

## Assessment of Dissolved/ Dispersed Aliphatic and Aromatic Hydrocarbon Pollution in Seawater at the Clifton Beach on the Karachi Coast

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**Summary:** Sea water samples collected at different depths from 10 stations along the Clifton beach of Karachi Coast were analyzed for dissolved/dispersed polyaromatic and aliphatic hydrocarbons (PAHs and AHs) by GC and GC-MS. Sampling was performed in August 2003, just after the *Tasman Spirit* oil spill and in August 2005. Levels of total polycyclic aromatic hydrocarbon (PAHs) and aliphatic hydrocarbons (AHs) were 8.20-26.35  $\mu\text{g L}^{-1}$  ( $\Sigma$  16 PAHs) and 5.23-21.005  $\mu\text{g L}^{-1}$  ( $\Sigma$  16 AHs), respectively. Values decreased later on till lower than 0.02-0.25  $\mu\text{g L}^{-1}$  possibly representing the background level of the region, exhibited lower concentrations that ranged from 0.02-0.05  $\mu\text{g L}^{-1}$  (Av. 0.15  $\mu\text{g L}^{-1}$   $\Sigma$  16 PAHs and 0.05-0.2  $\mu\text{g L}^{-1}$   $\Sigma$  14 aliphatic hydrocarbons) with decreasing to 0.07  $\mu\text{g L}^{-1}$  of PAHs with depth in the water. In September 2005, the concentrations were rather uniform, averaging 0.05  $\mu\text{g L}^{-1}$  ( $\Sigma$  16 PAHs) and 4.08  $\mu\text{g L}^{-1}$   $\Sigma$  14 aliphatic hydrocarbons.

### Introduction

On July 27, 2003, the *Tasman Spirit*, transporting 65,000 tons of Iranian crude oil from Iran to Pakistan, ran aground before entering the harbor channel at the Karachi seaport (Pakistan); after two weeks, 37,000 tons of oil spilled into the sea (Fig. 1), Oil-spill from *Tasman Spirit* polluted the Clifton beach of Karachi, (Photo-1) and the ship was

broken into two halves. (Photo-2) which was considered by experts to be the largest environmental disaster in Pakistan's history [1]. Advance research is being suggested to foresee the extension of the environmental damage and to develop effective restoration strategies. Oil on the coastal areas and fumes in the air raised serious health concerns.

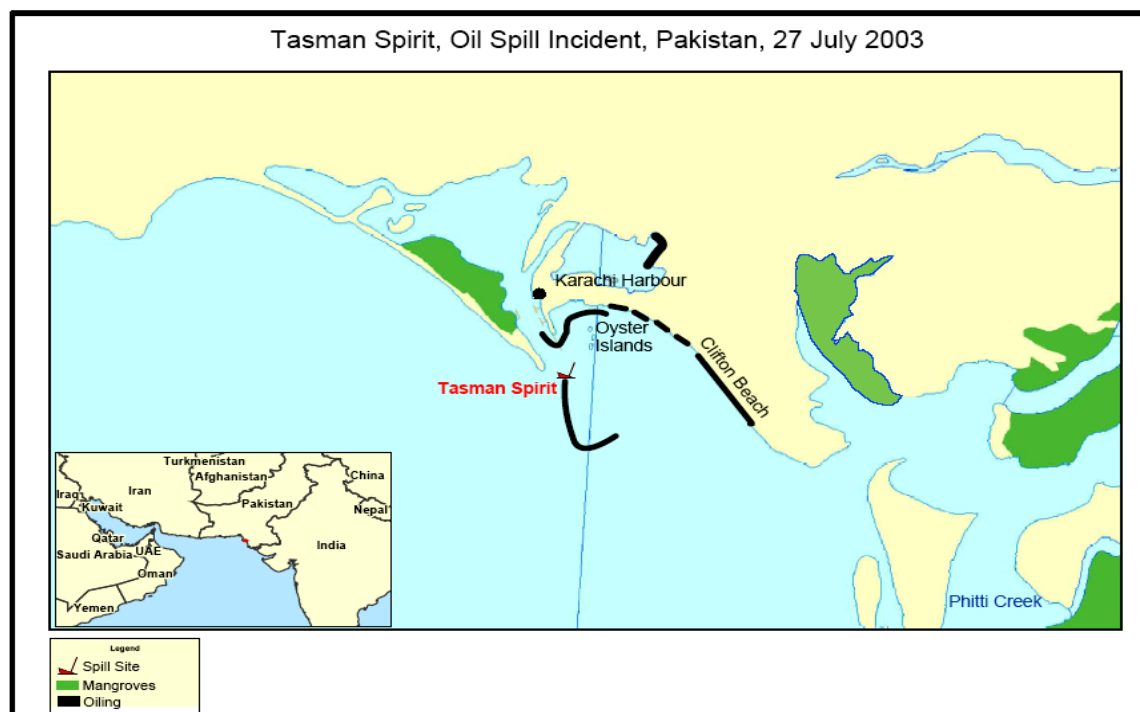


Fig. 1: Sampling location at Clifton Beach.

