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Polycyclic Aromatic Hydrocarbons in Water from the Menderes River, Turkey

Hatice Ardag · Mustafa Z. Ozel · Alaattin Sen

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Abstract This study was undertaken to determine the possibility of potential impact of PAHs on the aquatic biota. First, we had developed a new method for measuring 13 different priority pollutant PAHs in waste water samples. Then, eight different water samples collected from different sites along the Menderes River were analysed by this method involving SPE extraction and reverse-phase HPLC. The method presented here is suitable for rapid and accurate determination of PAH in surface waters and the PAH recoveries are practically quantitative. The levels of PAH in the analyzed samples range from 1.8 μg/L to 24.9 μg/L and industrialized areas were found to be highly polluted.

Keywords Polycyclic aromatic hydrocarbons · Surface water · River · Reverse-phase HPLC · Monitoring

PAHs are one of the main organic pollutants found in water. More than 100 PAHs have been found in nature; however, only 16 have been selected as priority pollutants by the United States Environmental Protection Agency (EPA) on the basis of their occurrence and carcinogecity (Barranco et al. 2003; Busetti et al. 2006). The most well-known of these is benzo[a]pyrene (BaP). Upon exposure to PAHs they are readily absorbed from the lung, gut and skin of mammals due to their high lipid solubility. They exert their mutagenic and carcinogenic activity through biotransformation to chemically reactive intermediates, which bind covalently to cellular macromolecules such as DNA. Extensive and systematic studies on the tumorigenicity of individual PAH metabolites in animals have led to the conclusion that vicinal or so called bay-region diol epoxides are the ultimate mutagenic and carcinogenic species of PAHs although not necessarily the only ones (Macek et al. 2000; Harvey et al. 2002; Rezek et al. 2008). Many PAHs have shown to be capable of producing tumors in experimental animals. When administered by the oral route, BaP and several other PAHs produced tumors in several rodent tissues such as the stomach, liver, lungs and mammary glands (Tolun et al. 2006).

The study presented in this paper is an evaluation of the contamination of a rural aquatic environment by selected compounds from the PAH group. Samples of surface water were analyzed for the presence of 13 PAHs belonging to the group (anthracene, benzo(a)anthracene, benzo(a)fluorene, benzo(b)fluorene, benzo(e)pyrene, benzo(g,h,i)-perylene, chrysene, dibenzo(a,c)anthracene, dibenzo(a,h)anthracene, fluoranthene, fluorene, phenanthrene and pyrene). We attempted to demonstrate a correlation between the levels of PAHs in water contaminated mainly by textile factories.

Materials and Methods

Acetonitrile, methanol, dichloromethane and hexane from Merck (Darmstadt, Germany) were of HPLC grade. Double distilled water was used throughout for dilution. The following PAHs were from Sigma (Missouri, ABD): fluorene (F), anthracene (A), phenanthrene (Pa), fluoranthene (Fl), pyrene (P), benzo[a]anthracene (Ba), chrysene (Ch),

H. Ardag · M. Z. Ozel
Chemistry Department, Faculty of Arts and Sciences, The University of Pamukkale, Denizli, Turkey

A. Sen (✉)
Department of Biology, Faculty of Arts and Sciences, The University of Pamukkale, 20070 Denizli, Turkey
e-mail: sena@pamukkale.edu.tr

benzo[a]fluoranthene (BaF), benzo[b]fluoranthene (BbF), benzo[e]pyrene (BeP), benzo(ghi)perylene (BghiP), dibenzo(a,c)anthracene (DBacA), dibenzo(a,h)anthracene (DBahA). All other chemicals were of analytical reagent grade and were provided by Merck (Darmstadt, Germany). C-18 SPE cartridges (500 mg/unit) were purchased from Whatman (Clifton, New Jersey, USA).

Water samples were collected in during September–November, 2003 from the Menderes River, Denizli (Turkey). The Menderes River (the ancient Maeander) runs through southwestern Turkey. It empties into the Aegean Sea after a course of 363 mi (584 km). Eight sampling stations were chosen to characterize the PAHs pollution range in such a way that river water was sampled before and after selected textile factories (Fig. 1). Briefly, water samples from 0.5 m below the surface, were collected at all eight stations. Water samples were transported to the laboratory and filtered through a glass fiber filter within 12 h of collection. The samples were stored at 4°C in dark brown glass bottles and used within two weeks. Several samples were collected between September and November in 2004.

