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Accumulation, release, and depuration of crude oil-in water emulsions by the bivalve Corbicula fluminalis Müller (Molluscs: Bivalia: Eulamellibranchiata: Corbiculidae) from Shatt Al-Arab river, Basrah, Iraq

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Abstract

The bivalve C. fluminalis was exposed to a single doses of sublethal concentrations (20, 60, and 120 ppm) of Basrah regular crude oil-in water emulsions for a month period, under the laboratory conditions. The pattern of accumulation and release of petroleum hydrocarbons, the depuration time (biological half-life, TB_{50}), and the mechanisms of transport of petroleum hydrocarbons into the bivalve were determined. The bivalve accumulated the highest amount of petroleum hydrocarbons during the first week of exposure to crude oil emulsions. The accumulated petroleum hydrocarbons decreased each week as the petroleum hydrocarbons content of the exposure water decreased. The capillary gas chromatography analysis revealed that the dominant petroleum hydrocarbons accumulated by the bivalve after the four weeks of exposure to crude oil emulsions were naphthalene, phenanthrene, C_{12}, C_{13}, and C_{14}. The depletion of petroleum hydrocarbons from the exposure water, and the accumulation and release of petroleum hydrocarbons by the C. fluminalis involved formation a complex of mucus and crude oil by the bivalve. The depuration period was began when the bivalve was transferred to a clean river water for two weeks subsequent to the month exposure to crude oil emulsions. The accumulated petroleum hydrocarbons were rapidly, although incompletely released. At the end of the depuration period, the capillary gas chromatography analysis showed that the petroleum hydrocarbons retain in the bivalve was naphthalene. The biological half-life (TB_{50}) for depuration of the bivalve calculated were 55 days, 14.5 days, and 18 days for 20 ppm, 60 ppm, and 120 ppm respectively.

1- Introduction

Chemical analysis have shown that various of petroleum hydrocarbons are accumulated within marine invertebrates (Neff, 2002). Farid (2007), Maioli etal. (2010), and Veerasingam etal. (2011) have reported the occurrence of petroleum hydrocarbons in bivalve molluscs taken from the environment. These molluscs were sampled from areas which had either been exposed to oil spills or from areas considered industrially contaminated. The uptake of petroleum hydrocarbons by bivalve molluscs had also been shown to occure under laboratory conditions (Neff, 2002). The laboratory studies of the accumulation of petroleum hydrocarbons by bivalve molluscs were investigated by Ganning etal. (2003), Boehm and Quinn (2004), and Marigonez etal. (2006). Their works indicated that various of petroleum hydrocarbons can be accumulated and retained by bivalves. Neff (2002) documented that the bivalves, which filter large volumes of water while feeding, can take up and concentrate petroleum hydrocarbons from the water, either from solution or adsorbed to suspended particles. Gill tissues of bivalve molluscs have a micellar layer on their surfaces which are responsible for the absorption of hydrophobic compounds, such as hydrocarbons (Dame, 1996).

The bivalve Corbicula fluminalis is a common molluscs in the water of Shatt Al-Arab river and is a dominant member of its benthic macrofauna (Farid, 2007). As with many molluscs species their growth rates are slow and the animals are long lived. The C. fluminalis, occur frequently in the areas receiving acute and chronic oil exposures and the oils continues to be a serious problem to the animal in the water of Shatt Al-Arab river. Field studies of oil spill and its effects on C. fluminalis and the occurrence of oil in the bivalve subsequent to environment pollution of Shatt Al-Arab river was reported by Al-Saad and DouAbul (1984). The C. fluminalis are often considered as an important part of food web in the Shatt Al-Arab river (Farid, 2007). This study was therefore performed to determine experimentally, the pattern of accumulation and release of petroleum hydrocarbons, the depuration time (biological half-life, TB_{50}), and the mechanism of transport of petroleum hydrocarbons into the bivalve C. fluminalis exposed to Basrah regular crude oil-in water emulsions.

The crude oil was chosen in this study because it is commonly in Shatt Al-Arab river water, used industrial installations around the river and has already been

involved in well documental spill. The crude oil was added in an emulsified form to simulate a potential naturally occurring condition. Fingas etal. (1996) and (2000) have reported the formation of oil emulsions in the water by various mechanisms. In the event of an oil spill, it is probable that much of the oil would be dispersed and emulsified in the water column through turbulent action of waters. Emulsions tend to be relatively stable and a mechanism for the accumulation of emulsified oil by the bivalve molluscs has been reported by Farid etal. (2008).

