EUROPEAN ACADEMIC RESEARCH
Vol. V, Issue 3/ June 2017

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Bacterial and hydrocarbons contamination in the water and bivalve (*Corbicula fluminalis*) of Shatt Al-Arab river, Iraq

ASEEL NADHIM KADHIM AL-SALMAN
Department of Pathology and Poultry
Veterinary College, University of Basrah, Iraq

Abstract:

The bacteria and hydrocarbons in Shatt Al-Arab river water and bivalve (Corbicula fluminalis) were studied. The total heterotrophic and coliform bacteria counts ranged from $5.6x10^4$ to $8.9x10^4$ cfu ml^{-1} and $2.0x10^3$ to $6.7x10^3$ cfu ml^{-1} respectively in the water samples, and from $4.2x10^5$ to $9.6x10^5$ cfu g^{-1} and $4.4x10^4$ to $7.8x10^4$ cfu g^{-1} respectively in the bivalve samples. The bacterial isolates were Aeromonas hydrophila, Bacillus cereus, Vibrio cholera, Vibrio parahaemolyticus, Pseudomonas aerugnosa, Listeria monocytogenes, Campylobacter jejuni, Campylobacter coli, Micrococcus spp. (heterotrophic), Escherichia coli, Edwardsiella tarda, Citrobacter braakii, Salmonella typhi, Shigella ssp., Klebsiella pneumonia, Enterobacter coloacae, Serratia marcescens, Yersinia enterocolitica, and Plesiomonas shigeloides (coliform) which are of health significance. In both water and bivalve samples, the n-alkanes from C_{14} to C_{33} and PAHs including naphthalene, biphenyl, acenphtaylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, perylene, benzo (a) pyrene, indeno (1,2,3-cd) pyrene, and benzo (g,h,i) perylene were detected. The concentrations of total hydrocarbons, n-alkanes, and PAHs varied from 4.63 to 20.36 µg l^{-1} dw, 4.20 to12.05 µg l^{-1} dw, and 1.92 to 7.55 ng l^{-1} dw respectively in the water, whereas ranged from 10.22 to 28.73 µg g^{-1} dw, 7.23 to 16.53 µg g^{-1} dw, and 3.55 to11.90 ng g^{-1} dw respectively in the bivalve. The bioconcentration factors of individual n-alkanes and PAHs in the bivalve ranged from 0.71 (C_{23}) to 6.33 (C_{30}) and from 1.48 (pyrene) to 1.84 (biphenyl)

respectively. The calculated hydrocarbons indices indicated that these compounds were generally from both biogenic and anthropogenic sources. The hydrocarbon concentrations in the bivalve could biomagnify through the food chain with adverse toxicological effects on the biota. The bivalve (C. fluminalis) could be used as a bioindicator of bacterial and hydrocarbons contamination in aquatic ecosystem.

Key words: bivalve, coliform bacteria, hydrocarbons pollution, PAHs, n-alkanes.

INTRODUCTION

Shatt Al-Arab River is the most important river in southern of Iraq, because of its economical, social and ecological values. It is the main source of surface fresh water in Basrah City and pours about $5x10^9$ m^3 nutrient rich water into the Arabian Gulf each year (Ali *et al*., 2013). The river has an area of million km^2 occupied extensively by trees and aquatic grasses. The aquatic fauna and flora are unique assemblages representatives diverse and delicately balanced ecosystem. The Shatt Al Arab River region is with a large area utilized for agriculture, grazing, fishing, urban, and industry (Abdullah, 2013). Development pressures to make Shatt Al-Arab River are provides easy access to small tankers. Many heavy industries are also located along the Shatt Al-Arab River shore. So, the river suffers constant threat of petroleum hydrocarbons pollution from tanker activities as well as chronic inputs from sources such as boating, refinery and other industrial outfalls, and domestic sewage. Moreover, the river suffers from fecal contamination due to the municipal solid and water wastes in open drains and surface runoff which are discharged directly into its water (Farid, *et al*., 2014). These pollutants may have severe impact on the water quality of the river and eventually upon living organisms.

A large variety of pathogenic microorganisms can contaminate water. The main source of these microorganisms are the feces of human and animals which are bring to aquatic environments through of wastewater effluents, soil runoff and leaching (Ouattara *et al*., 2011). Fecal contamination may cause degradation of aquatic systems, affect water quality and seriously affects human health (Pandey *et al*., 2014). Ouattara *et al*. (2011) reported that the detection and enumeration of all pathogenic microorganisms present in aquatic systems is impossible due to the large diversity of the pathogens, the low abundance of each species and the absence of standardized and low cost methods for the detection of each of them. Therefore, for evaluate the level of microbial water, indicators of fecal pollution were much needed. The most widely accepted indicators of fecal contamination in water have been the coliform group of bacteria (Rompre´*et al*., 2002).

Hydrocarbons are chemical compounds composed mainly of the elements carbon and hydrogen and found in crude oil and oil refinery products (NRC, 2003). There are several sources of petroleum hydrocarbons, however, human activities such as oil transportation and spills, shipping, industrial, storm-water and domestic discharges are believed to be an important influence on hydrocarbons in the aquatic environment (Shi *et al*., 2008). Petroleum hydrocarbons are highly toxic to aquatic organisms at low concentrations. The degree of toxicity of hydrocarbons depends on their solubility in water and volatility. The toxicity of various hydrocarbons increases successively as follows: paraffins with straight chains, naphtalene, olefine, cyclic paraffins and aromatic compounds. In addition to direct lethal toxicity, petroleum hydrocarbons can exert sublethal effects on aquatic organisms. Hydrocarbons are biopersistent, bioaccumulative and can cause deleterious effects to aquatic fauna and flora as well as to human (Wattayakorn and Rungsupa, 2012).

For provide an optimal use of aquatic resources, monitoring programs are ordinarily used to evaluate pollution state of aquatic environment and implement strategies for aquatic resources protection. In this regard, different aspect could be used: water, sediment and organisms. The measuring pollutants in water present some disadvantages such as the low concentrations and the random spatial and temporal variations. In sediment, the concentrations of pollutant are higher than in water, but contaminants are not always bioavailable for organisms. Moreover, the heterogeneity of particle size and organic matter of sediment could make comparison between sites difficult. This is why the use of living organisms called biomonitors is the best to estimate pollutants (MRC, 2014).

Molluscos such as oysters, mussels, cockle and clams have been widely employed as biomonitors for pollution in marine and freshwater environments because of their abundance, sedentary habits, long lifespan, ease of collection, ability to bioconcentrate many environmental pollutants to levels that greatly exceed those present in surrounding environment and limited ability to metabolize accumulated contaminants (Hamza-Chaffai, 2014). They have been used in different international monitoring programs such as the Mussel Watch (USA) and the RNO (France) (Stellio and Cédric, 2006). The wide use of shellfish reflects not only the high capacity of these organisms to bioaccumulation of contaminants and their widespread distribution, but also their importance, because shellfish represent an important source of protein for aquatic communities (Sousa *et al.*, 2008). It has been predictable, for instance, that over 90 % of human health exposure to several contaminants occurs through diet primarily seafood and meat (Vega Corrales *et al.*, 2013). The use of bivalves as biomonitors of microorganisms and petroleum hydrocarbons has been widely reported. This is due to their characteristics from ecological and biological points of view which are advantageous for biomonitoring (Miller *et al.*, 2005).

The bivalve *Corbicula fluminalis* is sedentary filter feeding bivalve that is widely available in Shatt Al-Arab River and satisfying criteria required for good biomonitors of pollution. it's prospective as a biomonitor has been reported by Al–Saad and DouAbul (1984) and (Farid *et al.* (2008). Hence, the present study was to estimate the bacteriological load and concentrations of hydrocarbons in tissues of Shatt Al-Arab river *C. fluminalis* and surrounding water.

MATERIALS AND METHODS

Five stations on the Shatt Al-Arab River were chosen for this study. The locations of these stations were shown on the map (Figure 1) and were geo-located with global positioning system (GPS) to ensure consistency as given in Table (1). Samples of water and the bivalve *C. fluminalis* (Müller) [Molluscs: Bivalia: Eulamellibranchiata: Corbiculidae] were collected from the study stations during June of 2016. The water collector device was employed to collect the samples of water. The bivalve specimens (adult and uniform size individuals) were collected with the hand. The water and bivalve samples were then transferred to laboratory by closed and sterile bottles and glass containers respectively.

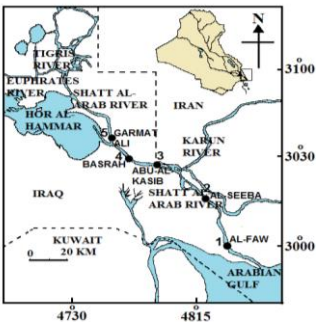

Fig 1: Map of sampling stations.

