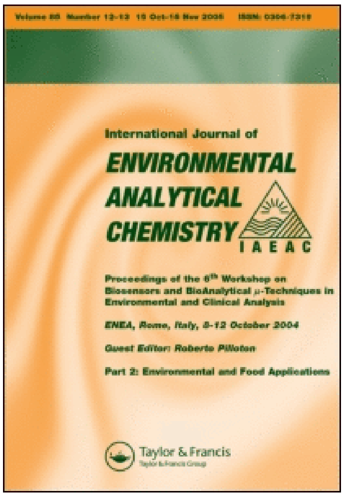

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The Effect of Temperature on the Composition and Relative Toxicity of the Water-Soluble Fraction of Kuwait Crude Oil (Export) in the Seawater

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THE EFFECT OF TEMPERATURE ON THE COMPOSITION AND RELATIVE TOXICITY OF THE WATER-SOLUBLE FRACTION OF KUWAIT CRUDE OIL (EXPORT) IN THE SEAWATER

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The effect of temperature on the composition of the water-soluble fraction (WSF) of Kuwait crude oil was investigated. The results showed that the WSF consisted of mostly monoaromatic (BTEX) compounds. The total concentration of volatiles in the WSF was about 7.5 mg/l. The increase in the temperature from 15°C to 25°C caused an increase of about 10% and to 35°C did not significantly affect the total concentration. GC-MS analysis of the concentrated extract of the WSF resulted in the identification of nine PAHs and six methylated PAHs. The total concentration of the PAHs ranged from 0.217 mg/l to 0.634 mg/l. The PAHs increased significantly with increasing temperature.

Naphthalene and methylated naphthalenes constituted about 95% of the total PAHs. Microtox assay of the WSF showed that the relative toxicity increased when the temperature was increased from 15° to 25°C. Increasing temperature further to 35°C did not effect the relative toxicity.

Keywords: Kuwait crude; water-soluble fraction; composition; temperature; relative toxicity

INTRODUCTION

Arabian Gulf is one of the most important bodies of water as far as the production and transport of crude oil and refined petroleum products are concerned. The countries bordering the Arabian Gulf supply about 40% of the world oil supply. About 60% of all the crude oil shipped worldwide passes through the gulf[1]. In addition, most of the countries of the region are also exploiting the offshore oil reservoirs. It is, therefore, not surprising that the Arabian Gulf is considered one of the most polluted bodies of water[2]. Literathy and Foda[3] estimated that the

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northern part of the Arabian Gulf alone receives about two million barrels of oil and related petroleum products each year. In addition to this large input, there have been large inputs, occurring somewhat regularly (oil-tanker wrecks, offshore well blowouts and oil well fire fallout). Nowruz oil spill (1983) and Gulf War oil spill (1991) released millions of barrels of oil into the Gulf. The impact of these stresses on the marine ecosystem of the Arabian Gulf has been the subject of many studies[4,5].

Petroleum oil (crude oil and refined petroleum products) spilled in marine environment undergo many diverse processes (evaporation, dissolution, photolysis, oxidation, adsorption on to particulates, sedimentation, biodegradation and uptake by biota) which collectively determine the fate of the oil and its impact on the environment. However, the dissolution is of special importance and significance especially from toxicity point of view. The amount of oil that dissolves in water is generally small (less than 1%), it is this fraction that contacts and impacts the marine organisms and is, therefore, an important determinant of the oil toxicity. Dissolution of crude oils has been studied extensively[6-10]. The composition of the WSF depended on the composition of the oil, temperature, water salinity and on the ratio of the volumes of water and oil that are brought into contact[11]. The toxicity of WSF was primarily due to the compounds that dissolved from the oil into the aqueous phase. Studies indicated that the water-soluble fraction (WSF) of crude oils was a complex mixture ranging from pentane to polycyclic aromatic hydrocarbons, phenols and nitrogen- and sulfur-containing heterocyclic compounds. Aromatics are the main class of hydrocarbons found in WSF. Carls and Rice[12] reported that total mononuclear aromatics constitute about 89% of the total WSF. BTEX (benzene, toluene, ethylbenzene and xylenes) represent 87.6% of the WSF.

