Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1073 (2005) 323–330
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of polynuclear aromatic hydrocarbons in marine samples of Siokolo Fishing Settlement

Chimezie Anyakora[a,*], Anthony Ogbeche[a], Pete Palmer[b], Herbert Coker[a]

[a] *Department of Pharmaceutical Chemistry, University of Lagos, Lagos, Nigeria*
[b] *Department of Chemistry and Biochemistry, San Francisco State University, CA, USA*

Abstract

Analysis for the presence of 16 priority polynuclear aromatic hydrocarbons (PAHs) was carried out in fish, sediment and water samples of a fishing settlement in the Niger Delta region of Nigeria which is supposed to be extensively polluted by seepages from oil discharge terminals. The determination and quantification of PAHs in water, fish and sediment samples were done by GC–MS with the aid of isotopically labeled internal standards. The 16 priority PAHs, namely naphthalene, acenaphthylene, acenaphthene, flourene, phenanthrene, anthracene, flouranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]flouranthene, benzo[k]flouranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene, were found to be present in significant amount in all three samples.

Keywords: PAH; GC–MS; Marine samples

1. Introduction

Polynuclear aromatic hydrocarbons (PAHs) are well-known environmental pollutants at low concentrations and are included in the European Union and US Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties [1]. They are generated by incomplete combustion of organic materials arising in part from natural combustion such as forest fires and volcanic eruptions [2]. Anthropogenic sources such as industrial production, transportation and waste incineration generate significant levels of PAHs [3–5]. Petroleum production, import and export of petroleum products also contribute a lot to the extent of PAH contamination especially in the marine samples [3,4,6,7]. Several PAHs are known to be potential human carcinogens; these include benz[a]anthracene, chrysene, benzo[b]flouranthene, benzo[k]flouranthene, benzo[a] pyrene and benzo[ghi]perylene [8]. The health hazard posed by these compounds has been studied extensively by several authors [9–13]. The presence of these compounds in marine samples has also been studied by numerous authors [14,15]. Fig. 1 shows the structures of 16 PAHs considered as priority by EPA.

Siokolo Fishing Settlement is a small island in the Niger Delta region of Nigeria where crude oil exploration is widely done. Its proximity to oil production facilities and seepages from oil discharge terminals have rendered the environment very polluted. Fishing is widely done in the area and fishes from there are consumed by a great percentage of the population. These facts have necessitated this study. Fat binding PAHs are capable of accumulating in the food chain [16,17]; therefore, the amount of PAHs per gram of fish consumed is a very important data to help advise on the long-term implication on human health.

So far, there has been practically no recorded investigation of PAH contamination in this environment. The objective of this work is to assess the effect of pollution on the PAH concentration of marine samples such as fish, water and sediment.

* Corresponding author. Tel.: +234 8023131333.
E-mail address: chimezie@email.com (C. Anyakora).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.10.014