The performance of the SPE method was tested with a model solution prior to its application to the waste water samples. For this, 500 mL of a model solution containing 10 µg/L each of the 13 PAHs was used. A C-18 SPE cartridge was used to concentrate the known and unknown PAH samples. The sample was loaded onto a 500 mg/unit cartridge previously washed with 5 mL of 1:1 (v/v) water–methanol and dried completely by means of a vacuum. A 30 min pause was then taken to ensure thorough drying had occurred before PAHs were eluted with 5 mL of hexane. The collected eluent was concentrated under a nitrogen stream with 1 mL of 1:1 (v/v) methanol-dichloromethane before analysis using HPLC.

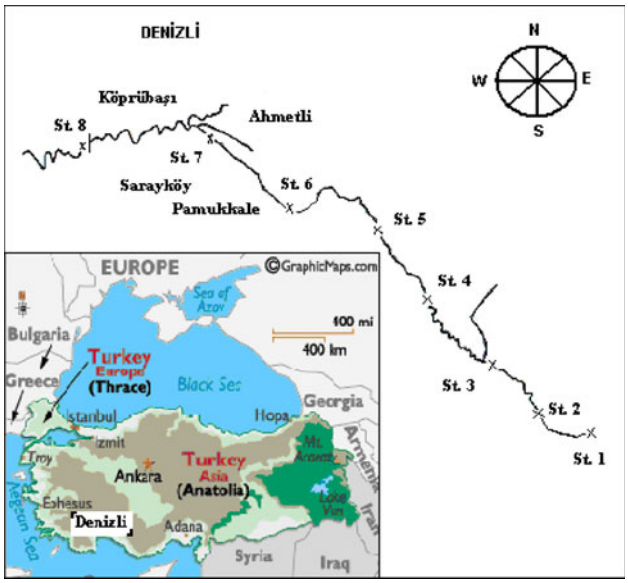

Fig. 1 Water sampling stations in the Menderes River, Denizli

HPLC was used for the determination and quantification of each PAH. The HPLC system (Thermo Quest Instruments 2215 Grand Avenue Parkway, Austin, TX 78728) consisted of a P1500 dual-pistol high pressure gradient pump, a 125 × 4.60 mm Environ Sep column (Phenomenex) and a UV detector. An oven was used to maintain the temperature of the column constant at 25°C. The solvents that constituted the mobile phase were A (acetonitrile) and B (water). The elution conditions applied were 0–5 min, 60% A; 40% B (isocratic) and 5–30 min, 100% A. The flow rate was 0.4 mL/min and the injection volume was 50 µL.

Standard deviations of the results of triplicate samples from this studies were calculated using the Microsoft Excel Spreadsheet Program.

Results and Discussion

The optimal SPE conditions were determined upon by using a known amount of 13 PAHs. For this experiment, 1 L of a model solution containing 1 µg/L each of the 13 PAHs in aqueous solution was used. Quantitative recoveries (92–102%) of 13 PAHs were achieved. The mean absolute recoveries were good, except for the lower molecular mass PAHs (F, Pa, A, Fl), which are more volatile and are therefore partially lost during the evaporation step (Table 1).

Table 1 Recoveries and standard deviations of PAHs in a model solution

PAH	Recovery (%)	Standard deviation
F	93	5
Pa	94	4
A	92	7
Fl	94	3
P	99	4
BaF	97	6
BbF	96	6
BaA	102	4
Ch	102	3
BeP	99	7
DB(a,c)A	100	6
DB(a,h)A	98	3
BghiP	96	5

Results expressed as areas under the peaks and are the average of three identical experiments

Recoveries and standard deviations of PAHs in a model solution: PAH: F, Pa, A, Fl, P, pyrene; BaF, BbF, BaA, Ch, BeP, DB(a,c)A, DB(a,h)A, BghiP

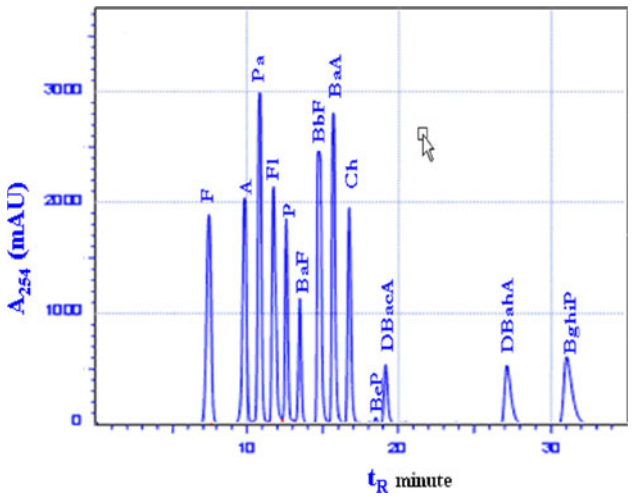

Fig. 2 Chromatogram for a standard mixture (13 PAHs)

Gradient elution with acetonitrile–water using a C_{18} column is the preferred method of HPLC separation of PAHs. Figure 2 shows the chromatogram for a standard mixture recorded by a UV–vis detector. All compounds were base-line separated with a very good resolution. 13 PAHs were selected because only these 13 PAHs were seen in the 8 water samples. Limit of detections and limit of quantifications were given in Table 2.