The composition of the WSF of Kuwait crude oil has been the subject of many studies. Anderson et al.[6] described the chemical composition. Recently, Ali et al.[13] reported on the same subject. Both of these studies used the so-called "Standard Kuwait Crude Oil" and water temperature of about 20°C. Anderson et al.[6] correctly pointed out that all oils bearing the same name do not necessarily have identical chemical composition.

Secondly, the temperature and the salinity used in the preparation did not reflect the actual values in the northern Gulf. The objective of the present study was to study the chemical composition of the WSF of Kuwait crude oil (export) under the local conditions (temperatures and salinity) and assess the changes in the chemical composition of the WSF due to different seasonal temperatures of the seawater. The effect of changes in the composition of the WSF on the relative toxicity (by using Microtox assay) was also assessed.

MATERIALS AND METHODS

Authentic sample of crude oil (export) was obtained from Kuwait Oil Company (KOC). The specific gravity of the oil was 0.897. The oil was kept in an airtight container and stored in a freezer at −20°C. The seawater used in the preparation was from an offshore intake well. The water was filtered through a series of filters to remove any biota and particulate matter and tested for the presence of any organic compounds.

Preparation of water-soluble fraction

The setup used for the preparation of WSF was that proposed by Ali et al.[14]. Figure 1 shows the schematic representation of the set up. It consisted of a 2-liter bottle that was kept in a water bath. The temperature of the bath was controlled by circulated water from a water-chiller/heater circulator. The temperature of the water-bath was monitored by a thermometer. The circulator controlled the set temperature to ±0.1°C. The water level in the bath was adjusted so that water and oil layers in the bottle were below the water level of the bath. The bath was placed on a magnetic stirrer. A Teflon-coated stirring bar (2 cm long) was placed in the bottle. The stirring speed was adjusted so as not to form a large vortex. The oil sample was introduced slowly on top of the water layer through a glass tube with the help of a syringe. The headspace was purged with nitrogen through another tube to expel air. WSF sample was collected by applying a slight nitrogen pressure that caused the WSF to rise in a glass delivery tube. The stirring was continued for 24 hr. after which the WSF was removed immediately for analysis or extraction.

Preparation of concentrated extract for GC-MS analysis

About 1.5 liter of the water soluble fraction was taken in a separatory funnel and extracted three times with 10 ml portions of 3:7 mixture of hexane: dichloromethane. The combined extracts were dried over anhydrous sodium sulfate, filtered and concentrated on a rotary evaporator to about 5 ml and then under a stream of nitrogen to dryness. The residue was taken up in 100 µl of dichloromethane.

Purge and Trap-gas chromatographic analysis

The volatile compounds in the WSF were analyzed by purge & trap-GC using flame ionization detector. Varian-3600 GC was linked to a Tekmar-3000 Purge and Trap Concentrator. A Tekmar cryofocusing module was employed to refocus

