Polycyclic Aromatic Hydrocarbons in the Chao Phraya Estuary, Thailand

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Solid-phase extraction (SPE) using a BAKERBOND C_{18} membrane disk (Empore™ extraction disk) coupled with high performance liquid chromatography (RP-HPLC) was used for analysis of sub-ppb levels of polycyclic aromatic hydrocarbons (PAHs) in water from the Chao Phraya estuary, Thailand. Recoveries of PAHs were found to be in the range of 60 – 88%. The method detection limits were in the range of 1-50 ng/L for a fluorescence detector and 5-200 ng/L for a photodiode-array detector. This method provides sufficient qualitative information for all compounds to be identified and accurate quantitative results in the range of concentration considered. Several PAHs, such as naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene, were identified in the water samples. The concentrations found were in the range of <5 – 1,500 ng/L. Total identified PAH concentrations were found to be higher during the wet season than the dry season. Using published data on river discharge and the concentrations measured in the water samples, tentative input rates into the Upper Gulf of Thailand for PAHs are calculated.

Key words: PAHs, hydrocarbons, Chao Phraya, Thailand.

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สารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอนในเอสทูรีแม่น้ำเจ้าพระยา

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วัฏภาคของแข็งแบบแผ่น (Empore™ Extraction Disk ชนิด BAKERBOND C_{18}) ประกอบกับเทคนิค RP-HPLC สามารถนำมาประยุกต์ใช้ในการวิเคราะห์สารโพลีไซคลิกอะโรมาติกไฮโดรคาร์บอน(PAHs)ในน้ำทะเลบริเวณเอสทูรีแม่น้ำเจ้าพระยาที่มีความเข้มข้นของสารในระดับต่ำกว่า ppb ได้ดี โดยมีค่าร้อยละของการคืนกลับอยู่ในช่วง 60–88% ค่าขีดจำกัดในการตรวจวิเคราะห์สาร PAHs อยู่ในช่วง 1-50 นาโนกรัม/ลิตรสำหรับตัวตรวจชนิดฟลูออเรสเซนซ์ และในช่วง 5-200 นาโนกรัม/ลิตรสำหรับตัวตรวจชนิดโฟโตไดโอดแอเรย์ เทคนิคนี้สามารถ วิเคราะห์สาร PAHs ได้ดีทั้งการแยกชนิดและการคำนวณปริมาณสารจากการศึกษาตรวจพบสาร PAHs หลายชนิดในตัวอย่างน้ำจากเอสทูรีแม่น้ำเจ้าพระยา เช่นแนบธาลีน อะซีแนบธีลีน อะซีแนบธีน ฟลูออรีน ฟีแนนธรีน และแอนธราซีน โดยค่าที่ตรวจพบอยู่ในช่วง <5 – 1,500 นาโนกรัม/ลิตร ปริมาณรวมของสาร PAHs ในช่วงฤดูน้ำหลากสูงกว่าช่วงหน้าแล้ง เมื่อนำค่าความเข้มข้นรวมของสาร PAHs มาคำนวณกับปริมาณน้ำที่ไหลออกสู่อ่าวไทยตอนบนในแต่ละช่วงฤดูกาล สามารถประเมินปริมาณสาร PAHs ทั้งหมดที่ถูกพัดพาออกสู่อ่าวไทยตอนบนได้

คำสำคัญ โพลีไซคลิกอะโรมาติกไฮโดรคาร์บอน PAHs ไฮโดรคาร์บอน เจ้าพระยา

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) result from incomplete combustion of organic material in industrial and private heaters, automobile exhaust fumes and tobacco smoke. They are widely distributed in the global environment and samples from areas throughout the world have been found to contain detectable concentrations of these compounds.[1-6] Estuaries receive input loads of PAHs from industrial and municipal sewage outfalls, river runoff, aerial fallout and dredged spoil disposal. Certain PAH compounds are known to be toxic, mutagenic and/or carcinogenic to mammals.[7] The U.S. Environmental Protection Agency (U.S. EPA) listed sixteen specific PAHs as priority pollutants in the monitoring and control of environmental pollution.[8-9]

The Chao Phraya River estuary is one of the major estuaries in Thailand. The Chao Phraya River rises in the southern slopes of the Himalaya mountains not far from Chiang Mai in northern Thailand, traverses several large cities and the main part of the agricultural land of the central provinces, and empties into the Gulf of Thailand. The river receives a considerable amounts of waste from point and non-point sources along its paths since many of the industrial sites and urban centers on the river banks tend to dispose of raw effluent to the nearest waterways as well as to the river.