Table 1: Location of sampling stations.

Station	Location	
1	29°58'28.6" N	48°29'09.5" E

2	**30°20'16.5" N**	**48°15'34.5" E**
3	**30°27'44.5" N**	**48°00'06.0" E**
4	**30°33'00.0"N**	**47°47'10.0"E**
5	**30°48'10.6" N**	**47°45'03.8" E**

The water samples for chemicals analysis were spiked with 5 ml of 1:1 HCl and stored in the dark at 4 °C with a maximum holding time of 2 h before extraction. A serial dilution of water samples were done for bacterial cultivated within a few hours of collection.

The bivalve shells were extensively washed with water and rinsed with normal saline to remove all surface contaminants. The tissues of the bivalves were then pooled and macerated in a sterile food liquidizer from which at least 10 g were freeze-dried, grounded and sieved through a 63 µ metal sieve for extraction. Another amount of tissues was transferred to a sterile blender for homogenization and serial dilution was then done to bacterial cultivated.

Enumeration of total heterotrophic and coliform bacteria was done by mixed 1 ml of water or 1 g of bivalve with 9 ml of sterile normal saline. The suspension was left to settle for 5–10 min. Six ten-fold dilutions were prepared and 0.1 ml from appropriate dilution was spreaded on to nutrient agar medium (Difco) (heterotrophic) and MacConkey agar medium (Oxoid) (coliform) by sterile spreader. The nutrient agar was supplemented with 300 mg l^{-1} of cycloheximide to prevent fungal growth. The plates were incubated aerobically for 48 h at 37 °C. Each type of colony appearing on the agar was then recorded and picked up. The grown colonies were purified, enumerated, and examined microscopically. Pure cultures were further inoculated onto tube slants containing nutrient agar media and were kept stock cultures. These cultures were maintained at 4 °C and subcultured every 6–8 weeks. Identification of bacteria was carried out according of their morphological characteristics and biochemical tests (Cowan and Steel, 1975; Holt *et al*., 1994).

To distinguish between the dissolved and particulate phases of water, it was suction filtered 5 h through preignited (450 °C) and preweight 0.45 μm pore size Whatman GF/F glass filter fiber. The fraction that passed through the filter was dissolved phase, whereas that retained on filter considered as particulate phase. The filter with particulate matter was wrapped in preheated aluminum foil and were freeze-dried.

The hydrocarbons were extracted from the dissolved phase of water following the procedure of Cripps (1989), 100 ml of 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 Na_2SO_4 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 vacuum evaporator. The reduced extracts were carefully pipette into a precleaned 10 ml volumetric flask, making sure that any residual particles of Na_2SO_4 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.

The method used to extract the hydrocarbons from the particulate phase and bivalve samples was based upon that of Grimalt and Olive (1993). The glass fiber filter with particulate matter or 10 g amount of dried bivalve were placed in soxhlet apparatus and extracted with 150 ml methanol: benzene mixture (1:1) for 24 hours. The extract was storage and the sample was further extracted with fresh solvents. The combined extract was then reduced in volume to 10 ml by a rotary vacuum evaporator and was sabonified for 2 hours with a solution of 4N KOH in 1:1 benzene: methanol. Then the extract was dried by anhydrous Na_2SO_4 and concentrated by a stream of nitrogen.

The water (dissolved and particulate phases) and bivalve extracts were divided into two portions. The first portion was used to determine total petroleum hydrocarbons using a Shimadzu RF-540 spectroflurometer. The hydrocarbons were quantified by measuring the emission intensity at 360 nm, with excitation set at 310 nm and monochromatic slits of 10 nm. The second portion of the extract was used to determine the aliphatic (n-alkanes) and aromatic hydrocarbons using a Allegiant capillary gas chromatography with flam ionization detector (FID). Prior to GC analysis the extracts were fractionated by passing them through column filled with 8 g of 5% deactivated alumina (100-200 mesh) on the top and silica (100-200 mesh) in the bottom. The samples then were eluted by 50 ml n-hexane (aliphatic) followed by 50 ml of benzene (aromatic). Both fractions were reduced to suitable volume and subjected to GC analysis. Helium gas was used as a carrier gas with a linear velocity of 1.5 ml min^{-1}. The fused silica capillary column (100 m x 250 µm x 0.5 µm) used was a wall coated open tubular (methyl silicone) (Agilent US2463233H DB-petrp). The operating temperatures for detector and injector were 280°C and 300°C (aliphatic) and 300°C and 320°C (aromatic) respectively. The temperature of column was held at 35 °C for 13 min then 5 °C min^{-1} to 280°C (aliphatic) and 50 °C for 8 min then 8 °C min^{-1} to 350°C (aromatic).

The individual n-alkanes and PAHs were identified based on the retention time of the authentic standards and concentrations of each n-alkane and PAHs were calculated based on the standard calibration curve of each corresponding standard compound.

For quality assurance and quality control, method blanks (solvent) and spiked matrixes (standards spiked into soil) were analyzed. None of the target compounds was detected. The recovery and relative standard deviation for C_{14} to C_{33} were in the range of 66.7–87% and 3.9–20.1%, respectively. The detection limits of the method range from 4.2

and 15.3 μg g^{-1}. All concentrations are expressed on a soil dry weight basis. For PAHs, 15 surrogate standards (naphthalene, biphenyl, acenphtaylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, perylene, benzo (a) pyrene, indeno (1,2,3-cd) pyrene, and benzo (g,h,i) perylene) were added to all samples to monitor matrix effects. The average recoveries of surrogate standards varied from 83.3% to 94%. In addition, the detection limit ranged from 0.13 to 0.8 ng g^{-1} dry weight (dw).

The data were statistically analyzed using SPSS (2006). The one way analysis of variance (ANOVA) was used to test for significant difference ($p > 0.05$). The concentrations of n-alkanes and PAHs detected were analyzed statistically using mean± standard division (SD). Sum of the n-alkanes and PAHs in samples represent the total n-alkanes and PAHs. The ratio of n-alkanes and PAHs were used to identify the possible n-alkanes and PAHs sources. The bioconcentration factor of petroleum hydrocarbons in the tissues of the bivalve in μg g^{-1} body mass was expressed as: Bioconcentration factor (Bf)= petroleum hydrocarbons concentration outside the tissues of bivalve/ petroleum hydrocarbons concentration inside the tissues of bivalve.

RESULTS AND DISCUSSION

The counts of total heterotrophic and coliform bacteria in the water of Shatt Al-Arab river ranged from $5.6x10^4$ to $8.9x10^4$ cuf ml^{-1} and $2.0x10^3$ to $6.7x10^3$ cuf ml^{-1} respectively. Whereas, the bacterial counts in bivalve varied from $4.2x10^5$ to $9.6x10^5$cuf g^{-1} and $4.4x10^4$ to $7.8x10^4$ cuf g^{-1} respectively (Table 2). The higher levels of bacteria in water and bivalve samples obtained from the station 4 and 5 (Fig. 2) which may be explained by that the water of this stations are more contaminated, thus presenting higher densities of bacteria. These stations proximity to the Basrah city, means that these water are subject to

contamination by illegal sewage discharge and/or by urban drainage waters, considered to be the main causes of the contamination of the river water. The bacterial counts in the bivalve samples were one magnitude higher than those in the water samples. Studies have shown that filter-feeding bivalve molluscs (shellfish) can bioaccumulate microorganisms to levels several times higher than those present in the surrounding water (Miller *et al*., 2005; Martinez and de Oliveira, 2010). In both the water and bivalve samples, the counts of bacteria were in the following trend heterotrophic > coliform. The same observation was found by (Eduok *et al*., 2010). The relationship between the bacterial number in water and bivalve is not straightforward and depends on several factors including the bivalve physiology and morphology, bacterial characteristics, and environmental conditions (Martinez and de Oliveira, 2010). However, the presence of positive correlation between the bacterial counts in the water and bivalve samples indicates that an increase of the bacterial counts in water directly influenced the load in the bivalve. The bacterial isolates recovered from recent water and bivalve samples were *Aeromonas hydrophila*, *Bacillus cereus*, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Pseudomonas aerugnosa*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Campylobacter coli*, *Micrococcus* spp. (heterotrophic), *Escherichia coli*, *Edwardsiella tarda*, *Citrobacter braakii*, *Salmonella typhi*, *Shigella ssp.*, *Klebsiella pneumonia*, *Enterobacter coloacae*, *Serratia marcescens*, *Yersinia enterocolitica*, and *Plesiomonas shigeloides* (coliform) (Table 3). The recovery of bacteria from water and shellfish samples has been widely reported and most of the studies have focused on fecal contamination, enteric pathogens and pathogenic species of *Vibrio* (Jalal *et al*., 2009). Kueh and Chan (1985) compared the microbiota of several species of bivalve molluscs to that of the surrounding seawater and found differences in both numbers and generic composition. The microorganisms detected from shellfish and seawater were

Pseudomonas, *Vibrio*, *Acinetobacter*, *Aeromonas*, coliform and coryneform bacteria. The isolation of bacteria from the samples (water and bivalve) taken from different parts of Shatt Al-Arab river regardless of the proximity to human settlements and river activities suggested a widespread distribution of these microbes in the river water. Such conclusion had also been reported by (Eduok *et al*., 2010). The detection of *E. coli* in the studied samples indicated human recent fecal contamination in Shatt Al-Arab river which together with other bacterial isolates had been responsible for various human infectious diseases. *E. coli* are the most frequently used indicators of fecal pollution as it was demonstrated by epidemiological studies that they were better indicators of the human risk associated with waters (João and Cabral 2010; Olaolu *et al*, 2014).