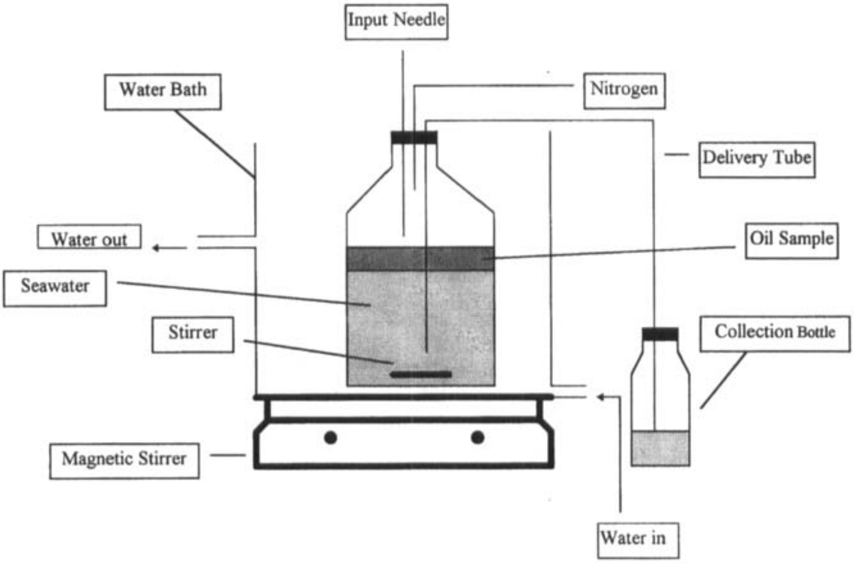

FIGURE 1 Experimental setup for the preparation of water-soluble fraction

the volatile desorbed from the trap. Data was acquired and reprocessed by Varian Star Chromatography Software. Sample of the WSF (3 ml) was introduced into the purging vessel with a Luer-lock syringe. A preset method (equivalent to EPA Method-502) was used for purging and trapping the volatiles. Briefly, the sample was purged for 11 min with helium. The purged volatile compounds were trapped on a Tenax trap. The trap was heated to 225°C to desorb and kept at the same temperature for 2 min during desorption. The desorbed compounds were refocused at the head of analytical column at −150°C after which the cryofocusing interface was heated to 180°C and the compounds introduced into the GC.

The conditions for gas chromatographic analysis were as follows: Fused silica capillary column, coated with SPB-624 (Supleco), 1.4 µm film thickness, 30 m long and 0.25 mm internal diameter. Helium was used as carrier gas at 2 ml/min. Initial column temperature was 40°C, maintained for 5 min and then programmed to 220°C at 4°/min. The final temperature was held for 10 min. Flame ionization detector used for detection. An external standard method was employed for quantitation.

P&T-GC-MS analysis

The identity of the peaks were ascertained by linking P&T-GC with a mass spectrometer. Tekmar-3000 purge and trap concentrator was linked to Shimadzu

GC-14A through a Tekmar cryofocusing module. GC was coupled with a Shimadzu QP-2000 quadruple mass spectrometer. The conditions used for GC analysis were same as given for P&T-GC. The mass spectra of unknown peaks were compared with those standards as well as with EPA/NIH mass spectral database.

GC-MS analysis of PAHs

Polycyclic aromatic hydrocarbons (PAHs) in the WSF were identified and quantified by the analysis of the concentrated extract of WSF on GC-MS operated in single ion monitoring (SIM) mode. The instrument used for the analysis was Shimadzu GC-14A/QP-2000 with split/splitless injector used in splitless mode. The GC conditions for the analysis were: fused silica capillary column, coated with SPB-5, 25 m long and 0.25 internal diameter. Initial temperature 100°C, held for 5 min and then programmed to 280°C at 6°C. The final temperature was held for 20 min. An external standard method was used for quantitation of PAHs. Commercially available mixture of PAHs (M-610, Supleco) was diluted after the addition of standard solutions of methylated PAHs. The response factors of the available methylated PAHs was used for the isomers for which the standards were not available. The response factor of 1-methyl naphthalene, for example, was used for the calculations of the quantities of all of methyl naphthalene isomers.

Microtox assay

The relative toxicity of the WSFs was assessed by employing Microtox Assay System (Model 5000) with Microtox data collection and reduction software (Microbics Corporation, Carlsbad, CA, USA). The assay was carried out according to the instructions of the manufacturer. Microtox reagent (freeze dried bacteria *Vibrio fischeri*) was reconstituted in 1 ml ultra pure water. The WSF (2.5 ml) and 0.25 ml osmotic solutions were mixed separately. This mixture was diluted serially in 4 separate cuvettes. The temperature was allowed to equilibrate for 5 min and the reagent was added to each of the cuvette, mixed thoroughly and allowed to stabilize for 15 min. Each cuvette was then read on the Microtox analyzer.

RESULTS AND DISCUSSIONS

The water-soluble fraction of Kuwait crude oil is a very complex mixture as reported by many authors[6,13]. Figure 2 shows the purge & trap/gas chromatographic profile of the WSF of Kuwait crude oil. The volatile compounds identified ranged from hexanes (C_6) to methyl indanes. Quantitatively, monoaromatics (BTEX) were dominating. Aliphatics ($C_6$and C_7) were present in appreciable concentrations. Straight chain aliphatic hydrocarbons (*n*-alkanes) were present in decreasing quantities. The solubilities of these compounds decreases as the number of carbon increases in the molecule[15].Many alicyclic compounds (cyclopentanes and cyclohexanes) were also identified though in small amounts.