Materials and Methods

Chemicals

Standard analytical grade chemicals used in this study were methanol, benzene, hexane, methylene chloride, and carbon tetrachloride were supplied from Scharlau, Fluka, Merck, and Burdick and Jackson laboratories, Inc. Standards of aliphatic (n-alkanes) and aromatic compounds and their related compounds were supplied by Ultra Scientific. Silica gel (100-200 mesh), alumina (100-200 mesh), sodium sulphate and potassium hydroxide were supplied by Supelco SA. The silica gel, alumina, and sodium sulphate were extracted with methylene chloride for 36 hours in a soxhlet. Followed the clean up extraction, they were dried in an oven at 130˚C for about 24 hours and deactivated with deionized water at the recommended percentage prior to use.

Crude oil

Basrah regular crude oil (medium-API gravity between 28-34) was used in this research was obtained from South Oil Company, Basrah, Iraq. The crude oil was transferred to the laboratory by blinding glass bottle closed tightly and kept in a cold and dark place till used. Six ppm of the crude oil was mixed with hexane for capillary gas chromatography analysis.

Preparation of crude oil-in water emulsions

The emulsions of crude oil were prepared by blending crude oil and water at the required quantity in a warring blender for two minutes according to a procedure developed by Bragin etal. (1994). The final three emulsions varied in color were made in this way with the following concentrations; 20 ppm, 60 ppm and 120 ppm.

Collection and acclimation of bivalve

Specimens of adult and uniform size individuals of the bivalves C. fluminalis were collected from Shatt Al-Arab river during 2008 (Figure 1). The bivalves 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.

Accumulation and depuration experiments

An exposure period of 30 days to Basrah regular crude oil-in water emulsions having

concentrations of 20, 60, and 120 ppm was utilized. Four 5 liters covered aquaria containing 4 liters of filtered water per aquaria (salinity= 1.6-1.8 ppt) were employed. The water was collected from clean location of Shatt Al-Arab river and filtered through a coarse plankton net to remove macro debris. One aquarium served as a control. Each of remaining aquaria received a sufficient volume of a stock crude oil emulsion to attain an initial concentration of either 20, 60, or 120 ppm of emulsified crude oil termed zero time. The water was continuously aerated and the temperature was maintained at 20±2°C. Sampling for petroleum hydrocarbons content of the exposure water and bivalves was performed every 7 days after zero time. The control was sampled at zero time and every 7 days subsequent to zero time. The depuration of the bivalves was determined for 14 days after the 30 days crude oil emulsions exposure. Each group of bivalves was removed from their aquaria and rapidly placed in clean 5 liters aquaria containing 4 liters of fresh coarse filtered Shatt Al-Arab river water maintained at the same temperature and salinity. The bivalves were transferred, the valves were wiped clean to remove deposited pseudofaeces, etc. Samples for chemical analysis were obtained on day 7 and day 14.

Collection of bivalve mucus

The mucus secreted by the bivalves was collected from the exposure aquaria after the four weeks crude oil emulsions exposure. It had a gray-green flocculent appearance and formed flocculent clump on the surface and in the exposure water column.

Extraction of petroleum hydrocarbons from bivalve mucus

The procedure of Grimalt and Oliver (1993) was used to extract the petroleum hydrocarbons bound or adsorbed to mucus of the bivalves exposed to 120 ppm emulsified crude oil. The mucus 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 into a strong flask. The sample was further extracted with a fresh solvent. The combined extracts were reduced in volume to 10 ml in a rotary vacuum evaporator. They were then saponified for 2 hours with a solution of 4 normality of potassium hydroxide (4NKOH) in 1:1 methanol:benzene. After extraction of the unsaponified matter with hexane, the extract was dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated by a stream of pure nitrogen (N_2).