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Photograph-1: Oil-spill from *Tasman Spirit* was polluting the Clifton beach of Karachi.



Photograph 2: Broken *Tasman Spirit*.

We have assessed the immediate environmental impact of this oil spill on the marine life of the affected coastline in Karachi, Pakistan. Selecting locations for sea water sampling scheme is

given in Table-1. The *Tasman Spirit* oil spill on the Karachi Coast caused enormous damage to the environment, marine life and humans, contaminating approximately 2062 km<sup>2</sup> of marine area as well as

affecting about 300,000 people [2]. The volunteers and the low cadre workers involved in the clean-up complained of dizziness had sudden bouts of vomiting, and finally lost consciousness. Local residents of Clifton complained of respiratory problems and itching eyes due to vaporization of hydrocarbons in the air (personal communication).

Table-1: Sea water sampling scheme for August 2003.

Clifton sample point	Location	Distance (km) From the affected zone	No. samples
1	Near the beach	5	20
2	Lat. 24.48.26° North Long. 66.59.48° East	47.7	6
3	Lat. 24.47.53° North Long. 67.01.43° East	50.7	10
4	Lat 29.47.36° North Long 57.01.43° East	10	12
5	Lat. 23.47.16° North Long. 64.59.24° East	28.08	9
6	Lat. 22.48.26° North Long. 57.59.48° East	22	6
7	Lat. 21.47.53° North Long. 66.01.43° East	18	13
8	Lat 30.47.36° North Long 77.01.43° East	30	8
9	Lat. 22.57.16° North Long. 60.59.24° East	15	11
10	Lat. 23.48.26° North Long. 67.59.48° East	8	7

A detailed understanding of the fate of any spilled oil is required in order to develop effective strategies to combat environmental damage. The first process affecting the fate of spilled oil is dispersion and dissolution. The composition and concentration of the soluble fraction will determine the early impact on marine biota. In this respect, aromatic and aliphatic hydrocarbons are of special concern, as they exhibit higher levels of solubility and greater toxicity in the aquatic environment. [3].

In order to evaluate the distribution of dissolved/dispersed Hydrocarbons (DDPH), two main approaches have been followed for the determination of dissolved/disperse and PAHs and AHs in sea water, namely Spectrofluorophotometer (RF-5301PC) and GC-FID for the analysis of total and individual hydrocarbons, respectively [4].

Greater levels of acute toxicity are generally associated with the lower molecular mass polycyclic aromatic (PAHs) and higher aliphatic hydrocarbons (AHs), whereas some of the higher molecular mass PAHs form carcinogenic active metabolites [5].

After the disaster, a number of attempts were made by The City Government and Clifton

Cantonment Board to clean the entire beach to protect marine life from further losses

## Results and Discussion

### Total Oil Content

The results of Spectrofluorophotometer analyses of the unfiltered water samples for total hydrocarbons are presented in Table-2.

Table-2: Total petroleum hydrocarbon concentration ( $\mu\text{g L}^{-1}$ ) in sea water determined by Spectrofluorophotometer.

Station	2003	2004	2005
1	1229.6	22.20	13
2	768.81	71.29	80
3	1592.14	253.18	20
4	2734.7	108.18	24
5	2695.48	300.60	18
6	1907.30	104.41	17
7	1229.6	22.20	3
8	768.81	71.29	10
9	1592.14	253.18	18
10	2734.7	108.18	12

In August 2003, concentrations of total polycyclic aromatic hydrocarbon (PAHs) and aliphatic hydrocarbons were ranged as 8.20-26.35  $\mu\text{g L}^{-1}$  ( $\Sigma$  16 PAHs) and 5.23-21.005  $\mu\text{g L}^{-1}$  ( $\Sigma$  16 AHs), respectively. Higher concentration was found in samples from the subsurface samples collected from the area around the incident location of *Tasman Spirit* oil Spill, where oil remained to be squeezed out for a longtime from broken oil tank of ship however, in general, the different station transects exhibited decreasing gradients far distance from the ship grounded and down through the water column.