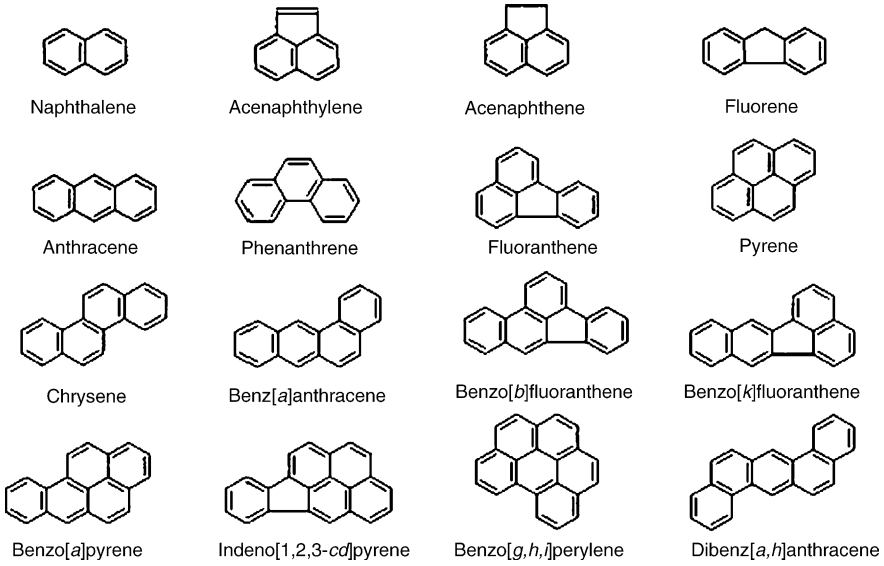

Fig. 1. The chemical structure of the 16 EPA priority PAHs.

2. Material and method

2.1. Sampling

The sediment samples were collected directly from the bed, air dried, wrapped in an aluminium foil and stored at a temperature of approximately 10 °C until ready for use. The fish samples were collected directly from the described environment, washed, wrapped in an aluminium foil and stored at a temperature of approximately 0 °C until ready for use. We restricted our fish samples to *Pseudomonas elongatus*, which is among the prevalent in this brackish water. The fishes weighed an average of 171.0 g with an average length of 25 cm. Water sample was collected from the same environment. Five hundred milliliters purified glass bottle was filled with water from this location, acidified with concentrated HCl and stored at low temperature until ready for use.

2.2. Reagents

All chemicals and reagents were of analytical grade and of highest purity possible. Dichloromethane used for the extraction was obtained from Fischer Scientific, New Jersey. Silica gel used in the cleaning up of the extract was supplied by BDH Labs (UK). A PAH standard mixture (NIST, Baltimore, MD, USA) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]flouranthene, benzo[k]flouranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene was used in this study. A mixture containing four isotopically labeled PAHs (ChemService, West Chester, PA, USA), namely [$^2H_{10}$]acenaphthene (acenaphthene-d_{10}), [$^2H_{12}$]chrysene (chrysene-d_{12}), [$^2H_{10}$]phenanthrene (phenanthrene-d_{10}) and [$^2H_{12}$]perylene (perylene-d_{12}), was used as an internal standard.

2.3. Sample preparation

2.3.1. Sediment

The sediment was extracted using a modified form of the EPA 3540 [18]. The Soxhlet apparatus consisted of a 250 ml round bottom flask, condenser and extractor tube, seated in a temperature-controlled heating mantle. A 20 g portion of the air-dried sediment sample was extracted with 150 ml of HPLC grade dichloromethane for 16 h.

2.3.2. Fish

Prior to extraction, 10 g of fish fillet was homogenized in a mortar with about 10 g of Na_2SO_4 [19] until a completely dry homogenate was obtained. The homogenate was extracted as the sediment above. The extract was passed through a silica gel column to clean off the lipids. The column was prepared by filling a 1 cm internal diameter chromatographic column with activated silica gel to the length of 5 cm and loading onto it about 1 cm of anhydrous Na_2SO_4. After conditioning, the extract which was concentrated to less than 1 ml was eluted using 10 ml of methylene chloride.

2.3.3. Water

A liquid–liquid extraction as stated in the EPA method 3510 [18] was employed in the extraction of PAHs from water sample. Before analysis, 0.5 μg each of the four internal standards, namely acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}, were added to all the samples and the volume reduced to 1 ml.

Table 1
List of molecular mass, retention time, limit of detection, limit of quantitation and *m/z* for the16 PAHs