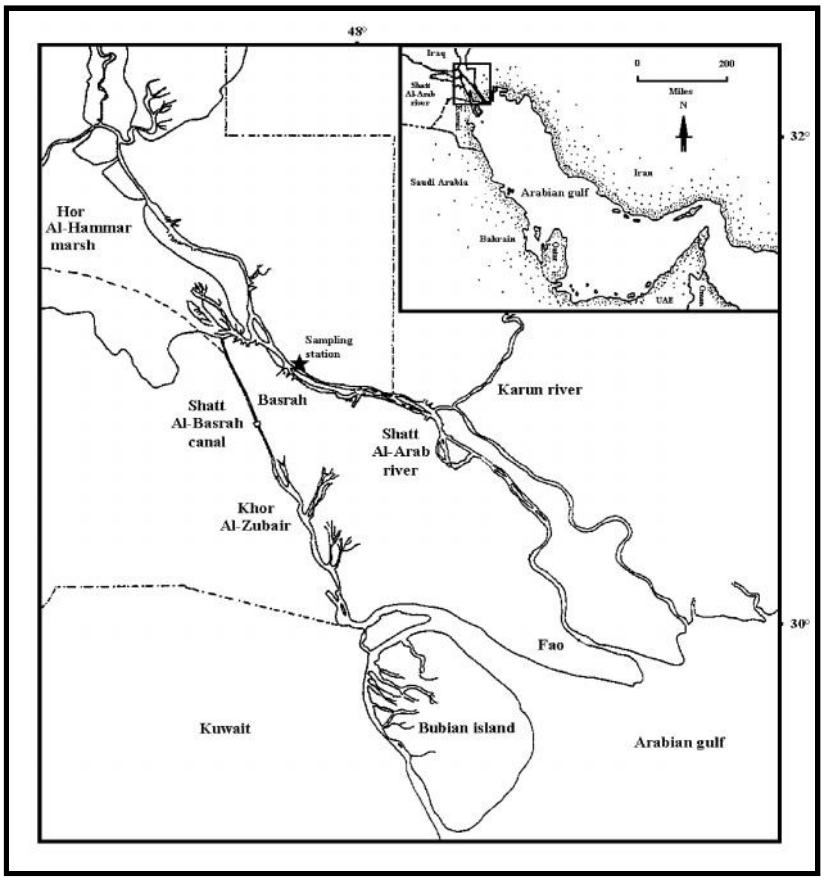

Figure 1. Map of sampling location on Shatt Al-Arab river.

Extraction of petroleum hydrocarbons from exposure water

The petroleum hydrocarbons were extracted from water following the procedure of UNEP (1989). According to which, 100 ml of nangrade carbon tetrachloride (CCl_4) were 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 was repeated several times before decanting the CCl_4. A small amount of anhydrous sodium sulphate (Na_2SO_4) were 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 extracts were carefully pipette into a precleaned 10 ml volumetric flasks, making sure that any residual particles of sodium sulphate were excluded and evaporated to dryness by a stream of pure nitrogen. The flasks were then rinsed with a fresh hexane. The rinsing was used to make the sample volume up to exactly 5 ml to analysis by spectroflurometer.

Preparation of bivalve tissues

The tissues of the bivalves were pooled and macerated in a food liquidizer from which at least 10 g were freeze-dried, grounded and sieved through a 63 µ metal sieve.

Extraction of petroleum hydrocarbons from bivalve tissues

The procedure used in the extraction of petroleum hydrocarbons from the bivalves tissues depending on Grimalt and Oliver (1993), was the same as that in the "Extraction of petroleum **hydrocarbons from bivalve mucus".** After extraction was over, the extracts of the bivalves tissues were dried over anhydrous sodium sulphate (Na_2SO_4) and concentrated by a stream of pure nitrogen (N_2) for analysis by spectrofl-urometer.

Spectroflurometer

This was Shimadzu RF-540 spectrofl-urometer equipped with a DR-data recorder, and was used to determine the total petroleum hydrocarbons. The basis quantitative measurements were made by measuring emission intensity at 360 nm, with excitation set at 310nm and monochromatic slits of 10 nm.

Fractionation

The concentrated extracts obtained from the bivalves mucus and tissues were cleaned up by columns chromatography. The columns filled with 8 g each 5 % water deactivated alumina (100-200 mesh) were placed at the top and silica gel (100-200 mesh) at the bottom. The extracts were then applied to the head of the columns and eluted with 50 ml of hexane to isolate the aliphatic fraction and 50 ml of benzene to isolate the aromatic one. The Both fractions were reduced to a suitable volume prior to analysis by capillary gas chromatography.

Capillary gas chromatography

This was a perkin-Elmer Sigma 300 capillary gas chromatography. It was equipped with flame ionization detector and splitless mode injection part. It was used to determine the aliphatic (n-alkanes) and aromatic hydrocarbons. Quantification of peaks and identification of hydrocarbons in the chromatograms was achieved by a Perkin-Elmer computing injection model LC-100. The fused silica capillary column used was a wall coated open tubular (WCOT) of 50 m x 0.25 mm Id SE (methyl silicone) (Perkin-Elmer). Helium was used as a carrier gas with a linear velocity of 1.5 ml/min. The operating temperatures for detector and injector were 350 and 320°C respectively. The column was operated under temperature programmed as follows: Initial temperature= 60°C (aliphatic fraction) and 70°C (aromatic fraction), initial time = 4 minutes (aliphatic fraction) and 0 minute (aromatic fraction), final temperature= 280°C (aliphatic fraction) and 300°C

(aromatic fraction), final time= 30 minutes (aliphatic and aromatic fractions), and rate= 4°C/minute (aliphatic and aromatic fractions).