The mean values of total oil content in the sea water samples declined from 0.93  $\mu\text{g L}^{-1}$ -0.54  $\mu\text{g L}^{-1}$  during 2006. Higher concentrations were found at sampling station 1, which was 5 km from the *Tasman Spirit*, and sampling station 3, which was 50 km away from the grounded.

There was a significant decrease of concentration with distance from the *Tasman Spirit*, with values from < 2.50 to 3.00  $\mu\text{g}$  of oil/km. Near the *Tasman Spirit*; however, concentrations were still high, up to 300.60  $\mu\text{g L}^{-1}$  in the surface water in March 2004. It is interesting to note that the higher values in this area were found near the bottom in November 2004, probably due to re-suspension of the accumulated oil in sediments. The level of oil pollution in sea water during 2003–2006 is shown in Fig. 2.

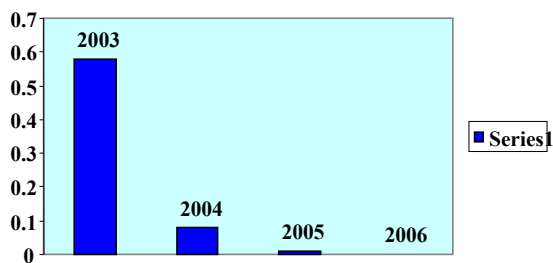


Fig. 2: Level of Oil Pollution in seawater during 2003-2006.

Mean values of total oil in the surface water declined from 80 µg L<sup>-1</sup> to 3 µg L<sup>-1</sup> on the Clifton

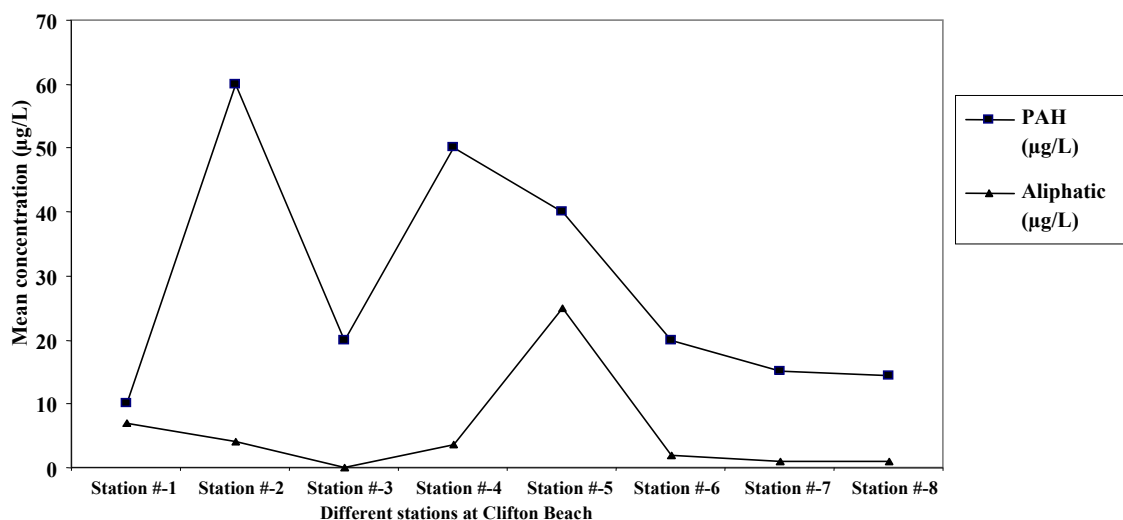


Fig. 3: Mean Concentration of Aromatic and Aliphatic Hydrocarbons in Seawater from Different Stations.