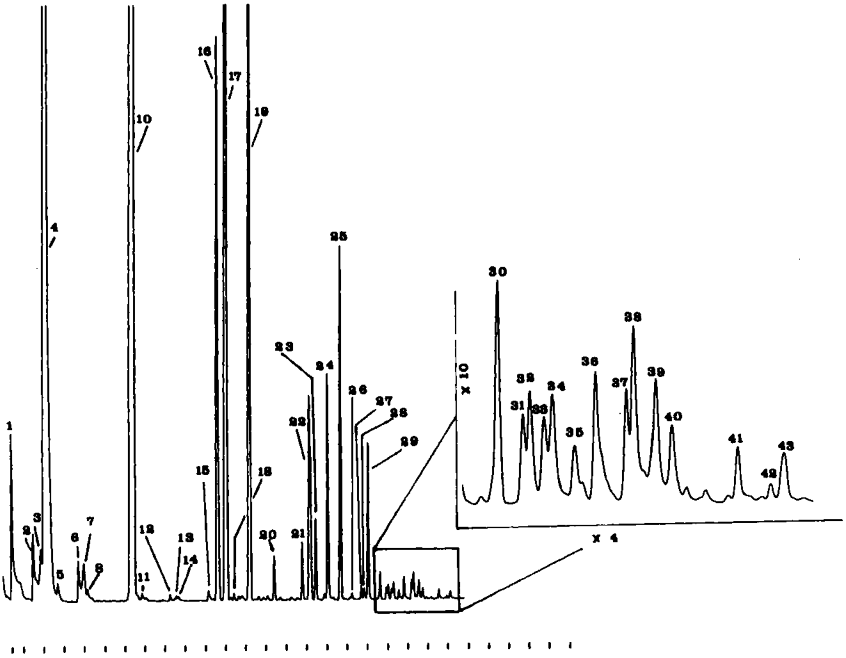

FIGURE 2 Purge & Trap/gas chromatogram of the WSF of Kuwait crude oil (See Table II for peak identification)

Aromatic compounds formed the bulk of the WSF in quantity as well as variety. A large number of isomers of C_3-benzene and C_4-benzenes were present. As the number of isomers of these compounds is very large and many of these isomers have similar mass spectra, many of these were identified as a group (C_3-benzenes and C_4-benzenes). In addition to benzene derivatives, other aromatic compounds were also identified. These included indane. Some compounds containing hetero-atom were also present in the WSF, although in small amounts. Thiophene homologs were detected along with methyl phenol. These compounds have not been reported in the WSF of Kuwait crude oil. These compounds may be important from toxicity point of view.

Table I shows the quantitative composition of the volatiles present in the WSF of Kuwait crude oil at different temperatures. A total of 43 compounds were identified and quantified. The total concentration of volatile compounds was around 8 mg/l. Anderson et al.[6] reported that WSF of Kuwait crude oil prepared at 20°C after 20 hr of stirring contained more than 21.65 mg/l of volatile organic compounds. Aromatic compounds comprised about half of the total (10.03 mg/l) In another study[12], the total concentration of the WSF of Kuwait crude oil prepared after 10 days of stirring was 9.4 mg/l (aromatics only) as determined by GC-MS single ion monitoring. These results show that total concentration of WSF was variable because of different samples of crude oil bearing the same name, and different methods of preparation of WSF. It seems that Kuwait crude oil produces WSF concentration of 7- 10 mg/l of volatile aromatic compounds depending on the sample of Kuwait crude oil.