Blank

Strenuous efforts were made to minimize the contamination of the samples; for such contamination would otherwise yield in erroneous results. Throughout the procedure, a great care was taken to ensure that samples were not contaminated; it was 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 were periodically determine. It was preferred to eliminate contamination sources rather than adjusting or correcting the data that were actually obtained according to the blank values.

Calibration

The fluorescence intensity of the sample analyzed was 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 look more than one day). The fluorescence of reference solution was measured at least once a day under identical instrumental conditions.

Calculation of the experimental concentration factor and the biological half-life (TB_{50}) for depuration

The calculated data of experimental concentration factors were achieved by subtraction of the petroleum hydrocarbons concentration of the exposure water column of the first week from the zero time value. This net petroleum hydrocarbons concentration in the exposure water column was then divided into the petroleum hydrocarbons value accumulated by the bivalves after the first week of crude oil emulsions exposure. The data of the biological half-life (TB_{50}) or residence time for depuration was calculated by regression analysis (Vaughan, 1973). The fourth week data were calculated as zero time for the depuration period. The significant of regression were analyzed by a t-test (AL-Rawi and Khalaf-Allah, 1980).

3-Results

Exposure water

The water of Shatt Al-Arab river before all tests and the water of the control treatment during the tests did not have detectable petroleum hydrocarbons. The spectrofluorometer analysis revealed that the concentrations of petroleum hydrocarbons in zero time varied from 5.80 ppm in the 20 ppm crude oil emulsion to 66.40 ppm in the 120 ppm crude oil emulsion. One week later, the detectable concentrations of petroleum

hydrocarbons in the column of the exposure water of the oiled aquaria were similar. The data ranged from 1.15 ppm in 20 ppm crude oil emulsion to 1.33 ppm in the 120 ppm crude oil emulsion. The spectrofluorometer also showed that the data of the second week were similar to those of the first week. They ranged from 0.93 ppm in the 20 ppm crude oil emulsion to 1.12 ppm in the 120 ppm crude oil emulsion. By the third week, the measurable concentrations of petroleum hydrocarbons had dropped further, where they varied from 0.44 ppm in the 20 ppm crude oil emulsion to 0.56 ppm in the 120 ppm crude oil emulsion. At the end of the fourth week, the petroleum hydrocarbons in the aquaria had fallen to almost lower concentrations. They ranged from 0.13 ppm in the 20 ppm crude oil emulsion to 0.27 ppm in the 120 ppm crude oil emulsion (Table 1). The range of dissolved oxygen and pH of the exposure water in all aquaria throughout the 30 days period of tests were about 8.5-10.1 mg/l and 7.1-7.8 respectively. The bacterial contamination was not apparent.

Bivalve mucus

Much of the crude oil was bound in mucus secreted by the bivalves which adhered to the aquaria walls or formed floating organic flocculent conglomerates. The capillary gas chromatography analysis demonstrated that the complex of mucus and crude oil contained predominantly naphthalene, biphenyl, fluorine, and phenanthrene with n-alkanes, mainly from C_{14} to C_{18}. Figure (2) illustrates the petroleum hydrocarbons gas chromatograms of Basrah regular crude oil and an extract of petroleum hydrocarbons from a mucus sample. A close correlation of peak heights was readily noticeable. The chromatograms pattern of the mucus was similar to crude oil but some of compounds are missing.

Bivalve of control treatment

The control bivalves were reactive to tactile stimuli and appeared to be feeding. There were no mortalities occurred among the bivalves of the control treatment. The gas chromatography analysis showed that there were no petroleum hydrocarbons to found in the tissues of the control bivalves (Table 3).

Bivalve exposed to 20 ppm crude oil emulsion

The mortalities of the bivalves in this crude oil emulsion concentration was 1 % (Table 2). The detectable total petroleum hydrocarbons concentrations in the tissues of bivalves exposed to 20 ppm crude oil emulsion were ranged from 36.55 µg/g dry weight in the first week to 19.69 µg/g dry weight in the fourth week. The capillary gas chromatography analysis of the four weeks samples revealed that the tissues of bivalves exposed to 20 ppm crude oil emulsion consisted of naphthalene (27.36 µg/g dry

weight in the first week to 14.42 µg/g dry weight in the fourth week), C_{12} (3.26 µg/g dry weight in the first week to 1.23 µg/g dry weight in the fourth week), and C_{13} (4.96 µg/g dry weight in the first week to 2.55 µg/g dry weight in the fourth week) (Table 3).