The purpose of this work is to contribute to the characterization of pollution loads, in particular polycyclic aromatic hydrocarbons, aiming at further implementation of a monitoring programme regarding the environmental assessment of the Chao Phraya River.

The experimental procedure is based on the U.S. EPA Method 550.1 of Bashe and Baker,[9] adapted for use with surface water. The principle of the method is adsorption of PAHs onto C_{18} membrane disks (Empore™ extraction disk) followed by desorption of the pollutants with acetonitrile and subsequent analysis by HPLC.

MATERIALS AND METHODS

Chemicals and Instrumentation

PAH standards were of analytical grade and purchased from Aldrich, ANSPEC, Chem Service and SIGMA. All solvents used were of HPLC grade, obtained from Baker and Merck. Empore™ extraction disks (BAKERBOND C_{18}) were purchased from Baker.

The HPLC measurements were carried out on a Hewlett-Packard liquid chromatograph model HP 1100 Modular HPLC system, with RP-C_{18} column (Vydac 201 TP, 5 μm, 25 cm x 2.1 mm ID stainless steel column) and with fluorescence and photodiode-array detection.

Procedure

Sampling and sample preparation

Subsurface water samples (1m depth) from the Chao Phraya estuary were collected, as grab samples in acid-washed amber glass bottles fitted with teflon screw caps, at ten stations along the length of the estuary (Figure 1), in October 1999 and February 2000. After collection, the samples were filtered through glass fiber filters (Whatman GF/C) to remove suspended matter. This is important in order to avoid plugging of the solid phase material as well as to analyze for

PAHs in the particulate fraction. The glass fiber filters and the filtered samples were stored in solvent-cleaned glass bottles at 4 °C prior to the extraction.

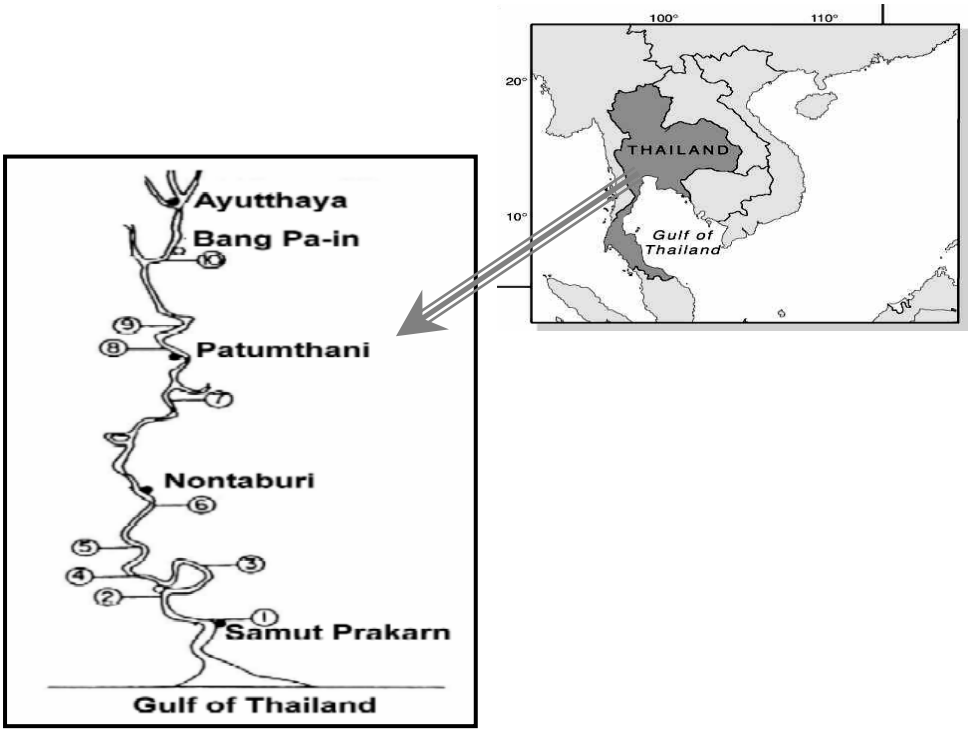

Figure 1. Sampling stations along the Chao Phraya estuary.

Extraction and concentration

Solid phase partitioning for the extraction of dissolved PAHs was carried out by processing a 500 ml of sample through a 25 mm C_{18} Empore™ extraction disk mounted in a side arm vacuum flask in approximately 30 minutes. The disks were then dried and desorption was performed by eluting with 4x5 ml of acetonitrile. The eluates were concentrated to 0.5 ml under a gentle stream of nitrogen.