TABLE I Composition of volatiles in the WSF (μg/l) of Kuwait crude oil

Peak No.	*Name*	*15°C (range)*	*25°C (range)*	*35°C (range)*
1.	Methylcyclopentane	292 (289–297)	370 (364–374)	229 (227–231)
2.	Cyclohexane	162 (159–163)	161(157–163)	106 (97–109)
3.	3-Methylhexane	60 (56–64)	50 (45–53)	18 (16–20)
4.	Benzene	2267 (2252–2275)	2391(2337–2426)	2284(2260–2295)
5.	*n*-Heptane	52 (49–54)	3 (1–5)	0 (0–1)
6.	Methyl cyclohexane	29 (28–29)	37 (36–40)	9 (8–13)
7.	C_8-Isomer	72 (69–75)	79 (74–83)	63 (60–65)
8	1,3-Dimethyl cyclopentane	26 (25–28)	23 (20–25)	82 (81–82)
9.	C_8-Isomer	8 (8)	3 (2–5)	15 (13–16)
10.	Toluene	2210 (2193–2224)	2407 (2385–2422)	2350 (2342–2356)
11.	*n*-Octane	7(6–8)	2 (2)	3 (1–5)
12.	Dimethyl cyclohexane	9 (8–10)	2 (1–4)	0 (0–1)
13.	Ethyl, methylcyclohexane	7 (7)	8 (7–9)	8 (7–8)
14	Methyl phenol	6 (6)	6 (5–6)	6 (5–8)
15.	Methyl thiophene	17 (12–21)	17 (15–21)	16(16–17)
16.	Ethylbenzene	306 (299–310)	337 (334–338)	336 (335–337)
17.	p- and m-Xylenes	719 (718–720)	803 (797–808)	805 (802–808
18.	Dimethyl thiophene	4 (1–7)	3 (1–5)	9 (9)

Peak No.	*Name*	*15°C (range)*	*25°C (range)*	*35°C (range)*
19.	o-Xylene	409 (404–410)	467 (466–470)	473 (471–474)
20.	Cumene	19 (16–21)	22 (19–24)	23 (22–24)
21.	*n*-Propylbenzene	24 (23–29)	27 (24–29)	29 (24–32)
22.	C_3-Benzene isomer	139 (138–141)	153 (150–155)	156 (150–161)
23.	Mesitylene	34 (33–35)	38 (37–38)	39 (38–39)
24.	C_3-Benzene isomer	97 (97–98)	109 (106–111)	115 (114–115)
25.	C_3-Benzene isomer	151 (147–154)	173 (171–174)	180 (177–182)
26.	2-Methyl, propylbenzene	3 (2–3)	3 (2–3)	4 (3–6)
27.	o-Cymene	6 (5–6)	6 (4–7)	7 (6–7)
28.	p-Cymene	6 (4–7)	5 (5–6)	7 (7)
29	C_3-Benzene isomer	59 (59)	67 (64–68)	75 (71–77)
30	Indane	7 (5–8)	13 (11–14)	15 (13–16)
31.	C_4-Benzene isomer	4 (3–5)	4 (4)	6 (6–7)
32.	3-Propyl, 1-methyl Benzene	6 (6)	6 (5–6)	7 (7)
33.	C_4-Benzene isomer	5 (5–6)	5 (3–6)	5 (4–5)
34.	*n*-Butylbenzene	7 (4–9)	7 (6–7)	10 (9–11)
35.	1,2-Diethylbenzene	5 (5)	3 (2–3)	5 (1–8)
36.	C_4-Benzene isomer	10 (9–13)	9 (8–10)	12 (9–14)
37.	C_4-Benzene isomer	5 (3–7)	5 (4–7)	9 (8–9)
38.	C_4-Benzene isomer	11 (10–13)	10 (9–10)	15 (13–6)
39.	C_4-Benzene isomer	9 (9–10)	9 (7–10)	13 (13–14)
40.	1-Methyl indane	5 (5)	4 (3–6)	6 (3–8)
41.	C_4-Benzene isomer	3 (3)	3 (2–5)	5 (4–5)
42.	2-Methyl indane	1 (0–1)	1 (1)	1 (0–1)
43.	4-Methyl indane	3 (2–3)	2 (1–3)	4 (4–5)
	Total	7283 (7234–7309)	7858 (7814–7905)	7566 (7508–7892)

The composition of different groups of compound in the WSF are given in Table II. It can be seen from these results that aromatics amounted to about 90% of the volatiles in the WSF. Aliphatics, higher than C_5, were only 2.14%. Naphthenes were significantly higher (7.79%). As mentioned earlier, BTEX constituted the bulk of the total WSF (about 90% of the aromatics). C_3-benzene collectively comprised about 8%. Volatile compounds other than discussed above were only 0.44% of the total.