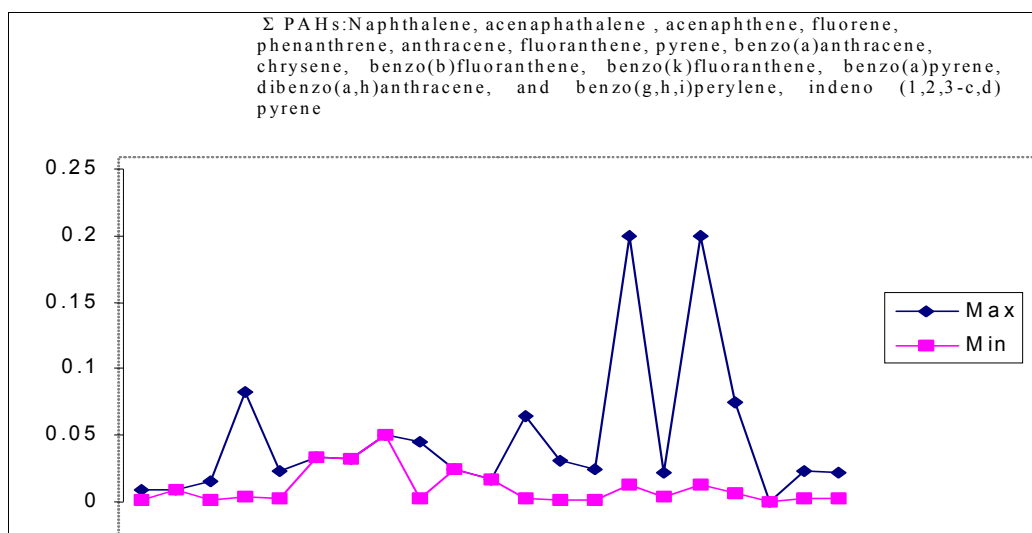


Fig. 4: Maximum and Minimum Concentrations of PAHs in Seawater.

coast ranged from 0.12 µg L<sup>-1</sup> to 0.17 µg L<sup>-1</sup> during 2005. Marrugo *et al.*, (1999) [6] reported values for oil of <1 µg L<sup>-1</sup> for deep sea water and above >4 µg L<sup>-1</sup> for coastal and estuarine water. Concentrations of oil in the range 1.29–2.9 µg L<sup>-1</sup> have been reported for UK inshore water, with values up to 14 µg L<sup>-1</sup> along the coastal area of the English Channel [7].

The concentration profile of aliphatic and aromatic hydrocarbons found at the stations around the *Tasman Spirit* is shown in Fig. 3. and the Maximum and Minimum Concentrations of PAHs in Seawater has shown in (Fig. 4).

In general, the concentrations found after the *Tasman Spirit* oil spill (August 2003) are in the higher range of those quoted for other polluted areas, where values up to 70–100  $\mu\text{g L}^{-1}$  have been reported in the coastal waters of Brittany, France after the Amoco Cadiz oil spill [8], in UK inshore waters, in Canada as *Fisheries Research Board of Canada* [9] and in the inner Gulf of Thailand. This is probably due to the heavy nature of the spilled product, which predominantly produces floating stable oil water emulsions and sea water concentrations of PAHs ranging up to 4.0  $\mu\text{g L}^{-1}$  [1]. After almost 25 months, wind carried patches of emulsified oil onto the shoreline, and the solubility of oil decreased with the number of aromatic carbon rings; the relative solubilities of the PAH aromatic compounds are given in Table-3. The higher molecular mass PAHs were found to be soluble in the range 0.1 – 0.4%.

Table-3: Relative solubility of PAHs.

Compound	Concentration	Solubility
Naphthalene	52.7	30.08
Methylene naphthalene	70.9	12.17
Dimethyl naphthalene	40.0	5.00
Trimethyl naphthalene	13.8	2.39
Acenaphthalene	1.74	5.22
Fluorene	1.60	3.65
Phenanthrene	2.83	1.65
Anthracene	0.36	1.58
Methyl phenanthrenes	2.91	1.20
Pyrene	2.29	0.48
Methyl pyrene	0.28	0.56
Dimethyl pyrene	0.67	0.38
Trimethyl pyrene	0.66	0.28
Diabenzothiophene	1.02	2.36
Methyl diabenzothiophene	2.60	1.74
Dimethyl diabenzothiophene	1.21	0.52
Tribenzothiophene	0.80	0.40
Chrysene	0.11	0.22
Methyl chrysene	0.26	0.16
Dimethyl chrysene	1.21	0.09
Perylene	0.02	0.07

#### Distribution of Aliphatic Hydrocarbons (AHs)

The distribution of individual aliphatic hydrocarbons along the Clifton coast was found to be in the range 5.071–29.34  $\mu\text{g L}^{-1}$ .