PAHs in the particulate fraction were isolated from the glass fiber filters with 4x5 ml of acetonitrile by ultrasonication during 30 minutes. The eluates were then centrifuged and concentrated to 0.5 ml under a gentle stream of nitrogen.

Chromatographic separation

HPLC analyses were performed on a reverse-phase Vydac 201 TP, 5-micron particle size, in a 25 cm x 2.1 mm ID stainless steel column. The solvent programme started with isocratic elution for three minutes using acetonitrile/ water (6:4), followed by a linear gradient to 90% acetonitrile in 7 minutes, and ended with 100% acetonitrile during 10 minutes at a flow rate of 0.42 ml/minute. Temperature of the column compartment was kept

at 20 °C. The injection volume was 1 μL.

Detection and identification

The PAHs were detected with a fluorescence detector by comparing the retention times of the peaks in the sample with those in a standard. In addition, a photodiode-array detector was also used for confirmation of compound identification, by comparing the UV spectra with those stored in a library.

Quantification

Concentrations of PAHs in a sample were determined by external standardization (peak area measurement).

The accuracy and precision of the methods were tested by spiking known amounts of the sixteen PAH standards, ranging between 20 and 500 μg/L, to 500 ml organic free water (HPLC grade) and synthetic seawater (salinity 5, 10, 20, 30 and 35 ppt) and by carrying out eight replicate analyses for each type of water. Extraction blanks were analyzed periodically to ensure the absence of contamination from glassware and filters.

RESULTS AND DISCUSSION

The retention time data of the sixteen PAHs are provided in Table 1. The PAHs can be effectively separated using the above HPLC conditions. The reproducibility of the peak retention time and peak area data for each PAH under the SPE-HPLC coupling technique was determined by carrying out eight replicates. The reproducibility of the retention time data was below 1% and that of the peak area was below 5% for all the sixteen PAHs considered. Method detection limits (MDL) were determined for both the fluorescence and photodiode-array detectors, and found to be in the range of 1-50 ng/L for the fluorescence detector and 50-200 ng/L for the photodiode-array detector. In general, the fluorescence detector is more sensitive to PAH molecules than the photodiode-array detector. However, using a diode array detector has the advantage of acquiring UV-spectra on-line and of monitoring signals of up to 5 wavelengths simultaneously. Thus for different classes of substances, the best selectivity can be selected by choosing the appropriate wavelength with maximum absorption. Substances that are not separated chromatographically, can be observed selectively if they have different absorbance maxima.

Table 1. Retention times and method detection limits of the sixteen PAHs.

Analyte	Detector	Retention Time (min)	Method Detection Limit (ng/L) [a]
Naphthalene	FLD	3.1	10
Acenaphthylene	DAD	3.5	100
Acenaphthene	FLD	4.3	5
Fluorene	FLD	4.6	8
Phenanthrene	FLD	5.4	10
Anthracene	FLD	6.5	10
Fluoranthene	FLD	7.8	7
Pyrene	FLD	8.6	3
Benzo(a)anthracene	FLD	11.5	10
Chrysene	FLD	12.1	20
Benzo(b)fluoranthene	FLD	14.1	5
Benzo(k)fluoranthene	FLD	15.2	1
Benzo(a)pyrene	FLD	16.0	15
Dibenzo(a,h)anthracene	FLD	18.1	20
Benzo(g,h,i)perylene	FLD	18.6	15
Indeno(1,2,3-cd)pyrene	FLD	19.9	50

[a] The MDL for acenaphthylene was determined using a photodiode-array detector. All others were determined using a fluorescence detector.

Table 2 gives the recoveries of PAHs from organic free water and synthetic seawater (salinity 20 ppt). Generally, recoveries above 70% were obtained for most of the PAHs studied. Recoveries of PAHs from synthetic seawater at other salinity levels (not shown) gave similar results to that at salinity 20 ppt. Hence, the presence of inorganic salts in the solution does not seem to seriously affect the recoveries of PAHs using SPE-HPLC coupling technique. The major loss appears to be related to the evaporation of organic solvent by nitrogen gas.