Denizli is an industrial, mainly textile, town in the west part of Turkey. The Menderes River flows through the industrial part of Denizli. Factories normally obtain their water supplies from the Menderes river and discharge their waste water into it. Paired water samples were taken from before the beginning of the industrialized area (Station 1), throughout the industrialized area (Station 2–7) and after the industrialized area (Station 8). The eight sampling stations were selected to characterize the range of PAH pollution in the Menderes River (Fig. 1) as affected by the textile factories.

Comparing the PAH levels detected at the 8 stations, those of stations 6, 7 and 8 were found to be higher than at the other stations (Table 3). PAHs that are not listed in Table 3 were not found in the samples. Almost all of the

Table 2 LODs and LOQs for the 16 PAHs determined in extracts from filtered liquid phases

PAH	LOD (ng/L)	LOQ (ng/L)
F	0.58	1.93
Pa	0.52	1.73
A	0.68	2.27
Fl	0.78	2.60
P	0.65	2.17
BaF	0.78	2.60
BbF	0.78	2.13
BaA	0.92	3.07
Ch	0.73	2.43
BeP	0.79	2.63
DB(a,c)A	0.98	3.27
DB(a,h)A	0.78	2.60
BghiP	0.92	3.07

The reproducibility of the system was tested by injecting 20 μL of the standard mixture ten times. The relative standard deviation was in a range of 0.9–3.3%

LODs Limit of Detections, *LOQ* Limit of quantifications

Table 3 Concentration of PAHs in the Menderes River water samples

PAH	PAH concentration in waste water samples (μg L^{-1}, $\bar{x} \pm$ SD), (N:3)							
	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8
F	0,4 ± 0,05	0,4 ± 0,03	0,5 ± 0,03	0,2 ± 0,03	0,5 ± 0,04	0,5 ± 0,05	0,9 ± 0,08	0,6 ± 0,06
Pa	0,2 ± 0,04	0,2 ± 0,01	0,2 ± 0,04	0,6 ± 0,07	0,4 ± 0,02	1,5 ± 0,08	1,8 ± 0,1	1,1 ± 0,17
A	nd	0,2 ± 0,03	0,4 ± 0,05	0,8 ± 0,09	0,7 ± 0,08	2,5 ± 0,10	1,2 ± 0,09	0,6 ± 0,07
Fl	0,6 ± 0,07	0,3 ± 0,01	0,1 ± 0,02	2 ± 0,10	0,4 ± 0,06	4,9 ± 0,20	4,5 ± 0.28	0,1 ± 0,04
P	nd	0,4 ± 0,02	0,1 ± 0,03	1,2 ± 0,20	0,6 ± 0,05	nd	4,5 ± 0,30	4,9 ± 0,38
BaF	nd	0,3 ± 0,03	nd	0,5 ± 0,02	0,3 ± 0,03	4,2 ± 0,15	2,3 ± 0.10	1,9 ± 0,45
BbF	nd	0,9 ± 0,06	nd	nd	0,8 ± 0,09	nd	6,6 ± 0.35	nd
BaA	nd	0,4 ± 0,03	0,2 ± 0,02	nd	0,2 ± 0,04	nd	1,2 ± 0,15	nd
Ch	0,1 ± 0,02	0,2 ± 0,02	0,2 ± 0,04	0,6 ± 0,05	0,3 ± 0,05	0,1 ± 0,04	1,0 ± 0,06	1,5 ± 0,36
BeP	0,2 ± 0,03	0,5 ± 0,03	0,9 ± 0,07	0,4 ± 0,04	4,2 ± 0,50	0,4 ± 0,06	0,2 ± 0,02	2,1 ± 0,43
DB(a,c)A	0,3 ± 0,05	0,3 ± 0,03	0,1 ± 0,03	0,3 ± 0,06	2,1 ± 0,90	2,2 ± 0,08	0,7 ± 0,05	2,1 ± 0,26
DB(a,h)A	nd	nd	nd	0,2 ± 0,03	nd	5,3 ± 0,25	nd	1,1 ± 0,32
BghiP	nd	nd	nd	0,3 ± 0,04	nd	0,7 ± 0,07	nd	0,2 ± 0,03
TOTAL	1,8	4,1	2,7	7,1	10,5	22,3	24,9	16,2

nd None detected, *SD* Standart derivation

Table 4 Literature data of PAH concentrations (ng/L)