Table 2: Bacterial counts in water and bivalve samples of Shatt Al-Arab River.

Sample	Station					
	Bacteria	1	2	3	4	5
Water	Heterotrophic	$5.6x10^4$	$6.9x10^4$	$7.6x10^4$	$8.9x10^4$	$8.2x10^4$
	Coliform	$2.9x10^3$	$2.0x10^3$	$4.6x10^3$	$6.7x10^3$	$5.3x10^3$
Bivalve	Heterotrophic	$4.8x10^5$	$4.2x10^5$	$6.8x10^5$	$9.6x10^5$	$7.5x10^5$
	Coliform	$4.4x10^4$	$5.1x10^4$	$6.0x10^4$	$7.8x10^4$	$6.3x10^4$

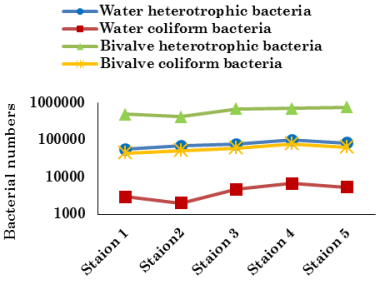

Fig 2: Numbers of bacteria in water (cuf ml^{-1}) and bivalve (cuf g^{-1}) samples of study stations.

Table 3: Bacterial species and genera isolated from water and bivalve of Shatt Al-Arab river.

Heterotrophic	Coliform

A. *hydrophila*	***E. coli***
B. cereus	***E. tarda***
V. cholera	***C. braakii***
V. parahaemolyticus	***S. typhi***
P. aerugnosa	***Shigella ssp.***
L. monocytogenes	***K. pneumonia***
C. jejuni	***E. coloacae***
C. coli	***S. marcescens***
***Micrococcus* spp.**	***Y. enterocolitica***
	P. shigeloides

The most diseases in the communities around Shatt Al-Arab river environment are gastrointestinal and bronchopulmonary disorders due to drinking the contaminated water, consumption of uncooked river foods thoroughly, and ingestion of water during the swimming in the river. This is in agreement with the findings of other workers (Rippey, 1994; Novotny *et al*., 2004). The waterborne diseases are widely distributed in the communities of Shatt Al-Arab river region due to low level of education, poor health conditions, historical neglect, apathy, pollution, substandard medical care, and extreme poverty level of residents who cannot afford clean and safe drinking water and medicare. Similar observations have been made by Eduok *et al*. (2010) in the Iboe river estuary communities of Nigeria Delta. The gastrointestinal diseases are prevalent in the Shatt Al-Arab river area ranging from a simple to severe diseases accompanied with symptoms such as abdominal cramps, diarrhea, vomiting, and dehydration. Despite the ability of preventing these diseases, they are often undiagnosed, life threatening and may be sometimes cause death (Wilson, 2005). The infections and poisoning cases in the present study as a result of drinking and consumption of contaminated water and river foods something to worry about because of the unhealthy environment, dealing directly with contaminated water and aquatic organisms, and eating river foods uncooked well. The microbial load in the Shatt Al-Arab river samples was higher than the standards prescribed by Ashbolt *et al*. (2001) for water

and molluscs. This may cause a health risks for the river resources consumers unless taken the necessary preventative procedures such as sterilization of river water before drinking and cooking the river foods thoroughly.

The hydrocarbons concentrations in the water samples of Shatt Al-Arab river were 1.74 to 9.62 μg g^{-1} (total hydrocarbons in dissolved water), 1.86 to 5.23 μg g^{-1} (*n*-alkanes in dissolved water), 0.74 to 3.64 ng g^{-1} (PAHs in dissolved water), 2.89 to 10.74 μg g^{-1} dw (total hydrocarbons in particulate water), 2.34 to 6.82 μg g^{-1} dw (*n*-alkanes in particulate water), and 1.18 to 3.91 ng g^{-1} dw (PAHs in particulate water).Whereas, the concentrations of hydrocarbons in the bivalve samples were 10.22 to 28.73 μg g^{-1} dw (total hydrocarbons), 7.23 to 16.53 μg g^{-1} dw (n-alkanes), and 3.55 to 11.90 ng g^{-1} dw (PAHs) (Table 4). Elevated concentrations of hydrocarbons in water and bivalve samples were relatively recorded at stations 1 and 2 (Fig 3) which associated with discharges of petroleum wastes. The comparison between the concentrations of hydrocarbons measured here and those of the other studies dealing with hydrocarbons contamination in water and bivalve samples were found in Table 5 and 6.

Table 4: Hydrocarbons concentrations in water and bivalve of Shatt Al-Arab River.

Sample	Hydrocarbon	Station				
		1	2	3	4	5
Water	Total hydrocarbons in dissolved water (μg l^{-1} dw)	6.95	9.62	2.43	4.09	1.74
	Total hydrocarbons in particulate water (μg l^{-1} dw)	8.53	10.74	2.94	5.84	2.89
	Total hydrocarbons	15.48	20.36	5.37	9.93	4.63
	Total n-alkanes in dissolved water (C_{14}-C_{33}) (μg l^{-1} dw)	3.94	5.23	1.86	3.34	1.94
	Total n-alkanes in particulate water (C_{14}-C_{33}) (μg l^{-1} dw)	4.35	6.82	2.34	3.92	2.77
	Total *n*-alkanes	8.29	12.05	4.20	7.26	4.71
	UCM in dissolved water (μg l^{-1} dw)	2.27	2.56	0.83	1.55	1.36
	UCM in particulate water (μg	3.03	3.59	1.47	1.98	1.88

	l^{-1} dw)					
	Total UCM	**5.30**	**6.15**	**2.30**	**3.53**	**3.24**
	Total 15 PAHs* in dissolved water (ng l^{-1} dw)	**2.48**	**3.64**	**0.74**	**1.79**	**0.99**
	Total 15 PAHs* in particulate water (ng l^{-1} dw)	**3.05**	**3.91**	**1.18**	**2.24**	**1.44**
	Total 15 PAHs	**5.53**	**7.55**	**1.92**	**4.03**	**2.43**
Bivalve	**Total hydrocarbons (µg g^{-1} dw)**	**24.54**	**28.73**	**10.22**	**18.59**	**11.08**
	Total n-alkanes (C_{14}-C_{33}) (µg g^{-1} dw)	**13.91**	**16.53**	**7.23**	**10.76**	**7.69**
	UCM (µg g^{-1} dw)	**3.27**	**2.81**	**1.72**	**2.98**	**2.53**
	Total of 15 PAHs* (ng g^{-1} dw)	**10.06**	**11.90**	**4.10**	**7.86**	**3.55**

*Naphthalene (Na), biphenyl (Bi), acenphtaylene (Ac), fluorine (Fl), phenanthrene (Ph), anthracene (An), fluoranthene (Flu), pyrene (Py), benzo (a) anthracene (Baa), chrysene (Ch), perylene (Pe), benzo (b) fluoranthene (Bbf), benzo (a) pyrene (Bap), indeno(1,2,3-cd) pyrene (Ip), and benzo (g,h,i) perylene (Bp).

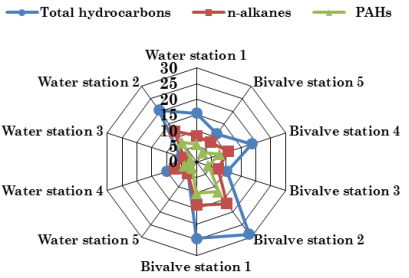

Fig 3: Concentrations of hydrocarbons in water and bivalve at study stations.

Table 5: Hydrocarbons concentrations in water of present study and other world water ecosystem.