Figure 2 shows the HPLC analysis of the acetonitrile extract of one of the surface water samples from the Chao Phraya estuary. In liquid chromatographic routine analysis usually the identification is made by comparing the retention times of the peaks in the sample with those in a standard. However, in environmental samples, the risk is high that other organic compounds are present and interfere with the compounds of interest. Therefore, each peak was checked by comparing the UV spectra with those stored in a library. Identified PAHs were dominated by the low molecular weight PAHs such as naphthalene, acenaphthylene and acenaphthene. The levels of three PAHs present in the water were between <5 - 1500 ng/L (Table 3). Results were corrected for the extraction efficiency based on the mean recovery values. Only a trace amount of fluorene, phenanthrene, anthracene and fluoranthene were present at the upper reach stations of the estuary. The levels found did not exceed the maximum allowed amount of PAHs in surface water as set by WHO[10] and the Ministry of Environment, Canada.[11]

The presence of these PAHs in the Chao Phraya Estuary can readily be attributed to anthropogenic inputs, particularly from fossil fuel burning, motor oils, asphalt particles and street dust.[12, 13] These source materials and the associated PAHs can reach the river via natural runoff from land during rainstorms, sewage outfalls, and transport by canals if they are not introduced directly. Motor oil may be directly deposited into the river by boating and marine transport activities.

Table 2. Recoveries of PAHs in 500-mL blank water samples.

Analyte	Organic free water		Synthetic seawater (20 ppt)	
	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	60.0	3.1	59.1	3.4
Acenaphthylene	71.2	3.5	72.4	10.8
Acenaphthene	70.4	3.1	72.4	8.9
Fluorene	85.6	3.4	78.8	3.2
Phenanthrene	88.7	4.1	87.3	3.1
Anthracene	64.2	5.3	61.4	0.6
Fluoranthene	85.3	3.6	85.1	1.7
Pyrene	77.5	6.5	94.4	3.0
Benzo(a)anthracene	83.0	2.0	99.5	2.3
Chrysene	87.5	2.3	98.9	3.6
Benzo(b)fluoranthene	85.2	6.4	103.8	1.7
Benzo(k)fluoranthene	83.6	3.0	103.5	0.2
Benzo(a)pyrene	88.3	2.8	80.2	1.0
Dibenzo(a,h)anthracene	84.5	4.0	83.8	1.0
Benzo(g,h,i)perylene	78.9	3.3	74.3	3.5
Indeno(1,2,3-cd)pyrene	75.3	7.4	65.2	5.2