Each component of AHs had a concentration different from those described in the literature after different oil spills. It is interesting to note that, besides the above concentration trends, the distribution profile of individual AHs also displayed a different pattern [10]. The profiles found in stations far from the oil spill were more depleted for low molecular mass compounds, *e.g.* undecane over heptadecane and octadecane over nonadecane, an indication of the predominance of prophetic inputs.

The concentration of tetradecane was the highest of all AHs in 70% of the samples collected during 2003–2005.

In general, GC analysis of sea water after the spill showed features common to the coastal environment with the predominant oil composition of Iranian crude oil, with *n*-alkanes based on C<sub>6</sub>–C<sub>19</sub>.

In the first round of sampling (August, 2003), just after the *Tasman Spirit* oil spill, the highest values were found at station 10 (Fig. 2), the most affected by the oil spill, with mean values for AHs of 16.07  $\mu\text{g L}^{-1}$  in surface water, 11.53  $\mu\text{g L}^{-1}$  at mid depth, and 6.40  $\mu\text{g L}^{-1}$  near the bottom.

The average values of AHs in surface water around the *Tasman Spirit* were 0.882  $\mu\text{g L}^{-1}$  in 2004 and 0.404  $\mu\text{g L}^{-1}$  in 2005. The values at mid-depth were 0.644  $\mu\text{g L}^{-1}$  in 2004 and 0.176  $\mu\text{g L}^{-1}$  in 2005. The values near the bottom were 0.489  $\mu\text{g L}^{-1}$  in 2004 and 0.122  $\mu\text{g L}^{-1}$  in 2005.

During 2006, the values for AHs at different depths ranged from 0.21  $\mu\text{g L}^{-1}$  to 1.56  $\mu\text{g L}^{-1}$ . In general, the concentration declined with distance from the ship and with depth in the water. Later, the values were lower, with averages ranging from 26.75  $\mu\text{g L}^{-1}$  to 0.621  $\mu\text{g L}^{-1}$  ( $\Sigma$  AHs 14), with some exceptions due to local situations during 2003–2005, but during 2006 the average value was 1.70  $\mu\text{g L}^{-1}$ .

During 2005–2006, the AH levels were found to be below the safety level for acute mortality of aquatic biota, and even just after the oil spill according to some toxicity models [11], although that study demonstrated that AHs were the major determinant of oil toxicity to aquatic organisms.

During 2005–2006, the AHs levels were lower than those in 2003–2004, and caused negligible acute mortality of the aquatic biota. Later, fish from Clifton beach did not show harmful or hazardous concentrations of aliphatic hydrocarbons.

#### Distribution of PAHs

The sea water samples collected along the Clifton beach during 2003–2006 were analyzed for individual PAHs, including parent and alkyl homologues.

In general, the distribution of PAHs paralleled those of total hydrocarbons, although some deviation associated with time was found at some stations.

In the first round of sampling (August. 2003), just after the *Tasman Spirit* oil spill, the highest subsurface values were between  $1.25 \mu\text{g L}^{-1}$  and  $8.20 \mu\text{g L}^{-1}$  ( $\Sigma 16$  PAHs) (Fig. 1).

In 2003, the maximum concentration of PAHs was  $0.888 \mu\text{g L}^{-1}$ , which decreased to  $0.716 \mu\text{g L}^{-1}$  in 2004 (Table-3). During 2005, the total concentration of  $\Sigma$  PAHs was increased slightly to  $0.81 \mu\text{g L}^{-1}$ , with more deposition of  $\Sigma$  PAHs at the bottom, where the concentration was  $0.452 \mu\text{g L}^{-1}$  presumably because of resuspension of oil from the sea bed.

During 2006, the level of  $\Sigma$  PAHs was  $0.24 \mu\text{g L}^{-1}$ , on average, which was lower than those measured in previous years, and not liable to cause acute mortality of the aquatic biota, even close to the *Tasman Spirit* (Table-4). Over all concentration ( $\mu\text{g L}^{-1}$ ) of 16  $\Sigma$ PAHs\* and 13  $\Sigma$ AHs were measured in sea water during the present study (Table-5) and (Table-6).

Later,  $\Sigma$  PAHs concentrations were in the range  $0.25\text{--}0.29 \mu\text{g L}^{-1}$ , with an average of  $0.18 \mu\text{g L}^{-1}$ . Like the PHAs, concentration declined with

distance from the site of the accident and with depth in the water (Table-7).

Table-4: Average concentration of individual aliphatic hydrocarbons ( $\mu\text{g L}^{-1}$ ) in sea water samples collected in 2003.