Hydrocarbon	Concentration	Location	Reference
Total hydrocarbons	**1007 to 3274 µg l^{-1}**	**Gulf of Campay, India**	**Reddy et al. (2005a)**
Total hydrocarbons	**657 to 3540 µg l^{-1}**	**Gulf of Campay, India**	**Reddy *et al.* (2005b)**
PAHs	**336 to 1565 µg l^{-1}**		
Total hydrocarbons	**23.7 to 508 µg l^{-1}**	**Bohai Bay, China**	**Li *et al.* (2010)**
Total hydrocarbons	**28.8 to 271.3 µg l^{-1}**	**Black sea sector, Romanian**	**Ţigănuş *et al.* (2016)**
Total hydrocarbons	**0.01-12.55 µg l^{-1}**	**Koh Sichang-Sfiracha, Thailand**	**Wattayakorn and Rungsupa, (2012)**

PAHs	**28.6 to 190.3 µg l^{-1}**	**Tema Harbour, Ghana**	**Gorleku *et al.* (2014)**
PAHs	**6.3 to 26.3 µg l^{-1}**	**Coastal Belt, Ghana**	**Essumang, (2010)**
n-alkanes	**0.045 to 0.281 mg l^{-1}**	**Taihu Lake, China**	**Ji-xianga and Jiab (2012)**
PAHs	**0.011to 0.034 mg l^{-1}**		
Total hydrocarbons	**0.106 to 1.168 mg l^{-1}**	**Taihu Lake, China**	**Guo *et al.* (2012)**
PAHs	**0.011 to 0.034 mg l^{-1}**		
n-alkanes	**0.045 to 0.281 mg l^{-1}**		
PAHs	**7.51 mg l^{-1}**	**Douglas Creek, Nigeria**	**Eduok *et al.* (2010)**
Total hydrocarbons	**1.74 to 9.62 µg g_{-1}**	**Shatt Al-Arab river, Iraq**	**Present study**
n-alkanes	**1.86 to 5.23 µg g_{-1}**		
PAHs	**0.74 to 3.64 ng g_{-1}**		

Table 6: Hydrocarbons concentrations in bivalve of present study and other world water ecosystem.

Hydrocarbon	Concentration	Location	Reference
n-alkanes	**4.5 to 849.8 ng g^{-1} ww (1)**	**Mediterranean Coast, Egypt**	**El-Sikaily *et al.* (2002)**
PAHs	**1219 to 46741 ng g^{-1} ww(2)**		
n-alkanes	**771 to 33,202 ng g^{-1} dw(3)**	**Gulf of Naples, Italy**	**Amodio-Cocchieri and Cirillo (2003)**
n-alkanes	**309-1461 ng g^{-1} dw(4)**	**Guanabara bay, Brazil**	**Azevedo *et al.* (2004)**
PAHs	**5.79 to1125.88 ng g^{-1} dw(5)**	**Morocco**	**Azdi *et al.* (2006)**
Even n-alkanes	**66.57 to 2196.33 ng g^{-1} ww(6)**	**Galicia, Spain**	**Carro *et al.* (2006)**
Ood n-alkanes	**22.89 to 2901.68 ng g^{-1} ww(7)**		
Even n-alkanes	**142.77 to 9692.11 ng g^{-1} ww(8)**		
Ood n-alkanes	**174.72 to 7887.26 ng g^{-1} ww(9)**		
PAHs	**1000 to 7780 µg kg^{-1} dw(10)**	**Galician coast, Spain**	**Soriano *et al.* (2006)**
n-alkanes	**570 to 2 574 ng**	**Jiaozhou Bay,**	**Ma *et al.* (2009)**

	g^{-1} dw(11)	**China**	
PAHs	**276 to 939 ng g^{-1} dw(12)**		
PAHs	**11.96 mg kg^{-1} dw(14)**	**Douglas Creek, Nigeria**	**Eduok *et al.* (2010)**
n-alkanes	**1 to 236 µg g^{-1} dw(13)**	**Mediterranean Coasts, France**	**Bouzid *et al.* (2011)**
PAHs	**134 to 342 µg kg^{-1} dw(15)**	**Pacific Coast, Japan**	**Onozato *et al.* (2016)**
PAHs	**268 to 351 µg kg^{-1} dw(16)**		
PAHs	**289 to 450 µg kg^{-1} dw(17)**		
Total hydrocarbons	**10.22 to28.73 µg g^{-1} dw(18)**	**Shatt Al-Arab river, Iraq**	**Present study**
n-alkanes	**7.23 to16.53 µg g^{-1} dw(19)**		
PAHs	**3.55 to 11.90 µg g^{-1} dw(20)**		

1 and 2 = Modiolus auriculatus+ Donax sp.; 3, 6, 7, 10 and 16 = Mussels (*Mytilus galloprovincialis*); 4 and 5 = Mussels (*Perna perna*); 8 and 9 = Cockle (*Cerastoderma edule*); 11, 12 and 15 = Clam (*Ruditapes philippinarum*); 13 = Oyster (*Crassostrea tulipa*); 14 = Cockles(*Acanthocardia tuberculata*); 17 = Oyster (*Crassostrea gigas*); 18, 19 and 20 = Clam (*Corbicula fluminalis*)

The n-alkanes with carbon numbers ranging from C_{14} to C_{33} were detected in the water and bivalve samples. The n-alkanes distribution of water samples are characterized by two patterns. The first is in the range C_{14} to C_{22} with dominance of even carbon n-alkanes (C_{14}, C_{16}, C_{18} and C_{20}) and short chain odd carbon n-alkanes (C_{15}, C_{17} and C_{19}). The second is in the range C_{23} to C_{33} with dominance of long chain odd carbon n-alkanes (C_{23}, C_{25}, C_{27}, C_{29}, C_{31} and C_{33}). For bivalve samples, the n-alkanes showed a bimodal distribution. The first model consist of predominance of short chain n-alkanes (C_{19} to C_{22}), and the second one ranged from C_{27} to C_{31} carbon n-alkanes (Fig 4).

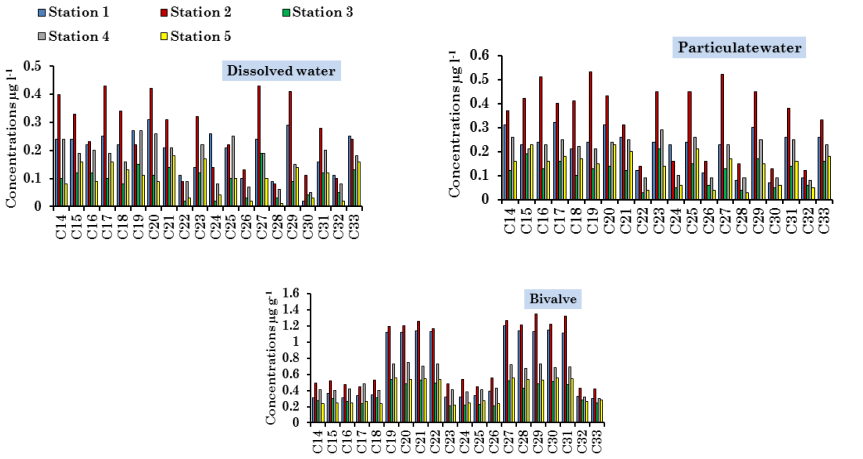

Fig 4. Chromatographic distribution of carbon chain lengths of the n-alkanes in water and bivalve of Shatt Al-Arab river.

The distribution of the individual PAHs in water and bivalve samples is reported in Fig (5). The PAHs appear divisible into two groups, the low molecular weight PAHs incorporating naphthalene, biphenyl, acenphtaylene, fluorene, phenanthrene and anthracene, and the high molecular weight including fluoranthene, pyrene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, perylene, benzo (a) pyrene, indeno (1,2,3-cd) pyrene and benzo (g,h,i) perylene. Fig (6) shows the percentages of individual PAHs to total PAHs in our samples, which suggests that there are no dominance of any PAH compound. The distribution patterns suggested that the n-alkanes and PAHs in Shatt Al-Arab river water and bivalve samples were from mixed sources.

The dominance of even carbon n-alkanes (C_{14}, C_{16}, C_{18} and C_{20}) indicates the contribution of aquatic microorganisms and petroleum inputs (Wang *et al.*, 2011), whereas the abundance of odd carbon n-alkanes (C_{15}, C_{17} and C_{19}) and (C_{23}, C_{25}, C_{27}, C_{29}, C_{31} and C_{33}) suggests the algal, phytoplankton, and terrestrial plants sources (Sonibare and Sojinu, 2009). The biogenic source is also confirmed by the presence of n-alkenes in

the range of C_{16} to C_{21} (Bouzid *et al.*, 2011). The predominance of C_{18}, C_{19} over C_{17} in some samples is often attributed to bacterial sources (Jeng and Huh, 2004).