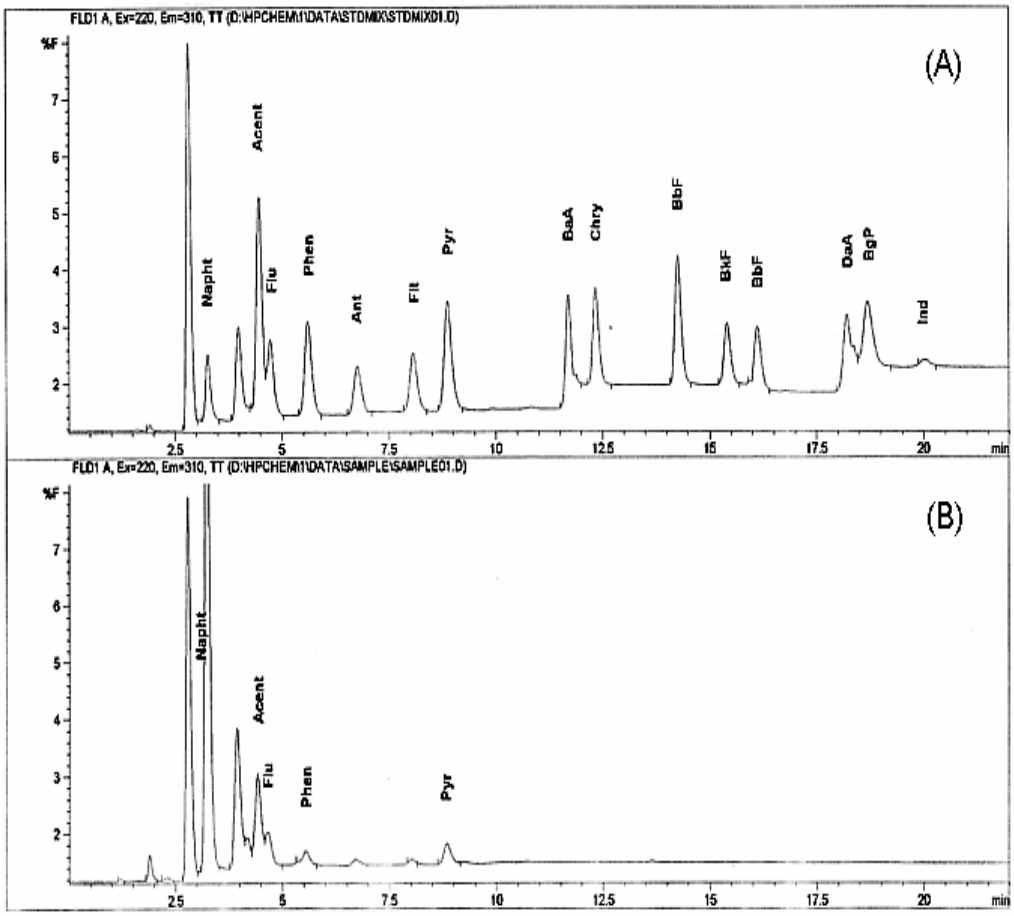

Figure 2. HPLC chromatograms of (A) standard PAHs (B) a water sample collected from the Chao Phraya estuary.

Table 3. Mean concentration of identified PAHs detected in the Chao Phraya estuary.

Analyte	Concentration (ng/L)			
	October 1999		February 2000	
	Dissolved	Particulate	Dissolved	Particulate
Naphthalene	688 (150-1533)[a]	75 (17-267)	178 (67-467)	83 (<10-150)
Acenaphthylene	573 (<100-768)	55 (<100-159)	157 (<100-232)	<100
Acenaphthene	185 (<5-378)	410 (<5-540)	171 (<5-310)	43 (<5-108)
Phenanthrene	67 (<10-128)	<10	<10	<10
Anthracene	64 (<10-121)	<10	17 (<10-30)	<10

Note: [a]( ) = Range

The area distribution of total PAHs in the Chao Phraya estuary samples is shown in Figure 3. Higher concentrations were found in samples from the upper reach of the estuary, particularly during the wet season period. This implies that besides industrial discharges which cause an increase in the PAHs content of the river, heavy rainfall and water runoff from farm lands and from the industrial estates of the Central Plain of Thailand can also strongly influence the PAHs content of the river. PAHs particularly those from motor oils, asphalt particles and street dust can reach the river via natural runoff from land during rainstorms. The comparatively lower level in the dry season (February 2000) could be explained by the almost complete absence of rainfall during that month.

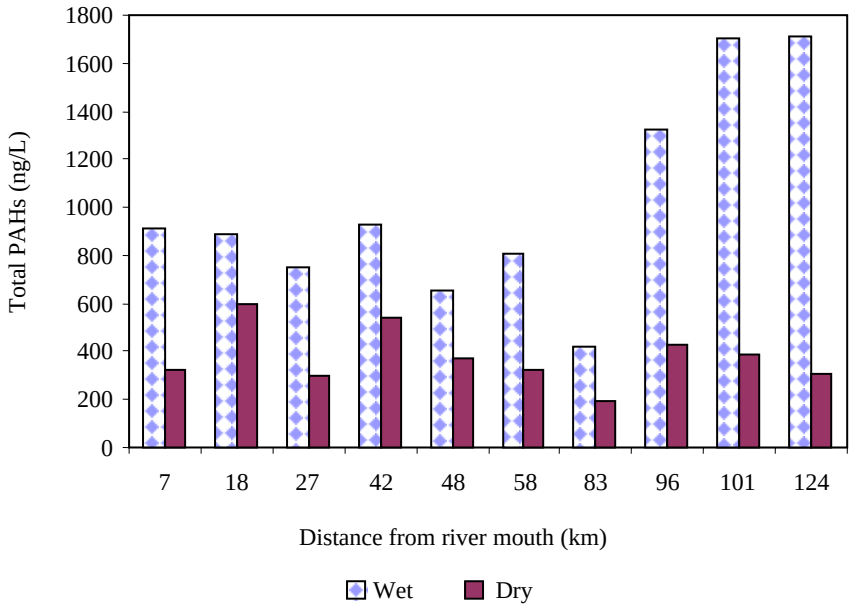

Figure 3. Distribution of PAHs in the Chao Phraya River estuary.

Using published data on river discharge and the concentrations measured in the water samples, tentative input rates of selected PAHs into the Upper Gulf of Thailand are calculated (Table 4). The water discharged by the Chao Phraya River during October 1999 was almost 25 fold that occurring in February 2000. Presumably, during that period a high input of PAHs to the Upper Gulf of Thailand took place. Approximately 120 Kg to 10 tons of PAHs were discharged from the Chao Phraya monthly.