TABLE II Group summary of the WSF of Kuwait crude oil at different temperatures

Group	*15°C*	*25°C*	*35°C*
Total Aliphatics (μg/l)	156	95	45
Total Naphthenes (μg/l)	567	643	487
Total Aromatics (μg/l)	6528	7098	6994
Total Others (μg/l)	32	3 1	40
Aliphatics %	2.14	1.2	0.6
Naphthenes %	7.79	8.18	6.44
Aromatics %	89.63	90.22	92.43
Others %	0.44	0.40	0.53
Benzene %	34.72	33.73	32.66
Toluene %	33.85	33.95	33.6
C_2-Benzenes %	21.96	22.66	23.09
C_3-Benzenes %	8.03	8.32	8.85
C_4-Benzenes %	1.00	0.86	1.22

Effect of temperature on volatiles

The effect of increasing temperature on the individual volatile compounds can be see in Table II. The solubilities of different groups were affected differently by the change in temperature of the water used for preparation of WSF. The concentrations of aliphatics, in general, decreased significantly. *n*-Heptane, for example, reduced drastically (from 52 μg/l to less than the detection limit) with increasing temperature. The same trend was observed for other aliphatic compounds although the decrease was variable. Naphthenes on the other hand, did not show a definite trend. The concentration of cyclohexane did not change much when the temperature was increased from 15°C to 25°C. However, a significant decreases was observed when the temperature was increased to 35°C. The levels of methyl cyclohexane increased appreciably when the temperature increased from 15° to 25°C but decreased considerably when the temperature was increased by a further 10°C.

The concentration of aromatics, which formed the bulk of the volatiles in the WSF, was significantly affected by the increasing temperature. As the temperature increased from 15°C to 25°C, the concentration of all of the aromatics increased. The increase in major components was about 10 – 14%. However, the increase in temperature from 25°C to 35°C, resulted in the overall decrease in the concentration of aromatics. A detailed examination of Tables I and II indicate that the decrease was mostly due to benzene and toluene. The higher aromatics

C_2-benzene and higher continued to increase with the temperature. The increase was more pronounced in the concentration of C_3-benzenes (from 8% to 8.9%) and C_4-benzenes (from 1% to 1.2%). The concentration of other compounds (hetero-atom containing) was not affected significantly by the change in temperature. It appears from these results, that if sufficient time is allowed for equilibration, the seawater gets saturated with the organic compounds from Kuwait crude oil, at about 8 mg/l level. Increasing temperature of the water causes higher molecular weight aromatic compounds to dissolve more replacing lower molecular weight aromatic and non-aromatic compounds.

Polycyclic aromatic hydrocarbons in the WSF

Polycyclic aromatic hydrocarbons (PAHs) and their methylated (MePAHs) derivatives are integral part of all of the crude oils. The concentration of these compounds in the crude oil is generally low (a few mg/kg) and their solubility in seawater is also low. However, these are important compounds from toxicity point of view. PAHs are known carcinogens, mutagens and neurotoxic. Moreover, these toxic compounds are semi volatiles and once introduced into the marine environment, are likely to persist in the seawater for much longer time than the volatile compounds. Many authors have reported their presence in the WSF of crude oils[6, 10, 13]. Due to the their low volatility and low concentration, PAHs and MePAHs are not easily determined in the WSF by purge & trap/GC. These difficulties were overcome by extracting relatively large volume (1.5 liter) of the WSF by a suitable solvent and injecting the concentrated extract into a GC-MS. This allowed the detection and quantitation of the PAHs present in very low concentrations.

Table III shows the results of the GC-MS analysis of concentrated extract of the WSF of Kuwait crude oil prepared at three different temperatures. In total nine PAHs were detected along with six MePAHs. The quantitative composition of this fraction was dominated by naphthalene and its methylated derivatives (about 95% of the total PAHs). The results showed that both isomers of methyl naphthalene were present. At least eight isomers dimethyl isomers were detected but not characterized individually and were determined together. Trimethyl naphthalene isomers were also present in significant concentration. The number of possible isomers for this compound is even higher than dimethyl naphthalene. At least 12 isomers of trimethyl naphthalene were detected but were not characterized individually. Naphthalene levels ranged from 20 to 74 μg/l while those of methyl naphthalenes were between 116 and 349 μg/l. Dimethyl naphthalenes were also present in appreciable concentrations (from 38 to 111 1 μg/l), followed by trimethyl naphthalenes (29 to 68 μg/l). The levels of naphthalenes are significantly different from those reported earlier in literature. Anderson et al.[6] reported levels of these compounds much lower than encountered in the present study. The levels of naphthalene were 20 μg/l, methyl naphthalenes were 28 μg/l; dimethyl naphthalenes were 20 μg/l and trimethyl naphthalene were only 3 μg/l. Ali[13] reported much higher levels of naphthalene (90 μg/l) and significantly lower levels of methylated naphthalenes (methyl napththalenes 60 μg/l; dimethyl naphthalenes 44 μg/l and trimethyl naphthalenes 11 μg/l).