Compound	Molecular mass	No. of rings	Retention time (min)	LOD (μg/ml)	LOQ (μg/ml)	*m/z*
Naphthalene	128.00	2	8.46	0.06	0.20	128, 115, 102, 87, 75, 63, 51
Acenaphthylene	152.00	3	13.00	0.02	0.06	152, 126, 98, 87, 76, 63, 50
Acenaphthene	154.00	3	13.26	0.02	0.06	154, 126, 102, 87, 77, 63, 50
Flourene	166.00	3	14.49	0.02	0.06	166, 139, 115, 83, 63, 50
Phenanthrene	178.00	3	17.14	0.03	0.09	178, 152, 126, 111, 99, 89, 76, 63, 50
Anthracene	178.00	3	17.22	0.02	0.06	178, 152, 126, 89, 76, 63,
Flouranthene	202.00	4	20.16	0.04	0.12	202, 174, 150, 122, 101, 87, 74, 50
Pyrene	202.00	4	20.49	0.04	0.12	202, 174, 150, 101, 88, 74, 50
Benz[a]anthracene	228.00	4	23.55	0.06	0.20	228, 200, 150, 113, 88, 63, 50
Chrysene	228.00	4	24.00	0.06	0.20	228, 202, 176, 150, 113, 101, 63
Benzo[b]flouranthene	252.00	5	26.30	0.10	0.30	252, 224, 174, 150, 126, 113, 86
Benzo[k]flouranthene	252.00	5	26.35	0.15	0.50	252, 224, 198, 150, 126, 74
Benzo[a]pyrene	252.00	5	27.18	0.15	0.50	252, 225, 161, 126, 74
Benzo[ghi]perylene	276.00	6	30.06	0.75	2.50	276, 248, 225, 207, 191, 138, 125, 97, 73
Dibenz[a,h]anthracene	278.00	5	30.17	0.90	2.70	278, 248, 225, 207, 191, 138, 125, 83, 73, 57
Indeno[1,2,3-cd]pyrene	276.00	6	30.55	1.70	5.00	276, 248, 225, 207, 191, 138, 111, 97, 73, 57

2.3.4. Preparation of calibration standards

Five standard solutions each containing 16 target compounds were prepared by diluting the standard mix (1647 mix from NIST) to desired concentrations with dichloromethane. To these were added 0.5 μg of the four internal standards, namely acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. These were transferred to a capped and sealed vial until ready for analysis.

2.3.5. GC–MS instrumentation and conditions

GC–MS analysis was carried out on a Finnigan Magnum instrument equipped with a CTC A200S autosampler and a 30 m, 0.25 i.d. DB-5ms fused silica capillary column (J & W Scientific, Folson, CA, USA). Helium was used as the carrier gas and the column head pressure was maintained at 10 psi (1 psi = 6894.76 Pa) to give an approximate flow rate of 1 ml/min. The injector and transfer line were maintained at 290 °C and 250 °C, respectively. All injection volumes were 1 μl in the splitless mode. The column temperature was initially held at 70 °C for 4 min, ramped to 300 °C at a rate of 10 °C/min, and then temperature was held at 300 °C for 10 min. The mass spectrometer was used in electron ionization mode and all spectra were acquired using a mass range of *m/z* 50–400 and automatic gain control (AGC).

2.3.6. Identification and quantification

The PAHs in the samples were identified by a combination of a retention time match and mass spectra match against the calibration standards. Quantitation was performed by the method of internal standardization using acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}. Acenaphthene-d_{10} was used as the internal standard for naphthalene, acenaphthylene, acenaphthene and flourene. Phenanthrene-d_{10} was used as the internal standard for phenanthrene, anthracene, flouranthene and pyrene. Chrysene-d_{12} was used for benz[a]anthracene and chrysene. Perylene-d_{12} was used for the rest of the PAHs.

3. Results and discussion

3.1. Analytical characteristics

Calibration curves were obtained using a series of standard solutions. All 16 calibration curves were linear with correlation coefficients from the linear regression ranging from 0.994 to 1.000. The relative standard deviation ($n = 3$) was mostly below 10%. Limits of detection and quantitation (LODs and LOQs) are provided in Table 1. The lowest LOD was 0.02 μg/ml for lower molecular mass compounds while indeno[1,2,3-cd]pyrene has the highest at 1.7 μg/ml. To evaluate the extraction efficiency for the target compounds, recovery studies were carried out using four isotopic PAH to represent two- and three-ring, four-ring, five-ring and six-ring PAHs, respectively. The recoveries ranged from 90.58% to 118% for sediment, 70.19% to 118.2% for fish, and those for water ranged from 64.78% to 91.94%. Table 1 also shows the retention time and important ions for 16 PAHs used in the quantification while Table 2 shows those for the internal standards.