Bivalve exposed to 60 ppm crude oil emulsion

The mortalities of the bivalves in 60 ppm crude oil emulsion were 2.5 % (Table 2). The concentrations of total petroleum hydrocarbons in the tissues of bivalves exposed to 60 ppm crude oil emulsion were varied from 138.24 µg/g dry weight in the first week to 94.50 µg/g dry weight in the fourth week. The capillary gas chromatography analysis of the four weeks samples revealed that the tissues of bivalves exposed to 60 ppm crude oil emulsion were comprised of naphthalene (81.32 µg/g dry weight in the first week to 54.84 µg/g dry weight in the fourth week), phenanthrene (22.19 µg/g dry weight in the first week to 14.64 µg/g dry weight in the fourth week), C_{12} (16.44 µg/g dry weight in the first week to 11.12 µg/g dry weight in the fourth week), and C_{13} (17.29 µg/g dry weight in the first week to 12.30 µg/g dry weight in the fourth week) (Table 3).

Bivalve exposed to 120 ppm crude oil emulsion

The mortalities of the bivalves in this concentration were 3.5 % (Table 2). The concentrations of total petroleum hydrocarbons in the tissues of bivalves exposed to 120 ppm crud oil emulsion were varied from 103.82 µg/g dry weight in the first week to 86.78 µg/g dry weight in the fourth week. The capillary gas chromatography analysis of the four weeks samples showed that the tissues of bivalves exposed to 120 ppm crude oil emulsion consisted of naphthalene (54.08 µg/g dry weight in the first week to 46.67 µg/g dry weight in the fourth week), phenanthrene (13.83 µg/g dry weight in the first week to 11.08 µg/g dry weight in the fourth week), C_{12} (10.67 µg/g dry weight in the first week to 8.11 µg/g dry weight in the fourth week), C_{13} (11.26 µg/g dry weight in the first week to 9.96 µg/g dry weight in the fourth week), and C_{14} (12.16 µg/g dry weight in the first week to 10.01 µg/g dry weight in the fourth week) (Table 3).

Depuration

During the 14 days depuration period, the 20 ppm bivalves seemed to recover their tactile irritability in comparison to the control bivalves. The 60 ppm and 120 ppm bivalves were sluggish and after shucking many smelled foul. Their visceral mass were less firm than the control bivalves and their color had paled from the normal color. Mortalities occurred in the bivalves previously exposed to the concentrations of 20, 60 and 120 ppm crude oil emulsion. The actual mortalities at the end of the depuration

period were 0 %, 5 %, 12 %, and 19 % in control, 20 ppm, 60 ppm, and 120 ppm respectively (Table 2). The concentrations of total petroleum hydrocarbons in the tissues of bivalves exposed to 20 ppm crude oil emulsion were from 12.35 µg/g dry weight in the first week of depuration to 10.83 µg/g dry weight in the second week of depuration. They were from 38.86 µg/g dry weight in the first week of depuration to 31.46 µg/g dry weight in the second week of depuration for the tissues of bivalves exposed to 60 ppm crude oil emulsion. Whereas, The concentrations were from 43.85 µg/g dry weight in the first week of depuration to 35.03 µg/g dry weight in the second week of depuration for the tissues of bivalves exposed to 120 ppm crude oil emulsion. The capillary gas chromatography analysis revealed that the samples of bivalves consisted of naphthalene and no n-alkanes at the end of the depuration period. The concentrations of naphthalene in the tissues of bivalves exposed to 20 ppm crude oil emulsion were from 11.25 µg/g dry weight in the first week of depuration to 9.18 µg/g dry weight in the second week of depuration. They were from 36.87 µg/g dry weight in the first week of depuration to 30.24 µg/g dry weight in the second week of depuration for the tissues of bivalves exposed to 60 ppm crude oil emulsion. Whereas, The concentrations were from 41.89 µg/g dry weight in the first week of depuration to 33.97 µg/g dry weight in the second week of depuration for the tissues of bivalves exposed to 120 ppm crude oil emulsion (Table 3).

Experimental concentration factor

The computed concentration factors were 7.8 ppm, 2.9 ppm, and 1.5 ppm for the concentrations of 20 ppm, 60 ppm, and 120 ppm respectively.