Table 4. Input of PAHs identified in Chao Phraya River water into the Gulf of Thailand.

Analyte	Input (tons)	
	October 1999	February 2000
Naphthalene	3.73	0.05
Acenaphthylene	3.07	0.03
Acenaphthene	2.91	0.04
Phenanthrene	0.33	-
Anthracene	0.31	0.003

CONCLUSIONS

The use of solid phase (bonded phases, Empore™) in PAH extraction is shown to be convenient with a simplified analysis process and good recovery results. The technique appears to be suitable for the study of PAH contamination in various aquatic environments. Compared with traditional liquid/liquid extraction (LLE), SPE provides some significant advantages, such as reduced sample volumes and small volumes of elution solvent.

The results of this study indicate that at least during the times of the study, PAHs have been found to present in all samples from the Chao Phraya River estuary, at concentrations that vary with sampling locations. Total identified PAH concentrations were found to be higher during the wet season compared to the dry season. The dominant PAHs found are naphthalene, acenaphthene and acenaphthylene. However, the concentration of these three dominant PAHs in the surface waters of the Chao Phraya estuary never reached the maximum admissible concentration established by the WHO and by the Government of Canada limits, allowing for an improvement in the quality of the water to be used for drinking.

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REFERENCES

1. Basu, D.K. and J. Saxena. (1978) "Polynuclear aromatic hydrocarbons in selected U.S. drinking waters and their raw water sources" *Environ. Sci. Technol*. **12**, 795-798.
2. LaFlamme, R.E. and R.A. Hites. (1978) "The global distribution of polycyclic aromatic hydrocarbons in recent sediments", *Geochim. Cosmochim. Acta*. **42**, 289–294.
3. Uthe, J. (1979) Polycyclic aromatic hydrocarbons in the marine environment. Fish. Mar. Ser. Tech. Report 914:iii, Dept. of Fisheries and Oceans, Canada, Ottawa. 30
4. Ehrhardt, M. (1987) "Photo-oxidation products of fossil fuel components in the water of Hamilton Harbour, Bermuda", *Marine Chemistry*. **22**, 85-94.
5. Ehrhardt, M., G. Wattayakorn and R. Dawson (1990) "GC/MS based analyses of individual organic constituents of Chao Phraya river water and estimated discharge rates into the Upper Gulf of Thailand", *Estuarine Coastal and Shelf Science*. **30**, 439-451.
6. Hoof, F.V. and T.V. Rompuy. (1991) "Polycyclic aromatic hydrocarbons in the river Meuse basin" In: Organic Micropollutants in the Aquatic Environment. G. Angeletti and A. Bjorseth (eds.), Kluwer Academic Publishers, London. 432-436.
7. Harvey, R.G. (1985) "ACS Symposium Series 283: Polycyclic aromatic hydrocarbons and carcinogenesis" *Washington, D.C., American Chemical Society.*
8. Eichelberger, J.W., T.D. Bekynmad and W.L. Budde. (1988) "Determination of organic compounds in Drinking water by liquid-solid extraction and capillary column gas chromatography/mass spectrometry", *U.S. EPA Method*. **525**, 325-356. Environmental Monitoring Systems Laboratory, Ohio, U.S.A.

9. Bashe, W.J. and T.V. Baker. (1990) "Determination of polycyclic aromatic hydrocarbons in drinking water by liquid-solid extraction and HPLC with coupled ultraviolet and fluorescence detection" *U.S. EPA Method* **550(1)**, 143-167. Environmental Monitoring Systems Laboratory, Ohio, U.S.A.
10. World Health Organization (WHO), (1971) "International Standards for Drinking Water, 3rd Edition" Geneva.
11. Ministry of Environment, Canada. (1996) "The Canadian (Federal) Guidelines in Water and Drinking Water Quality" "Generic Numerical Water Standards, 1996", Vancouver.
12. Wakeham, S., C. Schaffner, and G. Walter. (1980) "Polycyclic aromatic hydrocarbons in recent lake sediments-I" Compounds having anthropogenic origins. Geochim. *Cosmochim. Acta*. **44**, 403-413.
13. Zurcher, F., M. Thuer and J.A. Davis. (1980) "Importance of particulate matter on the load of hydrocarbons of motorway runoff and secondary effluents" In: Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment. B.K. Afghan and D. Mackay (eds.), Plenum Press, New York, 373-385.

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