 48 hours in clean river water, tissues burden had decreased to 0.22ppm of hydrocarbons. The bivalve remained contaminated with small concentrations of hydrocarbons for a long period (20 days). The complete depuration occurred in 26 days (Tables 3) and (Figure 3 and 6). Many of the uptake studies with marine bivalves involved depuration experiments. NRC (2003) reported that petroleum hydrocarbons accumulated by bivalves maintained under laboratory conditions generally have a short half–lives. This may be due to the high concentrations of petroleum used and the shorter exposure period. Fossato and Cononier (1976) exposed mussels for 14 days to 200–400 µg/l of diesel fuel and determined a half–life of approximately 3 days. Most depuration studies show an initial rapid discharge. However, there is a small concentration of petroleum hydrocarbons which retained for along period after the initial rapid discharge (Meardor *et al*., 1995). This conclusion is in agreement with the results of the present study. Mussel from oil polluted region retained 30 µg/g of petroleum hydrocarbons after 56 days of depuration (Widdows *et*

*al*., 1996), presumably held in tissues containing lipid stores. Paraffins are discharged at a faster rate than aromatics so that the latter are retained during long depuration experiments (Menon and Menon, 1999). Mussel exposed to fuel oil still retained paraffins after 35 days of depuration so aromatic hydrocarbons are presumed to persist in these animals for very long period (Widdows *et al*., 1995).

In conclusion, the bivalve *C. fluminea* accumulate the hydrocarbons more slowly with depuration taking many days.

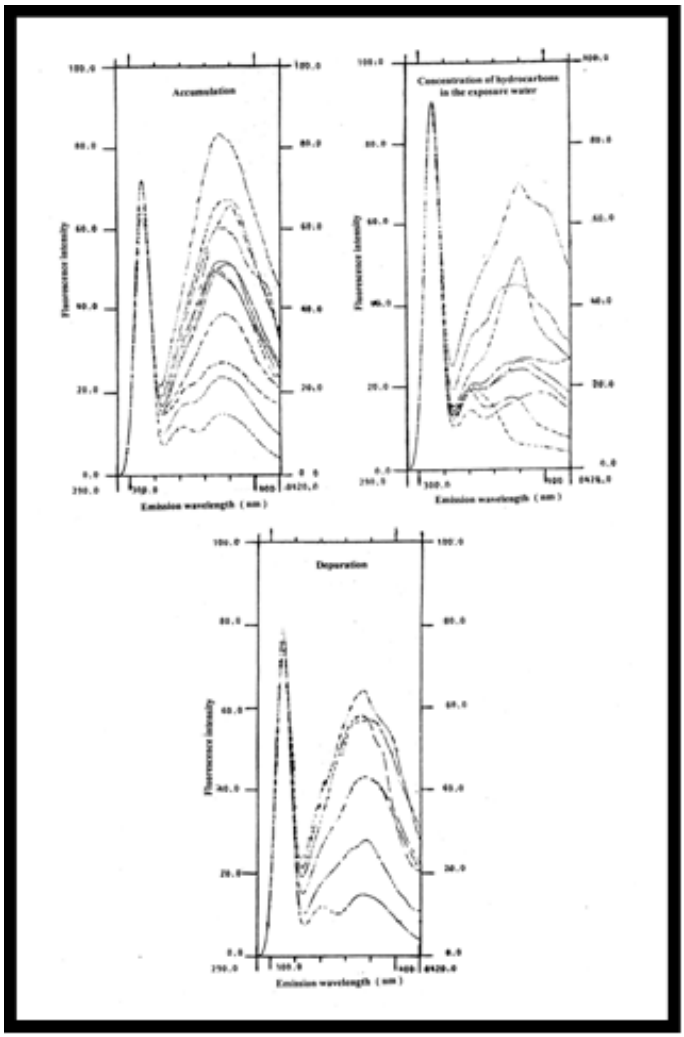


Figure 3. Fluoresence spectra of accumulation and depuration of hydrocarbons by the bivalve *C. fluminea* exposed to 5 ml/l of WSF of Nahran-Umar crude oil for 48 hours periods and the hydrocarbons concentration in the exposure water.