Biological half-life (TB_{50}) of depuration

The calculated biological half-life (TB_{50}) for depuration were 55 days, 14.5 days, and 18 days for the concentrations of 20 ppm, 60 ppm, and 120 ppm respectively.

Table 1. Concentration of total petroleum hydrocarbons (ppm) in the exposure water column of bivalve C. fluminalis exposed to different concentrations of Basrah regular crude oil–in water emulsions for a month period in addition to control treatment.

Exposure period	20 ppm crude oil emulsion	60 ppm crude oil emulsion	120 ppm crude oil emulsion	Control
Zero time	5.80	47.60	66.40	0
First week	1.15	1.28	1.33	0
Second week	0.93	0.99	1.12	0
Third week	0.44	0.48	0.56	0
Fourth week	0.13	0.20	0.27	0

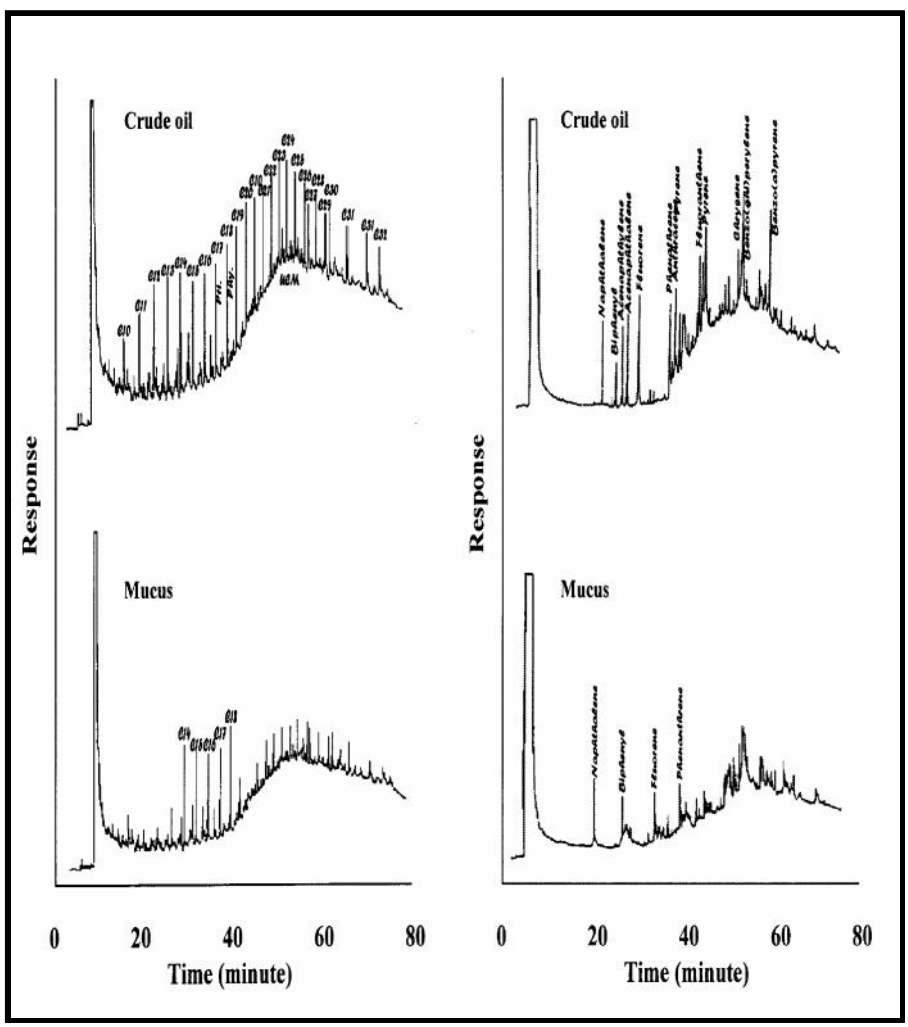

Figure 2. Capillary gas chromatograms of n-alkanes and aromatic hydrocarbons of Basrah regular crude oil and extract of petroleum hydrocarbons of the bivalve mucus.

Table 2. Percentage mortality of bivalve C. fluminalis in each concentration of crude oil emulsion and control treatment after the exposure period and at the end of depuration period.

Concentration	Mortality %	
	After exposure period	At the end of depuration period
20 ppm crude oil emulsion	1	5
60 ppm crude oil emulsion	2.5	12
120 ppm crude oil emulsion	3.5	19
Control	0	0

Table 3. Concentration of total petroleum hydrocarbons, C_{12}, C_{13}, C_{14}, naphthalene, and phenanthrene (µg/g dry weight) in the tissues of bivalve C. fluminalis exposed to different concentrations of Basrah regular crude oil-in water emulsions for a month period in addition to control treatment.