Compound	Concentration ( $\mu\text{g L}^{-1}$ )
<i>n</i> -Hexane	11.753
<i>n</i> -Heptane	20.000
<i>n</i> -Octane	17.235
<i>n</i> -Nonane	15.017
<i>n</i> -Decane	12.077
<i>n</i> -Undecane	5.071
<i>n</i> -Dodecane	7.048
<i>n</i> -Tridecane	10.046
<i>n</i> -Tetradecane	21.005
<i>n</i> -Pentadecane	6.024
<i>n</i> -Hexadecane	5.042
<i>n</i> -Heptadecane	8.037
<i>n</i> -Octadecane	10.039
<i>n</i> -Nonadecane	15.27

Mostly, the concentration was of a different order of magnitude for each component. It is interesting to note that, besides the above concentration trends, the distribution profiles of individual PAHs were different. The profiles found in stations far from the oil spill site were more depleted for low molecular mass compounds, *e.g.* benzene over pyrene and anthracene over chrysene.

Table-5: Concentration ( $\mu\text{g L}^{-1}$ ) of 16  $\Sigma$ PAHs\* found in sea water.

Station	Depth (m)	2003			2004			2005			2006		
		S	MD	B	S	MD	B	S	MD	B	S	MD	B
1	75	0.35	0.42	0.41	0.25	0.30	0.28	0.41	0.35	0.40	0.62	0.25	0.01
2	130	0.51	0.52	0.58	0.10	0.73	0.25	0.25	0.21	0.53	0.71	0.56	0.16
3	200	0.25	0.51	0.78	0.50	0.75	0.92	0.25	0.51	0.71	0.89	1.20	0.08
4	120	-	-	-	0.35	0.36	0.16	-	-	-	0.21	0.08	0.73
5	205	-	-	-	0.62	0.82	0.52	0.19	1.35	0.67	-	-	-
6	50	0.32	0.40	0.23	-	-	-	0.26	2.00	0.93	0.35	0.62	0.90
7	130	0.25	0.60	0.40	0.71	0.42	0.62	0.92	7.98	6.00	0.71	0.25	1.56
8	215	0.50	0.25	0.61	0.25	0.51	0.73	0.83	3.50	2.10	4.56	1.56	2.35
9	60	-	-	0.70	0.62	0.62	0.75	0.75	1.03	0.87	6.21	0.61	6.00
10	110	0.48	1.03	0.27	0.22	0.33	0.26	0.18	0.64	0.18	6.12	0.21	2.41

\*  $\Sigma$  PAHs naphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, indeno (1,2,3-c,d) pyrene

Table-6: Concentration ( $\mu\text{g L}^{-1}$ ) of 13  $\Sigma$ AHs\* found in sea water.

Station	Water depth (m)	2003			2004			2005			2006		
		S	MD	B	S	MD	B	S	MD	B	S	MD	B
1	75	30.35	18.42	13.41	1.25	1.30	1.28	0.41	0.35	0.20	0.22	0.25	0.23
2	130	19.51	9.52	5.58	1.10	0.93	0.85	0.25	0.21	0.53	0.26	0.56	0.16
3	200	13.25	10.51	6.78	0.90	0.75	0.62	0.25	0.51	0.21	0.89	1.20	0.08
4	120	<LOD	<LOD	<LOD	1.35	0.36	0.16	<LOD	<LOD	<LOD	0.21	0.08	0.73
5	205	<LOD	<LOD	<LOD	1.62	0.82	0.52	0.19	1.35	0.67	<LOD	<LOD	<LOD
6	50	33.32	30.40	10.23	<LOD	<LOD	<LOD	0.26	2.00	0.93	0.35	0.62	0.90
7	160	15.25	10.60	6.40	0.71	0.42	0.32	0.92	7.98	0.71	0.71	0.25	1.56
8	215	26.50	20.25	10.61	0.25	0.51	0.33	0.83	3.50	2.10	4.56	0.56	0.35
9	60	<LOD	<LOD	0.70	0.42	0.62	0.45	0.75	1.03	0.87	6.21	0.61	0.45
10	110	22.48	16.03	10.27	1.22	0.73	0.36	0.18	0.64	0.18	6.12	0.32	0.41

\**n*-Hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane, *n*-undecane, *n*-dodecane, *n*-tridecane, *n*-hexadecane, *n*-heptadecane, *n*-octadecane, and *n*-nonadecane

In 2003, anthracene accounted for 90% of total PAHs. During 2004–2005, naphthalene was the predominant PAH metabolite, and in 2006 it was pyrene. There was a change with time among homologous compounds due to seasonal variation (Table-7).