Table 1. Accumulation of hydrocarbons by the bivalve *C. fluminea* exposed to 5 ml/l of WSF of Nahran-Umar crude oil for 48 hours periods.

| Sampling time (hours) | Concentration of hydrocarbons (ppm) |
|---|---|
| 1 | 3.2 ± 0.4 |
| 2 | 4.8 ± 0.3 |
| 6 | 7.6 ± 0.4 |
| 10 | 11.2 ± 0.2 |
| 15 | 15.3 ± 0.3 |
| 20 | 17.7 ± 0.3 |
| 25 | 17.1 ± 0.3 |
| 30 | 15.6 ± 0.2 |
| 35 | 11.8 ± 0.2 |
| 40 | 10.2 ± 0.4 |
| 45 | 8.3 ± 0.5 |
| 48 | 7.4 ± 0.3 |

Table 2. Concentration of hydrocarbons in the exposure water of the bivalve *C. fluminea* exposed to 5 ml/l of WSF of Nahran-Umar crude oil for 48 hours periods.

| Sampling time (hours) | Concentration of hydrocarbons (ppm) |
|---|---|
| 1 | 0.42 ± 0.06 |
| 2 | 0.38 ± 0.05 |
| 4 | 0.30 ± 0.04 |
| 6 | 0.24 ± 0.05 |
| 10 | 0.16 ± 0.03 |
| 15 | 0.12 ± 0.02 |
| 20 | 0.09 ± 0.03 |
| 25 | 0.06 ± 0.04 |
| 30 | 0.02 ± 0.06 |
| 32 | 0 |

Table 3. Depuration of hydrocarbons by the bivalve *C. fluminea* (after 48 hours in clean river water) exposed to 5 ml/l of WSF of Nahran-Umar crude oil for 48 hours periods.

| Sampling time (hours) | Concentration of hydrocarbons (ppm) |
|---|---|
| 0 | 0.22 ± 0.07 |
| 4 | 0.20 ± 0.05 |
| 12 | 0.16 ± 0.05 |
| 24 | 0.13 ± 0.04 |
| 72 | 0.10 ± 0.04 |
| 288 | 0.07 ± 0.03 |
| 480 | 0.02 ± 0.02 |
| 576 | 0 |

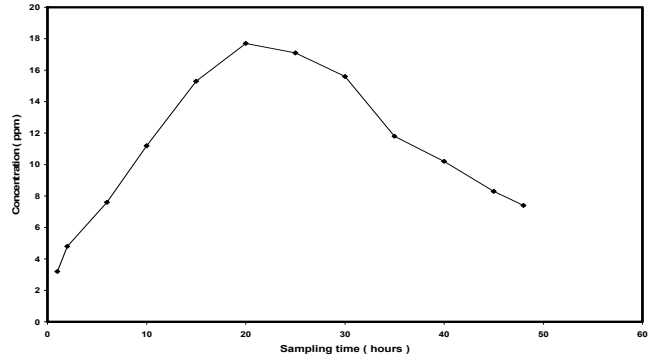


Figure 4. Accumulation of hydrocarbons by the bivalve *C. fluminea* exposed to 5 ml/l of WSF of Nahran–Umar crude oil for 48 hours periods.

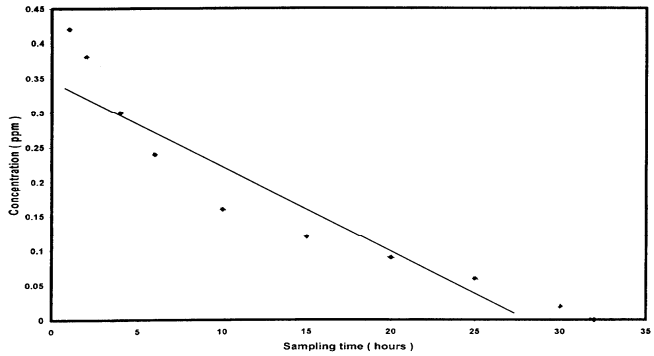


Figure 5. Concentration of hydrocarbons in the exposure water of the bivalve *C. fluminea* exposed to 5 ml/l of WSF of Nahran-Umar crude oil for 48 hours periods.