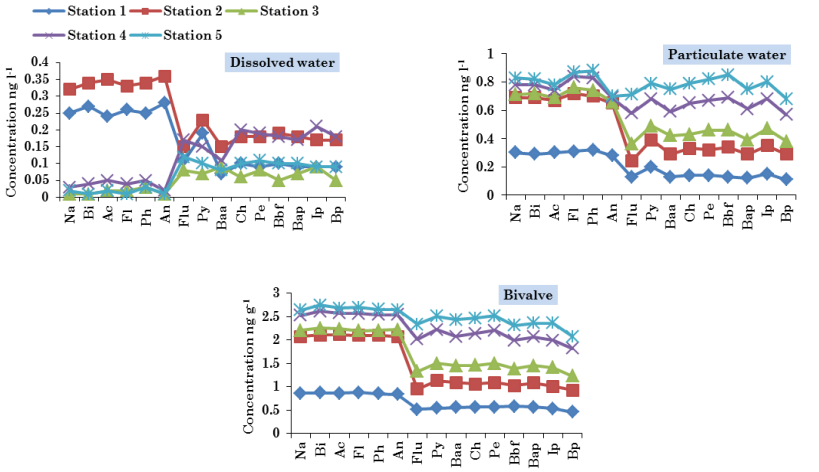

Fig 5. Distribution of PAHs in water and bivalve of Shatt Al-Arab river.

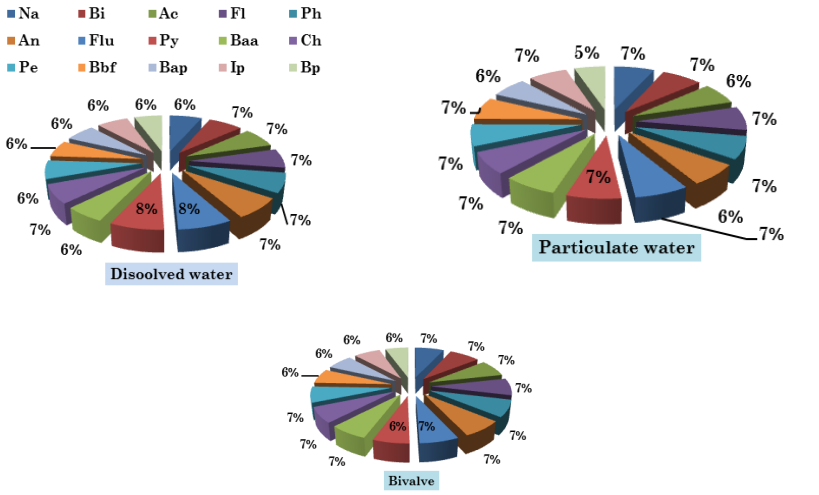

Fig 6: Percentages of individual PAHs in water and bivalve of Shatt Al-Arab river.

The presence of squalene in most bivalve samples supports the biogenic origin of n-alkenes in Shatt Al-Arab river (Bouzid *et al*., 2011) (Table 7). The pristane and phytane compounds are products of geological alteration of phytol and other isoprenoidyl natural products, and are not primary constituents of most terrestrial biota (Gao *et al*., 2007). However, pristane in the aquatic environment can be contributed by zooplankton and other higher aquatic animals while phytane is normal component of oil but also can be synthesized by the methanogenic and photosynthetic bacteria (Punyu *et al*., 2013). The pristane and phytane can also be produced from the phytyl side chain of chlorophyll *a* and *b* and therefore can indicate algal source (Peña *et al*., 2001). In our study, the pristane and phytane were detected in all water and bivalve samples (Table 7). The pristane/phytane ratio varied from 0.80 to 2.12 (dissolved water), 0.81 to 1.77 (particulate water), and 0.63 to 1.43 (bivalve). For uncontaminated site, pristane/phytane ratio varies from 3 to 5 whereas for petroleum contaminated sites the ratio is ≤ 1 (Punyu *et al*., 2013). The present values indicating petroleum inputs to the river. The C_{17}/ Pristane, C_{18}/ Phytane ratios are useful to identify the presence of freshly derived or degraded petroleum hydrocarbons (Harji *et al*., 2008). Low C_{17}/ Pristane and C_{18}/ Phytane ratios (<1) imply the presence of degraded petroleum hydrocarbons while higher ratios (>1) suggest the presence of less degraded or relatively fresh hydrocarbons. The C_{17}/ Pristane and C_{18}/ Phytane ratios varied from 0.52 to 2.38 and 0.72 to 1.62 (dissolved water), 1.00 to 2.77 and 1.05 to 1.81 (particulate water), and 0.71 to 1.26 and 0.64 to 1.20 (bivalve) respectively (Table 7), indicating the presence of degraded petroleum hydrocarbons in some stations of Shatt Al- Arab river and freshly derived petroleum hydrocarbons at others.

The CPI is a measure of homologous odd over even n-alkanes in a specified range of carbon numbers and is routinely used as a source indicator in waters and sediments. A CPI

value of 1 or near 1 indicates the presence of petroleum derived n-alkanes, whereas CPI values <1 indicates inputs from microorganisms including bacteria and diatioms (Ahad *et al*., 2011). A CPI value of 4 to 10 has been recorded for the terrestrial plants (Harji *et al*., 2008). The present CPI values varied from 1.34 to 2.59 (dissolved water), 1.45 to 2.00 (particulate water), and 0.50 to 1.12 (bivalve) (Table 7), suggesting the sources of petroleum and biogenic n-alkanes in Shatt Al Arab river.

The concentrations of UCM in water and bivalve samples ranged from 0.83 to 2.56 µg l^{-1} dw (dissolved water), 1.47 to 3.59 µg l^{-1} dw (particulate water), and 1.72 to 3.27 µg g^{-1} dw (bivalve) respectively (Table 4). The occurrence of the unresolved complex mixture (UCM) in most water and bivalve samples suggests a petrogenic pollution by weathered, degraded petroleum residues or bacterial degradation of natural organic inputs (Wang *et al*., 2011). The UCM is generally considered as a mixture of many structurally complex isomers and homologs of branched and cyclic hydrocarbons that cannot be resolved by capillary columns (Bouloubassi *et al*., 2001). The ratio of UCM/resolved *n*-alkanes (U/R) has been calculated for recent samples. U/R is used as a diagnostic criterion for petroleum pollution. Values of U/R > 2 suggest petroleum origin (Punyu *et al*., 2013). In our study, the U/R values varied from 1.45 to 3.87 (dissolved water), 1.27 to 3.53 (particulate water), and 1.78 to 4.65 (bivalve) (Table 7), which confirmed petroleum inputs in stations 1 and 2.

Typically, the low molecular weight PAHs (LMW PAHs) with 2 to 3 rings are from petrogenic source, while the high molecular weight PAHs (HMW PAHs)with 4 to 6 rings are from the pyrolysis of fossil fuels (Hu *et al*., 2015). Analysis of individual PAHs characteristics of recent water and bivalve samples showed the predominance of LMW PAHs in water and bivalve samples at stations 1 and 2 and HMW PAHs at stations 3, 4, and 5 (Fig 7). The presence of fluoranthene and pyrene in

water and bivalve samples indicates the importance of pyrolytic inputs (Zrafi *et al*., 2013). However, the presence of chrysene in all our samples indicates petroleum contamination input (Yang, 2000).

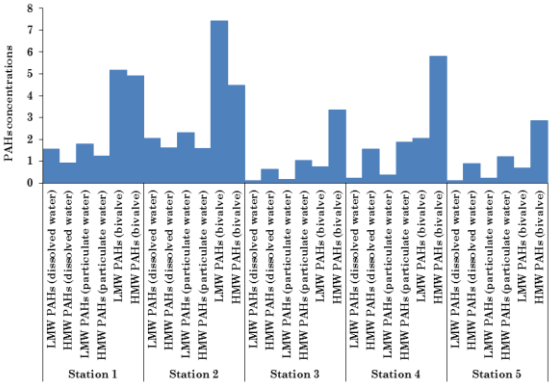

Fig 7: Composition profile of PAHs in water and bivalve of Shatt Al-Arab river.

In addition, some diagnostic ratios, such as phenanthrene / anthracene, fluoranthene / pyrene, anthracene / anthracene + phenanthrene, fluoranthene / fluoranthene + pyrene, and benzo(a)anthracene / benzo(a)anthracene+chrysene were used to distinct between the possible sources of PAHs. Phenanthrene/anthracene ratio less than 3 refers to the origin of pyrolytic and it shows the origin of petrogenic when more than 3. fluoranthene/pyrene ratio < 1 is characteristic of the source of petrogenic and > 1 characterize the source of pyrolytic. anthracene/anthracene+phenanthrene ratio < 0.1 are observed in the petroleum inputs or diagenesis sources, while the values > 0.1 are characteristic of combustion processes. fluoranthene/fluoranthene+pyrene ratio < 0.4 means the source of petrogenic as a property of fuel combustion (gasoline, diesel and oil crude), 0.4 to 0.5 means the combustion of petroleum, and > 0.5 means the combustion of coal, wood, kerosene, terrestrial plants and biomass (pyrolytic). and benzo (a) anthracene/benzo (a) anthracene+ chrysene ratio < 0.20 indicates petroleum input, 0.20 to 0.35 petroleum and oil

combustion, and > 0.35 combustion (Yang, 2000; Wang *et al*., 2011; Long *et al*., 2011; Zrafi *et al*., 2013; Tay and Biney 2013). The present ratios values suggest that the PAHs in Shatt Al-Arab river water and bivalve samples have originated from at least three different sources; pyrolytic, petrogenic, and biogenic (Table 7).