Concentration	Hydrocarbon	Exposure and depuration period					
		First week of exposure	Second week of exposure	Third week of exposure	Fourth week of exposure	First week of depuration	Second week of depuration
20 ppm crude oil emulsion	TPH	36.55	30.47	26.92	19.69	12.35	10.83
	C_{12}	3.26	2.93	2.62	1.23	---	---
	C_{13}	4.96	3.76	3.14	2.55	---	---
	Nap	27.36	23.26	19.54	14.42	11.25	9.18
60 ppm crude oil emulsion	TPH	138.24	117.39	101.41	94.50	38.86	31.46
	C_{12}	16.44	14.22	12.89	11.12	---	---
	C_{13}	17.29	15.57	12.64	12.30	---	---
	Nap	81.32	67.27	59.05	54.84	36.87	30.24
	Phe	22.19	19.44	15.25	14.64	---	---
120 ppm crude oil emulsion	TPH	103.82	95.17	90.63	86.78	43.85	35.03
	C_{12}	10.67	9.57	9.32	8.11	---	---
	C_{13}	11.26	11.12	10.03	9.96	---	---
	C_{14}	12.16	10.45	10.20	10.01	---	---
	Nap	54.08	51.36	48.43	46.67	41.89	33.97
	Phe	13.83	12.24	11.63	11.08	---	---
Control	TPH	0	0	0	0	0	0

TPH = Total petroleum hydrocarbons , Nap= Naphthalene, Phe= Phenanthrene

4- Discussion

The concentrations of petroleum hydrocarbons in the exposure water column began to decrease several hours after the addition of the crude oil emulsions. Several factors were probably responsible for this. At the end of the exposure period (30 days), the petroleum hydrocarbons in the aquaria had fallen to almost lower concentrations. Much of the crude oil was apparently removed from the exposure water column by the mucociliary feeding and ejection mechanisms of the bivalves. Large masses of mucus were ejected from the bivalves and were accumulated on the sides of exposure aquaria. Chemical analysis revealed a large content of petroleum hydrocarbons in the mucus of the bivalves. It was found that the mucus contained predominantly naphthalene, biphenyl, fluorine, and phenanthrene with n-alkanes, mainly from C_{14} to C_{18} chain. Earlier investigators reported that the crude oil can form droplets and micelles in the water, and that the major water soluble components were the aromatic hydrocarbons with some aliphatic compounds (Fingas etal., 1996 and 2000; Baussant etal., 2001). The accumulation of large amounts of petroleum hydrocarbons in the mucus may be a potential mechanism of concentrating and disseminating petroleum hydrocarbons in the environment.

The higher accumulation of petroleum hydrocarbons by the bivalves was reached one week after exposure, following by a gradual loss in accumulated petroleum hydrocarbons. The petroleum hydrocarbons accumulated were naphthalene, phenanthrene, C_{12}, $C_{13,}$ and C_{14}. The results of this study were comparable to those of Nunes and Benville (2003) they reported that oyster exposed to crude oil often accumulated naphthalene and paraffinic compounds in the C_{14} to C_{15} regions. Other studies also indicated that the petroleum hydrocarbons may be accumulated by bivalves (Al-Mudaffer etal., 1990; Benson etal., 2007 and Peteiro etal., 2007). The greater uptake of petroleum hydrocarbons by the bivalves exposed to 60ppm crude oil emulsion, may be due to a dose dependent narcosis. Maioli etal. (2010) reported that the water soluble fractions of crude oil could produce anesthetic effects on the ciliated epithelium of the bivalves gills. The concentration of 120 ppm crude oil emulsion may have reduced the filtration activity of the bivalves below that of the bivalves exposed to 60 ppm crude oil emulsion. The reduced filtration rate may account for the bivalves exposed to the higher concentration of crude oil emulsion (120 ppm) accumulating less oil than the bivalves exposed to the lower concentration of crude oil emulsion (60 ppm).

The depuration of accumulated petroleum hydrocarbons actually began as the petroleum hydrocarbons decreased in the exposure water column. When the bivalves were removed to fresh river water, depuration proceeded rapidly for the first week. The calculated biological half-life (TB_{50}) for depuration ranged from 14.5 to 55 days. The retention of petroleum hydrocarbons at the end of the depuration period indicated that after crude oil exposure, depuration will proceed rapidly to a low concentration after which depuration proceeds slowly. Other investigators have reported similar findings in other bivalves species (Farid, 2007; Ganning etal., 2003). However, the actual retention of petroleum hydrocarbons by the bivalves in this study was further substantiated by the observations of Nunes and Benville (2003) in which the bivalves were observed to eject petroleum hydrocarbons for at least one month after oil spill.