TABLE III Levels of PAHs (μg/l) in the extract of WSF of Kuwait crude oil

PAH	*15°C (range)*	*25°C (range)*	*35°C (range)*
Naphthalene	20.6 (18.3–21.8)	46.7 (43.2–48.1)	74.1
Methyl naphthalenes	116.8 (110.0–121.3)	208.4 (200.7–213.6)	348.7 (344.0–351.9)
dimethyl naphthalenes	38.4 (35.7–39.8)	66.5 (65.9–67.2)	110.5 (106.0–114.3)
Acenaphthylene	0.2 (0.1–0.3)	0.3 (0.2–0.4)	0.4 (0.4–0.5)
Acenaphthene	0.2 (0.0–0.3)	0.3 (0.3–0.4)	0.5 (0.4–0.6)
Trimethyl naphthalenes	29.4 (25.9- 32.4)	40.2 (37.9–41.8)	67.6 (65.4–68.6)
Fluorene	0.8 (0.7–0.9)	1.3 (0.9–1.5)	2.2 (2.0–2.3)
Methyl Fluorene	3.3 (3.0–3.5)	5.6 (4.5–6.2)	9.6 (8.7-10.1)
Phenanthrene	1.0 (0.7-1.2)	1.4 (1.1-1.7)	2.8 (2.7-2.9)
Anthracene	0.0 (0.0–0.1)	0.0 (0.0)	0.0 (0.0–0.1)
Methyl phenanthrenes	4.8 (4.1-5.1)	6.8 (5.6–7.6)	13.1 (11.9–13.7)
Dimethyl phenanthrenes	1.9 (1.5–2.2)	1.5 (1.2–1.9)	4.6 (4.3–4.8)
Fluoranthene	0.0 (0.0–0.1)	0.0 (0.0)	0.0 (0.0)
Pyrene	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.1(0.0–0.2)
Dimethyl anthracenes	0.0 (0.0–0.1)	0.0 (0.0)	0.0(0.0–0.1)
Benzo(a)anthracene	0.0 (0.0)	0.0 (0.0–0.1)	0.0(0.0)
Chrysene	0.0 (0.0)	0.0 (0.0–0.1)	0.0 (0.0–0.1)
Benzo(b&k)flouranthene	0.1 (0.1–0.2)	0.1 (0.0–0.2)	0.2 (0.2)
Total PAHs	217.4 (206.1-229.9)	379.1 (369.7–386.4)	634.3 (620.9–642.1)

Acenaphthylene and acenaphthene were detected in very small amounts (less than 0.5 μg/l). Fluorene was also present but only in trace amounts, however, the concentration of methyl fluorenes was significantly higher. Among three-ring PAHs phenanthrene was present in small amounts (about 3 μg/l) and anthracene was not detected. Methylated phenanthrenes were present in much higher amounts compared with phenanthrene. Single ion plot of ion 192 (methyl phenanthrene) showed the presence of at least four isomers of methyl phenanthrene. Dimethyl phenanthrenes were also detected but in much lower concentrations. Only four PAHs (fluoranthene, pyrene, chrysene and benzo(b)fluoranthene) higher than three-rings were detected but the concentrations were very low. Low concentrations of these PAHs were also reported by Anderson et al.[6].

The effect of increasing temperature of the seawater showed the same trend as mentioned earlier for higher molecular weight monoaromatic compounds. The concentration of all of the PAHs and their methylated derivative increased significantly as the temperature increased. Increase of 10° caused the overall concentration of PAHs to increase about 70%. The increase was more or less uniform for all of the major components. Naphthalene and its methylated derivatives constituted about 95% of all of the PAHs in the WSF prepared at 15°C. Their contribution to the total PAH burden of the WSF prepared at 25° and 35°C remained at about 95% level.