Many of petroleum hydrocarbons are toxic to aquatic life and wildlife and have the ability to accumulate and transfer through the food chain (Ma et al., 2009). Highly toxic petroleum can damage the systems of human body and consequently cause a wide range of diseases and disorders. They could negatively impact the biodiversity and cause the loss of ecosystems stability. The petroleum hydrocarbons could influence and negatively impact the biodiversity and densities of the biota and cause the loss of stability of ecosystems (NRC, 2003).

Table 7: Pristane, phytane, n-alkanes odd and even carbon numbers, total LMW and HMW PAHs, and n-alkanes and PAHs diagnostic ratios of water and bivalve of Shatt Al Arab river.

Sample	Hydrocarbon index	Station				
		1	2	3	4	5
Dissolved water	Pristane	0.16	0.18	0.19	0.08	0.17
	Phytane	0.18	0.21	0.11	0.10	0.08
	Pristane/Phytane	0.88	0.85	1.72	0.80	2.12
	C_{17}/ Pristane	1.56	2.38	0.52	2.00	0.94
	C_{18}/ Phytane	1.22	1.61	0.72	1.60	1.62
	Odd carbon numbers of n-alkanes	2.26	3.19	1.26	2.05	1.40
	Even carbon numbers of n-alkanes	1.68	2.04	0.60	1.29	0.54
	CPI	1.34	1.57	2.10	1.58	2.59
	U/R	3.87	3.65	1.45	1.98	1.55
	Total LMW PAHs	1.55	2.04	0.10	0.23	0.10
	Total HMW PAHs	0.93	1.60	0.64	1.56	0.89
	Phenanthrene/Anthracene	0.89	0.94	3.00	2.50	3.00
	Fluoranthene/Pyrene	0.57	0.65	1.14	1.13	1.20
	Anthracene/Anthracene+Phenanthrene	0.52	0.51	0.25	0.28	0.25
	Fluoranthene/Fluoranthene+Pyrene	0.36	0.39	0.53	0.53	0.54
	Benzo(a)anthracene/Benzo(a)anthracene+Chrysene	0.41	0.45	0.60	0.35	0.44
Particulate water	Pristane	0.19	0.23	0.16	0.09	0.17
	Phytane	0.20	0.25	0.09	0.11	0.12
	Pristane/Phytane	0.95	0.92	1.77	0.81	1.41
	C_{17}/ Pristane	1.68	1.73	1.00	2.77	1.05
	C_{18}/ Phytane	1.05	1.64	1.11	1.81	1.41
	Odd carbon numbers of n-alkanes	2.58	4.24	1.56	2.43	1.77
	Even carbon numbers of n-alkanes	1.77	2.58	0.78	1.49	1.00
	CPI	1.45	1.64	2.00	1.63	1.77
	U/R	2.78	3.53	1.43	1.88	1.27
	Total LMW PAHs	1.80	2.32	0.16	0.38	0.22
	Total HMW PAHs	1.25	1.59	1.02	1.86	1.22

	Phenanthrene/Anthracene	**1.14**	**1.02**	**4.00**	**3.00**	**5.00**
	Fluoranthene/Pyrene	**0.65**	**0.57**	**1.20**	**1.15**	**1.18**
	Anthracene/Anthracene+Phenanthrene	**0.46**	**0.49**	**0.20**	**0.25**	**0.16**
	Fluoranthene/Fluoranthene+Pyrene	**0.27**	**0.36**	**0.54**	**0.53**	**0.54**
	Benzo(a)anthracene/Benzo(a)anthracene+Chrysene	**0.48**	**0.45**	**0.56**	**0.43**	**0.53**
Bivalve	**Pristane**	**0.43**	**0.63**	**0.19**	**0.49**	**0.34**
	Phytane	**0.38**	**0.44**	**0.30**	**0.56**	**0.37**
	Pristane/Phytane	**1.13**	**1.43**	**0.63**	**0.87**	**0.91**
	C_{17}/ Pristane	**0.79**	**0.71**	**1.26**	**0.97**	**0.76**
	C_{18}/ Phytane	**0.92**	**1.20**	**1.03**	**0.71**	**0.64**
	Squalene	**0.35**	**0.42**	**0.52**	**0.34**	**0.44**
	Odd carbon numbers of n-alkanes	**7.36**	**8.71**	**3.77**	**5.57**	**4.03**
	Even carbon numbers of n-alkanes	**6.55**	**7.82**	**7.46**	**5.19**	**3.66**
	CPI	**1.12**	**1.11**	**0.50**	**1.07**	**1.10**
	U/R	**4.65**	**2.63**	**1.98**	**1.90**	**1.78**
	Total LMW PAHs	**5.16**	**7.43**	**0.75**	**2.04**	**0.70**
	Total HMW PAHs	**4.90**	**4.47**	**3.35**	**5.82**	**2.85**
	Phenanthrene/Anthracene	**1.01**	**1.00**	**0.78**	**1.03**	**1.09**
	Fluoranthene/Pyrene	**0.96**	**0.72**	**1.02**	**0.95**	**1.10**
	Anthracene/Anthracene+Phenanthrene	**0.49**	**0.49**	**0.56**	**0.49**	**0.47**
	Fluoranthene/Fluoranthene+Pyrene	**0.49**	**0.42**	**0.50**	**0.48**	**0.52**
	Benzo(a)anthracene/Benzo(a)anthracene+Chrysene	**0.49**	**0.51**	**0.54**	**0.48**	**0.52**

Susceptible human population in the Shatt Al-Arab river would be exposed to petroleum hydrocarbons primarily through the drinking of river water and the consumption of aquatic resources that are essentially an integral component of every diet of the people in this region. In Shatt Al-Arab river much of the petroleum hydrocarbons are released into the atmosphere from fuel flaring and eventually reaches the water by direct deposition in addition to inputs from sewage, routine tanker and shipping operations, electrical generating stations, oil refinery plants, transportation activities, and rural and urban run-off. Higher incidences of cancer and respiratory and upper gastrointestinal tract tumors were associated with exposures to these compounds. This research finding has strong public health implications although there are no records or data of body burden reflecting exposures of the populace to petroleum hydrocarbons or other bioaccumulative or toxic compounds in the Shatt Al-Arab river. Apart from microbial causes, gastrointestinal distress prevalent in the river area may not be unconnected with direct uptake of petroleum hydrocarbons through drinking of river water and the consumption of river aquatic resources. The surface water of many parts of Shatt Al

Arab river is darkened with an oily sheen as a result of discharges from oil installations and other sources which could have contributed to the accelerated mortality of the aquatic plants and animals. Several studies have indicated that bivalve mollusks and some other invertebrates are unable to efficiently metabolize petroleum hydrocarbons and excrete them presumably due to inefficient or missing mixed function oxidase systems. The bioconcentration factors of individual n-alkanes and PAHs in the bivalve ranged from 0.71 (C_{23}) to 6.33 (C_{30}) and from 1.48 (pyrene) to 1.84 (biphenyl) respectively (Table 8).