Table-7: Average individual PAHs pollution level in sea water 2003-2006.

Component	2003	2004	2005	2006
Naphthalene	1.098	0.112	0.061	0.002
Acenaphthylene	0.583	0.065	0.061	0.013
Acenaphthene	0.341	0.006	0	0.032
Fluorene	0.761	0.063	0.051	0.023
Phenanthrene	0.662	0.006	0.001	0.014
Anthracene	1.256	0	0.01	0.051
Fluoranthene	0.001	0.021	0.042	0
Pyrene	0.611	0.01	0	0.107
Benzo(a)anthracene + chrysene	0.962	0.002	0.021	0.003
Benzo(b)fluoranthene + benzo(k)fluoranthene	0.631	0	0.003	0.005
Benzo(a)pyrene + dibenzo(a,h)anthracene	0.674	0	0.009	0
Benzo(g,h,i)perylene + indeno(1,2,3-c,d)pyrene	0.621	0.002	0.001	0

In all samples, the PAHs levels were lower than those published for acute mortality of aquatic biota. Using several toxicity models, French (1998) estimated that LC50 (dose at which 50% of test animals die) for  $\Sigma$  PAHs ( $\Sigma$ 26PAHs: parent and alkyl homologues) in North Cape oil ranged from 15  $\mu\text{g L}^{-1}$  to 245  $\mu\text{g L}^{-1}$ , while Barron *et al.* [12] found that 2.2–9.2  $\mu\text{g L}^{-1}$  of  $\Sigma$  PAHs ( $\Sigma$ 110PAHs: parent and alkyl homologues) in the water contaminated with fractions of three oils was the LC50 for the mysid shrimp *Mysidopsis bahia* (Table-4), although this study demonstrated that PAHs were the major cause of oil toxicity to aquatic organisms.

## Experimental

### Materials and Methods

#### Chemical and Reagents

All glassware cleaned with concentrated  $\text{H}_2\text{SO}_4$  after washing with detergent followed by cleaning with water and Milli-Q water. Analysis grade *n*-hexane and dichloromethane were purchased from the sole agent of Merck (Germany), and chrysene (98%) was purchased from Sigma-Aldrich (Stein heim , Germany )

A PAH mixture containing naphthalene, accenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and benzo(g,h,i)perylene, indeno(1,2,3-c,d) pyrene of concentration 10  $\text{mg L}^{-1}$  in *n*-hexane and an aliphatic mixture containing ( $\text{C}_6$ - $\text{C}_{19}$ ) *n*-hexane, *n*-heptane, *n*-octane, *n*-nonane, *n*-decane,

*n*-undecane, *n*-dodecane, *n*-tridecane, *n*-tetradecane, *n*-pentadecane, *n*-hexadecane, *n*-heptadecane, *n*-octadecane and *n*-nonadecane purchased from Dr Ehrenstorfer Laboratory (Augsburg, Germany). The deuterated surrogate standards were obtained from Accusstandards, USA and from Dr. Ehrenstorfer, Germany)

### Sampling / Preliminary Step

The sampling locations for sea water shown in Fig. 1 and sampling plant in Table-1. Sampling was done at three depths, from August 2003-September 2006 on the affected area of Clifton Beach of Karachi Coast in collaboration with National Institute of Oceanography, Karachi, Pakistan, and assisted by fishermen, local divers and experts from the Scuba Diving Center, Karachi (Photo-3).

Subsurface seawater (01 m depth) was sampled with 2 L amber glass bottles held in stainless-steel frames and to avoid local contamination. Mid depth and bottom/deep sea (2-3 m above the sediment) sampling was done with Go-Flo bottles and immediately transferred to the 2 L glass bottles. Then, 30 mL of hexane and 1 mL of  $\text{HgCl}_2$  solution were added .The bottles were kept at 4  $^{\circ}\text{C}$  until extraction. For the analysis of individual PAHs the sea water was acidified with HCL, 6 N until  $\text{pH}<2$ , following the EPA recommendation.

### Spectrofluorometric Analysis for Total Hydrocarbons

The unfiltered seawater samples analyzed by Spectrofluorometer for dissolved /dispersed hydrocarbons. Two liters of seawater were extracted three times with 25 mL of hexane. The organic extracts were combined, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated to 5ml with a rotary evaporator. The fluorescence was measured using Shimadzu Spectrofluorometer (RF-5301 PC) with excitation wavelength of 310 nm and an emission wavelength of 360 nm.