Location	Sampling period	∑PAHsmean	Reference
Menderes River, Turkey	Summer 2002	∑P13PAHs1800	This study
Izmit Bay, Turkey	2002/2003	∑P15PAHs 3288	Pekey et al. (2007)
Lake Balaton, Hungary	Summer 2002	∑P13PAHs 96	Bodna′r and Hlavay (2005)
Ankara, Turkey	2001	∑P13PAHs 2416	Gaga and Tuncel (2003)
Trier, Germany (in town, trafficked area)	1999/2000	∑P17PAHs 71	De Rossi et al. (2003)
Gdan'sk, Poland	1996/1997	∑15PAHs (except NaP)5414	Polkowska et al. (2000)
Lake Balaton, Hungary	January–February 1995	∑P12PAHs 750	Kiss et al. (2001)

samples showed the presence of F, Pa, A, Fl, Ch, BeP and DB(c,c)A, and some also had a trace amount (or more) of P, BaF, BbF, BaA, DB(a,h) and BghiP.

The water from station 1, before the industrial area, contained only 6 PAHs and the maximum concentration found was 0.6 μg/L of Fl. The total concentrations of the 6 PAHs were 1.8 μg/L. This was an expected result, because the water had not yet reached the factories (the assumed polluters) (Fig. 1). There are some correlations between stations; F, Fl, Ch and DB(a,c)A were seen in all samples. However, BbF and BaA were only found at stations 2, 5 and 7. Also, DB (a,h)A and Bghi were found only in stations 4, 6 and 8. Apart from stations 5 (4.2 μg/L) and 8 (2.1 μg/L), the concentrations of BeP are in a similar range throughout all eight sampling stations. Some of the studies, in which PAH concentrations have been reported for many different areas throughout the world in precipitation samples are given in Table 4 for comparison. Volume weighted mean concentrations (ng/L) of pollutants in rain water are preferred for comparison to eliminate dilution effect.

The separation of PAHs from aqueous systems by SPE has been performed in many cases (Countway et al. 2003). It was reported that the PAHs were successfully removed from aqueous extract by SPE of C_{18} materials. Therefore, it was decided to use C18 materials for enrichment of PAHs from water samples.

The concentration of the organic solvent in the sample is a critical parameter, because, if it is too low, it may not be enough to solubilize the heavy PAHs, whereas if it is too high, the breakthrough volume for the light PAHs will be too low and they will not be retained on the SPE cartridge (Marce and Borrull 2000). Several solvents (acetonitrile, methanol, acetone and hexane) were studied. The best recoveries were obtained with hexane Lower molecular mass PAHs attained better recoveries with hexane due to its low vapour pressure, which reduces by volatilization during the evaporation step. The heavy PAHs have low polarities, so they were better eluted with apolar solvents. The order of extraction recoveries for the PAHs studied were hexane > acetonitrile > methanol > acetone. Taking into account these results, hexane was selected as the best eluting solvent, the recoveries being more than 94% for all PAHs. Barranco et al. (2003) have also reported the use of hexane as the elution solvent for extraction of PAHs using C_{18} SPE cartridges. The results are the mean of six identical experiments and the relative standard deviations were in a range of 2–8% (Table 1). The level of PAHs were found to rise gradually as water contamination increased due to industrial use after station 1 until station 7. The industrial area ends in Denizli just before station 8 (Table 3). The level of PAHs dropped at station 8 which is probably mostly due to this reason but also some natural dilution may have occurred as another two water courses join the river between stations 7 and 8 (Fig. 1). However, the level at station 8 is still very high compared with that of station 1, showing that significant pollution is occurring from the factories. This PAH polluted water then goes on to be used further downstream for agricultural purposes. Therefore, waste water treatment facilities of those factories and their discharges should be carefully regulated to prevent possible contaminations of agricultural product to which we are direct foods for both human and animals in this region. Total PAH concentrations calculated in this study were higher than the results reported in most of the data from literature given in Table 4. On the other hand, it was lower than the levels reported by Polkowska et al. (2000). However, one should be aware of the differences existing within any urban environment, where PAH levels could be significantly affected by the location of the sampling site and its proximity to emission sources. The sampling and analysis method is also a critical parameter influencing differences in observed concentrations of PAHs at different sites. In this study, the lower molecular weight (LMW) PAHs were expectedly predominant in the precipitation samples because of the proximity of the sampling point to the refinery, chemical and petrochemical industries, which represent the largest sources of lower molecular weight PAHs. The same patterns for the LMW PAHs in precipitation samples were also reported in the literature (Polkowska et al. 2000; Kiss et al. 2001).

In conclusion, the method presented here is suitable for rapid and accurate determination of PAHs in surface waters

and the PAH recoveries are practically quantitative. The levels of PAHs in the analyzed samples range from trace to 6.6 μg/L and industrialized areas were found to be highly polluted.

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