Table 8: Concentrations of individual n- alkanes and PAHs hydrocarbons in water and bivalve samples and their bioconcentration factor

n-alkanes	Concentration		Bf	PAHs	Concentration		Bf
	Water (µg l^{-1})	Bivalve (µg g^{-1} dw)			Water (µg l^{-1})	Bivalve (µg g^{-1} dw)	
C_{14}	2.28	1.72	0.75	Na	1.44	2.64	1.83
C_{15}	2.32	1.83	0.78	Bi	1.49	2.75	1.84
C_{16}	2.13	1.71	0.80	Ac	1.46	2.68	1.83
C_{17}	2.44	1.77	0.72	Fl	1.52	2.70	1.77
C_{18}	2.04	1.83	0.89	Ph	1.49	2.66	1.78
C_{19}	2.28	4.14	1.81	An	1.49	2.65	1.77
C_{20}	2.54	4.09	1.61	Flu	1.55	2.50	1.61
C_{21}	2.19	4.18	1.9	Py	1.56	2.32	1.48
C_{22}	0.76	4.06	5.34	Baa	1.36	2.44	1.79
C_{23}	2.30	1.64	0.71	Ch	1.44	2.47	1.71
C_{24}	1.14	1.71	1.5	Pe	1.41	2.52	1.78
C_{25}	2.19	1.70	0.77	Bbf	1.38	2.32	1.68
C_{26}	0.81	1.83	2.25	Bap	1.32	2.36	1.78
C_{27}	2.43	4.27	1.75	Ip	1.39	2.38	1.71
C_{28}	0.66	3.99	6.04	Bp	1.16	2.08	1.79
C_{29}	2.40	4.22	1.75				
C_{30}	0.65	4.12	6.33				
C_{31}	2.07	4.14	2.00				
C_{32}	0.76	1.62	2.12				
C_{33}	2.12	1.55	0.73				

CONCLUSIONS

Mankind through socio-economic activities introduces many pollutants such as the hydrocarbons and microbial contaminants to aquatic environment of Shatt Al-Arab river

where prohibitions existing on documents of regulatory agencies are poorly enforced. Petroleum and microbial pollution problems in water of Shatt Al-Arab river area appear to arise from intermittent discharges to the river area of crude oil and its refined products, ballast water and land-based wastewaters. Bivalves acts as pollutants accumulator and provide an integrated picture of the events taking place in the water column; hence they are an extremely good indicator of the magnitude of environmental contamination. The recent study presents a baseline distribution assessment of hydrocarbons and bacteria in the water and bivalve of Shatt Al-Arab river area; from the results of this work, it is evident that all sites are contaminated to some extent with petroleum hydrocarbons and bacteria. The overall levels of hydrocarbons in the water and bivalve of Shatt Al-Arab river when compared to selected aquatic areas elsewhere, revealed a moderate level of hydrocarbons pollution. However, owing to hydrocarbons bioaccumulative potential and toxicity to both aquatic organisms and human consumption of seafood, as well as impacting the composition and diversity of in faunal communities, periodical monitoring and assessment of water and tissues of various biota of Shatt Al-Arab river water is still necessary.

ACKNOWLEDGEMENT

The author wish to acknowledge the Marine Science Center, University of Basrah for providing the laboratory facilities.

REFRENCES

1. Abdullah, E.J. (2013). Quality Assessment for Shatt Al-Arab River Using Heavy Metal Pollution Index and Metal

Index. Journal of Environment and Earth Science, 3 (5): 141-120.

2. Ahad, J. M. E.; Ganeshram, R. S.; Bryant, C. L.; Cisneros-Dozale, L. M.; Ascough, P. L.; Fallick, A. E. and Slater, G. L. (2011). Sources of *n*-alkanes in an urbanized estuary: Insights from molecular distributions and compound specific stable and radiocarbon isotopes; Marine Chemistry, 126: 1-4.
3. Ali, W.A.; Farid, W. A. and Al-Eed, A. A. (2013). Hydrocarbons distribution in Shatt Al-Arab River bacteria and fungi. International Journal of Science and Modern Engineering (IJISME), 1 (12): 5-9.
4. Al-Saad , H. T. and A. A. Z. DouAbul (1984). Hydrocarbons concentration in the mussel *Corbicula fluminalis* from Shatt Al–Arab river. In Fate and fluxes of oil pollutants in the Arabian Gulf (A. A. Z. DouAbul and H. M. Bedair, eds.) Basrah University Press, 291-303.
5. Amodio-Cocchieri, R. and Cirillo, T. (2003). Aliphatic hydrocarbons in biota from the Gulf of Naples (Italy). Marine Pollution Bulletin, 46 (3): 362-377.
6. Ashbolt, N. J.; Grabow, O. K.; Snozzi, M. (2001). Indicators of microbial water quality. In Water quality: Guidelines, standards and health; Fewtrell, L. and Bartram, J., eds., World Health Organization (WHO), IWA Publishing: London, UK, pp. 289-316.
7. Azdi, M.; Moukrim, A.; Burgeot, T.; Budzinski, H.; Chiffoleau, J. F.; Kaaya A.; Zekhnini, A.; Narbonne, J. F. and Guarrigues, Ph. (2006). Hydrocarbon pollution along Moroccan coast and BPH activity in the mussel *perna perna*. Polycyclic Aromatic Compounds, 26: 265-282.
8. Azevedo, L. A.; De Andrade Brüning, I. M. R. and Moreira, I. (2004). Hydrocarbon contamination in mussels from Guanabara Bay, Marine Pollution Bulletin, 49 (11-12): 1109-1126.

9. Bouloubassi, I.; Fillaux, J. and Saliot, A. (2001). Hydrocarbons in surface sediment from the Changjiang (Yangtze River) estuary, east China Sea. Marine Pollution Bulletin, 42 (12): 1335-1346.
10. Bouzid, S.; Khannous, S.; Bouloubassi, I.; Saliot, A. and Er Raioui, H. (2011). Assessment of the Moroccan Mediterranean coasts contamination by hydrocarbons (Non aromatic hydrocarbons, aromatic hydrocarbons and linear alkylbenzenes). International Journal of Geosciences, 2: 562-572.
11. Carro, N.; Cobas, J. and Maneiro, J. (2006). Distribution of aliphatic compounds in bivalve mollusks from Galicia after the Prestige oil spill: Spatial and temporal trends. Environmental Research, 100 :339-348.
12. Cowan, S. T., and Steel, K. J. (eds.) (1975). Manual for the identification of medical bacteria. 2nd, Cambridge University Press, Cambridge, London, 124 p.
13. Cripps, G. C. (1989). The extraction and analysis of hydrocarbons in marine samples. British Antarctic, Cambridge, 18 p.
14. Eduok, S. I.; Ebong,, G. A.; Udoinyang, E. P.; Njoku, J. N. and Eyen, E. A. (2010). Bacteriological and polycyclic aromatic hydrocarbon accumulation in mangrove oyster (*Crassostrea tulipa*) from Douglas Creek, Nigeria. Pakistan Journal of Nutrition, 9 (1): 35-42.
15. El-Sikaily, A.; Khaled, A.; El-Nemr, A.; Said, T. O. and Abd-Allah, A. M. A. (2002). Determination of hydrocarbons in bivalves from the Egyptian Mediterranean Coast. Mediterranean Marine Science, 3/2: 123-131.
16. Essumang, D. K. (2010). Distribution, levels, and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in some water bodies along the Coastal Belt of Ghana. The Scientific World Journal, 10: 972-985.
17. Farid, W. A.; Al-Eed, A. A.; Shihab, L. A. and Al-Saad, H. T. (2014). Distribution, sources, and seasonal variations of

hydrocarbons in Shatt Al-Arab River. Journal of International Academic Research for Multidisciplinary, 2 (2): 729-740.

18. Farid, W. A.; Al–Saad, H. T. and Al–Adhub, A. Y. (2008). Monitoring of hydrocarbons in Shatt Al-Arab River by using some species of mollusks. Marina Mesopotamica, 23 (1): 305-319.
19. Gao, X.; Chen, S.; Xie, X.; Long, A. and Ma, F. (2007). Non-aromatic hydrocarbons in surface sediments near the Pearl River estuary in the South China Sea. Environmental Pollution, 148 40-47.
20. Gorleku, M. A.; Carboo, D.; Palm, L. M. N; Quasie, W. J., Armah A. K. (2014). Polycyclic aromatic hydrocarbons (PAHs) pollution in marine waters and sediments at the Tema Harbour, Ghana. Academy Journal of Environmental Sciences, 2 (7): 108-115.
21. Grimalt, J.O. and Oliver, J. (1993). Source input elucidation in aquatic systems by factor and principal component analysis of molecular marker data. Analytica Chimica Acta, 278: 159-176.
22. Hamza-Chaffai, A. (2014). Usefulness of Bioindicators and Biomarkers in Pollution Biomonitoring. International Journal of Biotechnology for Wellness Industries, 3: 19-26.
23. Harji, R. R.; Yvenat, A. and Bhosle, N. B. (2008). Sources of hydrocarbons in sediments of the Mandovi estuary and the Marmugoa harbour, west coast of India. Environment International, 34: 959-965.
24. Holt, J. G.; Krieg, N. R.; Sneath, P. H.; Staley, J. T. and Williams, S. T. (eds.) (1994). Bergeys manual of determinative bacteriology. 9th ed. Williams and Wilkins, Baltimore, 787 p.
25. Hu, G.; Chen, S.; Shi, W.; Zhang, B.; Zhang, Y.; Huang, J.; Chen, J., Giesy, J. P. and Yu H. (2015). Identification of polycyclic aromatic hydrocarbons in soils in Taizhou, East

China. Environmental Geochemistry and Health, 37: 429-439.