The calibration was made with a hexane solution of chrysene and crude oil from the *Tasman Spirit* (between 0.01 and 1000  $\mu\text{g L}^{-1}$  and 0.1 and 20,000  $\mu\text{g L}^{-1}$  of chrysene and crude oil, respectively). The detection limit ( $DL = \text{average} + 3SD$ ) was 0.05  $\mu\text{g L}^{-1}$  and 0.1  $\mu\text{g L}^{-1}$  equivalent of chrysene and crude oil, respectively. The method was tested for reproducibility using six samples from different sites producing results with coefficients of variables ranging from 10%-20%.



Photograph 3: Experts from the Scuba Diving Center, Karachi helped in Sampling.

#### *Gas Chromatographic Analysis for Individual PAHs*

The unfiltered sea water samples were spiked with acenaphthene- $d_{10}$ , phenanthrene- $d_{10}$  and chrysene- $d_{12}$  as internal standards, and a mixture of 14 AHs as surrogate standard. The samples were extracted consecutively with 50 mL of hexane and 25 mL of dichloromethane. The organic extracts were combined, dried over anhydrous  $Na_2SO_4$ , rotary-evaporated and fractionated in a glass column filled with neutral alumina (1 g) and silica gel (1 g). Two fractions were eluted, the first with 2 mL of hexane, which contained the aliphatic compounds, and the second with 10 mL of dichloromethane/hexane (30:70, v/v), which contained the PAHs.

Both fractions were analyzed by Gas Chromatograph (Perkin Elmer, Clarus-500) equipped with fused silica capillary column (Elite-1:30m long 0.53 mm id, 1.5  $\mu$ m film thickness). FID was used as detector.

For Polycyclic Aromatic Hydrocarbons (PAHs) analysis, the oven temperature program was set at 80 °C (initial time :05 min) to 140 °C at a rate of 25 °C /min and held for 10-minutes, 140 °C to 270 °C at a rate of 15 °C / min for 04- minutes. Finally ramped to 280 °C at a rate of 3 °C /min for 5 minutes. Inlet and detector temperature were 270 °C and 285 °C, respectively.

For Aliphatic Hydrocarbons (AHs) analysis the oven temperature programme was set at 20 °C (initial time: 03 min) to 150 °C at a rate of 20 °C/min and held for 12 min. Finally ramped at 200 °C at 20 °C/min for 04 min. Inlet and detector temperature were 225 °C and 275 °C, respectively.

The recoveries of spiked PAH standards ranged from 90%-92% (average value 85%); the detection limits ranged from 0.5 ng L<sup>-1</sup> to 10.0 ng L<sup>-1</sup> fluorene to dibenzene(a, h) anthracene, respectively.



### Standardization of Methods for Analysis

Quantification was carried out by comparison with naphthalene and normal hexadecane as internal standards for the aliphatic and aromatic fractions, respectively, as well as with an external standard. A spiked water sample of known concentration ( $1.00 \mu\text{g L}^{-1}$ ) was analyzed according to this procedure; the results were within the confidence intervals for both aliphatic and polyaromatic hydrocarbons. The average extraction recovery was  $92.8 (\pm 4.0) \%$  for the PHA fractions and  $90.4 (\pm 2.9) \%$  for the PAH fractions.

### Analytical Quality Control / Quality Assurance

The quality of data was assured through analysis of the appropriate Certified Reference Materials. Deuterated surrogates and internal standards were used to compensate for loss during sample extraction and work-up. The surrogate standard was used to monitor method performance for each sample, while the internal standard was used to compensate for variation in the final extract volume, injection volume, and instrument sensitivity [13]. Analysis of blank samples was carried out with each batch. All compounds were identified by retention times. Values for % RSD, LOD and LOR are given in Table-3.

### Conclusion

Studies have demonstrated that levels of PAHs and AHs as low as  $1 \text{ ng L}^{-1}$  in sea water cause toxic, long-term effects. This detailed study has taken about two years, with the main objective of monitoring the levels of PAHs and AHs from July 27, 2003 up to December 2006 after an oil spill at sea. On the basis of the findings of this study, it has been concluded that no harmful oil pollution persists at present (2006), due to considerable bioremedial measures that were taken in this area, but regular monitoring for at least the next 10 years is recommended.

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