The petroleum hydrocarbons retention can have several deleterious effects. Long term low level petroleum hydrocarbons contamination may interferer of weaken the ability of the bivalves to withstand further environmental stresses such as those of temperature, salinity, spawning, disease and insult from other contaminants. Furthermore, oil retained in the sediment and bivalves may also be passed to predators, including man.

5-Conclusion

The bivalve C. fluminalis accumulated large amounts of petroleum hydrocarbons after initial exposure to petroleum. The amount and type of petroleum hydrocarbons accumulated were related to the initial dose, time following initial exposure and the relative solubilities of the various hydrocarbons. Naphthalene, phenanthrene, C_{12}, C_{13} and C_{14} were the most common compounds concentrated by the bivalve.

The depuration by the bivalve of accumulated petroleum hydrocarbons may actually begin as soon as hydrocarbons level drop in the exposure water column. When the bivalve placed in fresh hydrocarbons free river water, depuration proceeds rapidly within the first week after which the rate slows. Naphthalene were still present in the bivalve after two weeks in fresh river water.

Chemical analysis of the bivalve mucus extract demonstrated that the bivalve can concentrate and ingest or release to the environment extremely large amounts of petroleum hydrocarbons by means of their mucus-oil binding mechanism. Analysis of petroleum hydrocarbons present in the mucus revealed them to be naphthalene, biphenyl, fluorine, and phenanthrene with n-alkanes (C_{14} to C_{18}). The types of compounds bound in the mucus were

probably related to differential solubilities of petroleum constituents.

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تراكم وطرح واسترجاع مستحلبات النفط الخام والماء من قبل ثنائية المصراع Corbicula fluminalis (Müller) (شعبة: النواعم، صنف: ثنائية المصراع، رتبة: حقيقية الغلاصم، عائلة: الكوربيكيولدي) من نهر شط العرب، البصرة، العراق

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الخلاصة

عرضت ثنائية المصراع C. fluminalis إلى جرع مفردة من تراكيز تحت مميتة (20 جزء بالمليون و60 جزء بالمليون و120 جزء بالمليون) لمستحلبات نفط خام البصرة الاعتيادي والماء لمدة شهر واحد، تحت الظروف المختبرية. حدد نموذج تراكم وطرح الهيدروكاربونات النفطية من قبل ثنائية المصراع، ومتوسط الزمن الحيوي لعملية الاسترجاع، وميكانيكية انتقال الهيدروكاربونات النفطية إلى ثنائية المصراع. راكمت ثنائية المصراع أعلى كمية من الهيدروكاربونات النفطية خلال الأسبوع الأول من التعرض إلى مستحلبات النفط الخام والماء. انخفضت الهيدروكاربونات النفطية المتراكمة بعد ذلك في كل أسبوع كنتيجة لانخفاض محتوى الهيدروكاربونات النفطية في ماء التعريض. بين التحليل بجهاز الغاز كروماتوغرافي بان الهيدروكاربونات النفطية السائدة المتراكمة في ثنائية المصراع بعد أربعة أسابيع من التعرض إلى مستحلبات النفط الخام والماء هي النفثالين والفينانثرين والكاربون 12 والكاربون 13 والكاربون 14. تضمن انخفاض الهيدروكاربونات النفطية في ماء التعريض وتراكم وطرح الهيدروكاربونات النفطية من قبل ثنائية المصراع C. fluminalis تكوين معقد من المخاط والنفط الخام بواسطة ثنائية المصراع. بدات عملية الاسترجاع بعد نقل ثنائية المصراع إلى ماء نهر نظيف لمدة أسبوعين بعد تعرضها لمدة شهر إلى مستحلبات النفط الخام والماء. كان تراكم الهيدروكاربونات النفطية سريعا من قبل ثنائية المصراع، على الرغم من عدم حصول طرح كامل للهيدروكاربونات النفطية خلال عملية الاسترجاع. في نهاية عملية الاسترجاع، بين التحليل بجهاز الغاز كروماتوغرافي بان الهيدروكاربونات النفطية المحتبسة في داخل ثنائية المصراع كانت النفثالين فقط. كان متوسط الزمن الحيوي المحسوب لعملية الاسترجاع 55 يوم، و14.5 يوم و18 يوم لتركيز 20 جزء بالمليون و60 جزء بالمليون و120 جزء بالمليون على التوالي.