26. Jalal, K. C. A.; Najiah, M.; Fathiyah, M.; Kamaruzzaman, Y.; Nor Omar, M.; Nurul Amin, S. M. and Jaswir, I., (2009). Bacterial pollution in molluscs arch clam, *Orbicularia orbiculata* and blood cockle, *Anadara granosa* of Pahang Estuary, Malaysia. Journal of Biological Sciences, 9: 841-850.
27. Jeng, W. L. and Huh, C. A. (2004). Lipids in suspended matter and sediments from the east China Sea shelf. Organic Geochemistry, 35 (5): 647-660.
28. Ji-xianga, G. and Jiab, F. (2012). The Distribution of n-alkanes and polycyclic aromatic hydrocarbons in water of Taihu Lake. Procedia Environmental Sciences, 12: 258–264.
29. João, P. S. and Cabral, J. P. S. (2010). Water microbiology. Bacterial pathogens and water, International Journal of Environmental Research and Public Health, 7: 3657-3703.
30. Kueh C. S. and Chan, K. Y. (1985). Bacteria in bivalve shellfish with special reference to the oyster. Journal of Applied Microbiology, 59 (1): 41-7.
31. Li,Y.; Zhao, Y.; Peng, S.; Zhou, Q.; Lena and Maa, Q. (2010). Temporal and spatial trends of total petroleum hydrocarbons in the seawater of Bohai Bay, China from 1996 to 2005, Marine Pollution Bulletin, 60: 238-243.
32. Long, Y.; Chi, G.; Qing, H.; Dai1, T.; Wu, Q. (2011). Sources of polycyclic aromatic hydrocarbons in street dust from the Chang-Zhu-Tan region, Hunan, China. Journal of Environmental Protection, 2: 1331-1340.
33. Ma, H.; Song, Q. and Wang, X. (2009). Accumulation of petroleum hydrocarbons and heavy metals in clams (*Ruditapes philippinarum*) in Jiaozhou Bay, China. Chinese Journal of Oceanology and Limnology, 27: 887.
34. Martinez, D. I. and de Oliveira, A. J. C. (2010). Faecal bacteria in *Perna perna* (Linnaeus, 1758) (Mollusca:

Bivalvia) for biomonitoring coastal water and seafood quality. Brazilian Journal of oceanography, 58 (special issue IVSBO): 29-35.

35. Miller, W. A.; Atwill, E. R.; Gardner, I. A.; Miller, M. A.; Fritz, H. M.; Hedrick, R. H.; Melli, A. C.; Barnes, N. M. and Conrad, P. A. (2005). Clams (*Corbicula fluminea*) as bioindicators of fecal contamination with *Cryptosporidium* and *Giardia* spp. in freshwater ecosystems in California. International Journal for Parasitology, 35: 673-684.
36. MRC (2014). Multi-media (water, sediment, biota) monitoring and assessment report. MRC Technical Paper No. 41. Mekong River Commission, Vientiane, 134 pp.
37. Novotny, L.; Dvorska, L.; Lorencova, A.; Beran, V. and Pavlik, I. (2004). Fish: a potential source of bacterial pathogens for human beings. Veterinary Medicine–Czech, 49 (9): 343-358.
38. NRC (National Research Council) (2003). Oil in the sea III. Input, fates and effects. Washington. D. C., National Academic Press, 265pp.
39. Olaolu, T. D.; Akpor, O. B. and Akor, C. O. (2014). Pollution indicators and pathogenic microorganisms in wastewater treatment: Implication on receiving water bodies. International Journal of Environmental Protection and Policy, 2(6): 205-212.
40. Onozato, M.; Nishigaki, A. and Okoshi, K. (2016). Polycyclic Aromatic Hydrocarbons in Sediments and Bivalves on the Pacific Coast of Japan: Influence of Tsunami and Fire. PLoS ONE, 11(5): 1-13.
41. Ouattara, N.K.; Passerat, J. and Servai, P. (2011). Faecal contamination of water and sediment in the rivers of the Scheldt drainage network. Environmental Monitoring and Assessment, 183(1): 243-257.
42. Pandey, P. K.; Kass, P. H.; Soupir, M. L; Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. AMB Express, 4: 51.

43. Peña, E.; Astroga-España, Ma. S. and Montelongo, G. (2001). Chemical fingerpringting applied to the evaluation of marine oil pollution in the coasts of the Canary Islands (Spain). Environmental Pollution, 111(2): 117-187.
44. Punyu, V. R.; Harji, R. R.; Bhosle, N. B.; Sawant, S. S. and Venkat, K. (2013). n-Alkanes in surficial sediments of Visakhapatnam harbour, east coast of India. Journal of Earth System Science, 122 (2): 467–477.
45. Reddy, M. S.; Basha, S.; Joshi, H. V. and Ramachandraiah, G. (2005a). Seasonal distribution and concentration levels of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons in marine environment at Alsng[-]Sosiya ship scrapping yard, Gulf of Cambay, India. International Oil Spill Conference Proceedings, May 2005, 2005 (1): 149-160.
46. Reddy, M. S.; Basha, S.; Joshi, H. V. and Ramachandraiah, G. (2005b). Seasonal distribution and contamination levels of total PHCs, PAHs and heavy metals in coastal waters of the Alang–Sosiya ship scrapping yard, Gulf of Cambay, India Chemosphere 61 1587-1593.
47. Rippey, S. R. (1994). Infectious diseases associated with molluscan shellfish consumption. Clinical Microbiology Reviews, 7 (4): 419-25.
48. Rompre´, A.; Servais, P.; Baudart, J.; de-Roubin, M. and Laurent, P. (2002). Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. Journal of Microbiological Methods, 49 : 31-54.
49. Shi, H.; Zhang, L.; Yue, L. and Zheng, G. (2008). Petroleum hydrocarbon contamination in surface sediments of Beiluohe Basins, China. Bulletin of Environmental Contamination and Toxicology, 81: 416-421.
50. Sonibare, O. O. and Sojinu, O. S. (2009). Chemical composition of leaf lipids of angiosperms: Origin of land plant-derived hydrocarbons in sediments and fossil fuel. European Journal of Scientific Research, 25: 192-199.

51. Soriano, J. A.; Viñas, L.; Franco, M. A.; González, J. J.; Ortiz, L. ;Bayona, J. M. and Albaigés J. (2006). Spatial and temporal trends of petroleum hydrocarbons in wild mussels from the Galician coast (NW Spain) affected by the Prestige oil spill. Science of the Total Environment, 370: 80-90.
52. Sousa, R.; Antunes, C. and Guilhermino, L. (2008). Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. Annales de Limnologie-International Journal of Limnology, 44 (2): 85-94.
53. Stellio, C. and Cédric, B. (2006). Modelling trace metal (Hg and Pb) bioaccumulation in the Mediterranean mussel, *Mytilus galloprovincialis*, applied to environmental monitoring. Journal of Sea Research, 56 (2): 168-81.
54. Tay, C. K. and Biney, C.A. (2013). Levels and sources of polycyclic aromatic hydrocarbons (PAHs) in selected irrigated urban agricultural soils in Accra, Ghana. Environmental Earth Sciences, 68: 1773–1782.
55. Ţigănuş, D.; Coatu, V.; Lazăr, L. and Oros, A. (2016). Present level of petroleum hydrocarbons in sea water associated with offshore exploration activities from the Romanian Black Sea Sector. Cercetări Marine, 46: 98-108.
56. Vega Corrales, L.A. ; Marín Vindas, C.; Pacheco Prieto, O. and Zúñiga Calero, G. (2013). Preliminary assessment of small scale bacterial depuration of *Crassostrea gigas* and *Anadara* spp., Gulf of Nicoya, Costa Rica, Rev. Mar. Cost., 5: 107-117.
57. Wang, C.; Wang, W.; He S.; Due, J. and Suna, Z. (2011). Sources and distribution of aliphatic and polycyclic aromatic hydrocarbons in Yellow River Delta Nature Reserve, China. Applied Geochemistry, 26: 1330-1336.
58. Wattayakorn, G. and Rungsupa, S. (2012). Petroleum hydrocarbon residues in the marine environment of Koh Sichang-Sriracha. Thailand, Costal Marine Science, 35 (1): 122-128.

59. Wilson, M. (2005). Microbial inhabitants of humans. Their ecology and role in health and disease; Cambridge University Press: Cambridge, UK.
60. Yang, G. (2000). Polycyclic aromatic hydrocarbons in the sediments of the South China Sea. Environmental Pollution, 108: 163-171.
61. Zrafi, I.; Hizem, L.; Chalghmi, H.; Ghrabi, A.; Rouabhia, M.; Saidane-Mosbahi, D. (2013). Aliphatic and aromatic biomarkers for petroleum hydrocarbon investigation in marine sediment. Journal of Petroleum Science Research, 2 (4): 145-155.