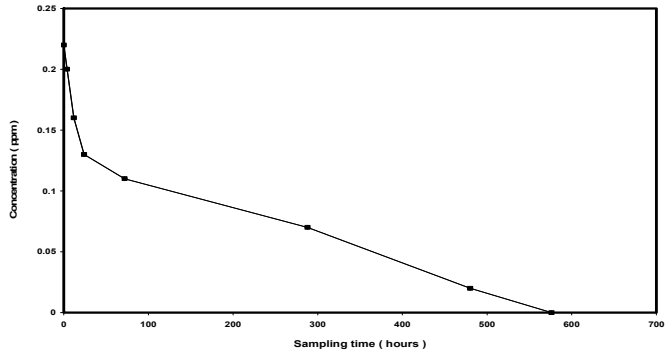


Figure 6. Depuration of hydrocarbons by the bivalve *C. fluminea* exposed for 48-hours to 5 ml/l water–soluble fractions (WSF) from Nahran-Umar crude cil.

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# الأخذ والاسترجاع للأجزاء الذائبة بالماء من النفط الخام لثنائية المصراع(Muller) *Corbicula fluminea* المتواجدة في نهر شط العرب

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**المستخلص -** تضمنت الدراسة الحالية تجارب للأخذ والاسترجاع، ففي تلك التجارب عرض المحار *C. fluminea* إلى 5 مليلتر من الأجزاء الذائبة بالماء من نفط خام نهران عمر لكل لتر من ماء النهر لمدة 48 ساعة في نظام مستقر للاختبار. أخذت عينات من ماء التعريض والمحار على فترات زمنية مختلفة لقياس تركيز الهيدروكاربونات الكلية بواسطة جهاز التفلور. بعد انتهاء فترة التعرض نقلت كائنات الاختبار إلى أوعية تحتوي على ماء نهر نظيف (لا يحتوي على الهيدروكاربونات النفطية) وتركت لمدة 48 ساعة لغرض الاسترجاع. لقد بينت التجارب عدم وجود وفيات في محار *C. fluminea* بسبب التعرض إلى الأجزاء الذائبة بالماء من النفط الخام. وان المحار يبقى فعالا خلال 12–16 ساعة من فترة التعرض. كما بينت بان تركيز الهيدروكاربونات ينخفض ببطئ في ماء التعريض بشكل خطي بحيث لم تبقى تراكيز من الهيدروكاربونات يمكن الكشف عنها بعد مرور 32 ساعة من التعرض. كما تراكمت الهيدروكاربونات في أنسجة المحار ببطئ خلال الساعات الأولى من التعرض. وبعد مرور 2 ساعة من التعرض، كان مستوى تركيز الهيدروكاربونات في ماء التعريض بحدود 0.38 جزء بالمليون تقريبا، بينما كان تركيز الهيدروكاربونات في داخل أنسجة المحار بحدود 4.8 جزء بالمليون. في حين بعد مرور 20 ساعة من التعرض، كان تركيز الهيدروكاربونات في ماء التعريض بحدود 0.09 جزء بالمليون، بينما كان تركيز الهيدروكاربونات في أنسجة المحار بحدود 17.7 جزء بالمليون. وبعد

مرور 25 ساعة من التعرض، انخفض تركيز الهيدروكاربونات في أنسجة المحار بحدود 15.6 جزء بالمليون. وفي نهاية فترة التعرض (48 ساعة) احتوت أنسجة المحار على تركيز 2.4 جزء بالمليون من الهيدروكاربونات تقريبا. وبعد مرور 48 ساعة من وجود المحار في ماء نهر نظيف لغرض الاسترجاع، انخفض عبئ أنسجة المحار من الهيدروكاربونات بحدود 0.22 جزء بالمليون، وبقيت أنسجة المحار تحبس تراكيز قليلة من الهيدروكاربونات لمدة 20 يوم. في حين حصل الاسترجاع الكامل بعد مرور 26 يوم.