The levels of PAHs found in this study makes interesting comparison with those reported in literature. Anderson et al. [6] reported that the WSF of Kuwait crude oil contained naphthalene and methylated naphthalenes along with fluorene and phenanthrene and their methylated homologs. The amount of naphthalene found was comparable with that found in the present study. The levels of other compounds were much lower than being reported here.

Relative toxicity of the WSF

In the present investigations, the toxicity of the WSF of Kuwait crude oil was assayed using a toxicity assay system that has found wide acceptance over the last decade. The test organism is highly sensitive to a broad range of toxicants. The suspension used in the test contains about one million microorganisms. Monitoring the response of such a large population gives the system high resolution compared to other bioassays. The WSFs of Kuwait crude oil prepared at different temperatures were assayed for their relative toxicity by Microtox. The results are showed that the WSF of Kuwait crude oil was not highly toxic. The EC_{50} (effective concentration of the sample that will reduce the light emission by 50%) was 38.2% for the WSF prepared at 15°C. The relative toxicity of the WSF increased when the temperature was increased (EC_{50}= 24.2%) to 25°C. However, increasing the temperature to 35°C did not affect the relative toxicity of the WSF (EC_{50} = 24.5%). It appears that the toxicity of the WSF increased as the temperature increased from 15°C to 25°C. Increasing the temperature further of the water did not show any increase. The same trend was observed for the volatiles present in the WSF.

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References

[1] R. Golob and R. Brus. IMCO/UNEP International workshop on combating marine pollution from oil exploration, exploitation, and transport in the Kuwait Action Plan Region. Manama, Bahrain, 6–10 December, 1980. Cambridge, Massachusetts: World of Information Systems.

[2] O. Linden, M. Y. Abdulraheem, M. A. Gerges, I. Alam, B. Behbehani, M. A. Borhan and L. F. Al-Kassab. State of marine environment in the ROPME sea area. UNEP Regional Sea Report. Study No. 112 (1990).

[3] P. Literathy, and M. Foda. KISR activities on Nowruz oil slick. Kuwait Institute for Scientific Research, Report No. KISR1827, Kuwait (1985).

[4] S.W. Fowler, J.W. Readman, B. Oregioni, J.P. Villeneuve and K. Mckay. *Environ. Int.*, **27**, 171–182 (1993).

[5] M. Sadiq and J.C. McCain. *The Gulf War Aftermath. An Enivronmental Tragedy*, (Kluwer Academic Publishers, Dordrecht 1993) 5–58 pp.

[6] J.W. Anderson, J.M. Neff, B.A. Cox, H.E.Tatem and G.M.Hightower. *Marine Biol.*, **27**, 75–88 (1974).

[7] C.C. Boylan and B.W. Tripp. 1971. *Nature*, **230**, 44–47 (1971).

[8] C.C. Lee, W.K. Craig and P.J. Smith. 1974. *Bull. Environ. Contam. Toxicol.*, **12**, 212–215 (1974).

[9] D. Mackay, and W.Y. Shiu. *Bull. Environ. Contam. Toxicol.*, **15**, 101–109 (1976).

[10] W.Y. Shui, M. Bobra, A. Maijanen, L. Sunito and D. Mackay. 1990. *Oil Chem. Pollution*, **7**, 57–84 (1990).

[11] W.Y. Shui, A. Maijanen, A.L.Y. Ng and D. Mackay. *Environ. Toxicol. Chem.*, **7**, 125–137 (1988).

[12] M.G. Carls, and S.D. Rice. 1990. *Fishery Bull.*, **88**, 29 –37 (1990).

[13] L.N. Ali. The dissolution and photodegradation of Kuwait crude oil in seawater. Ph.D. Thesis. University of Plymouth, Plymouth, UK. 1994.

[14] L.N. Ali, R. F. C. Mantoura and S.J. Rowland. *Marine Environ. Res.*, **40**, 1–17 (1995).

[15] C. McAuliffe. *J. Phy. Chem.*, **70**, 267–1275, (1966).