3.2. GC–MS separation and identification

Prior to analyzing the samples, the efficiency of GC–MS for analysis of the target compounds was tested with a standard mixture of 16 PAHs (target compounds). Fig. 2 shows the total ion chromatogram for this analysis. A baseline separation was achieved using the EPA method TO-13A [20]

Table 2
A list of the important *m/z* values for the four internal standards

Compound	*m/z*
Acenaphthene-d_{10}	164, 132, 108, 84, 66, 51
Phenanthrene-d_{10}	188, 160, 132, 94, 80, 66, 51
Chrysene-d_{12}	240, 208, 156, 120
Perylene-d_{12}	264, 236, 207, 180, 132, 118, 86

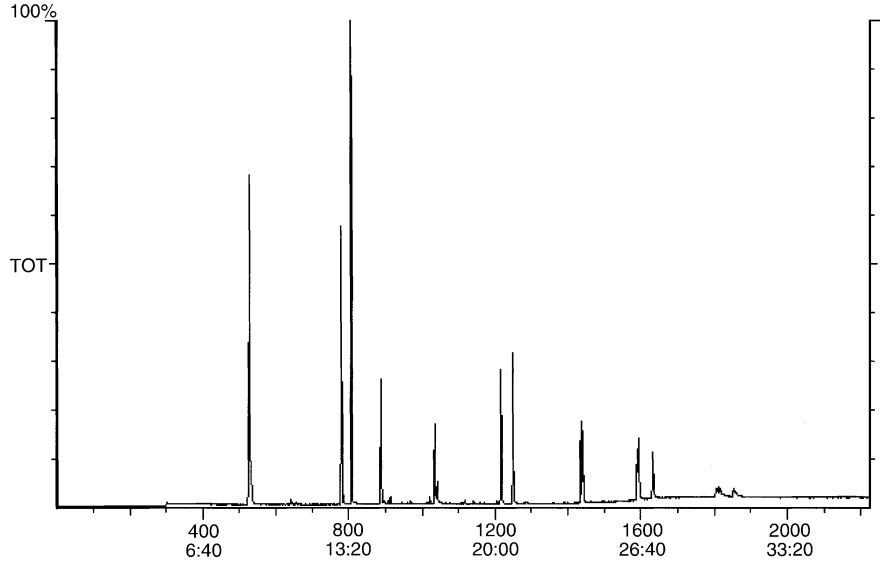

Fig. 2. Total ion chromatogram of the 16 PAHs, namely naphthylene, acenaphthylene, acenaphthene, flourene, phenanthrene, anthracene, flouranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]flouranthene, benzo[k]flouranthene, benzo[a]pyrene, benzi[ghi]perylene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene.

in 37 min. The identities of these compounds were established by combining the retention time data and the individual mass spectra. Since the target compounds are numerous and have significantly different chemical properties and retention times, four isotopic internal standards were used to monitor the 16 compounds. Acenaphthene-d_{10} with a retention time of 13.23 min was used for the two and three aromatic ring-containing PAHs within the retention time window of 8–15 min. Phenanthrene-d_{10} with a retention time of 17.10 min was used for the PAHs within the retention time range of 17–21 min. Chrysene-d_{12} was used for chrysene and benz[a]anthracene. Perylene-d_{12} was used for the remaining PAHs. Figs. 3 and 4 show the selected ion chromatograms illustrating how the internal standards effectively cover the different PAH compounds. The separation and quantitation of PAHs in the samples were achieved using the same GC–MS conditions as the standards. PAHs were quantified using internal standardization.

3.3. PAH distribution

Table 3 shows PAH concentration in water, fish and sediment samples. Figs. 5–7 show graphical distribution of these compounds in water, fish and sediment samples

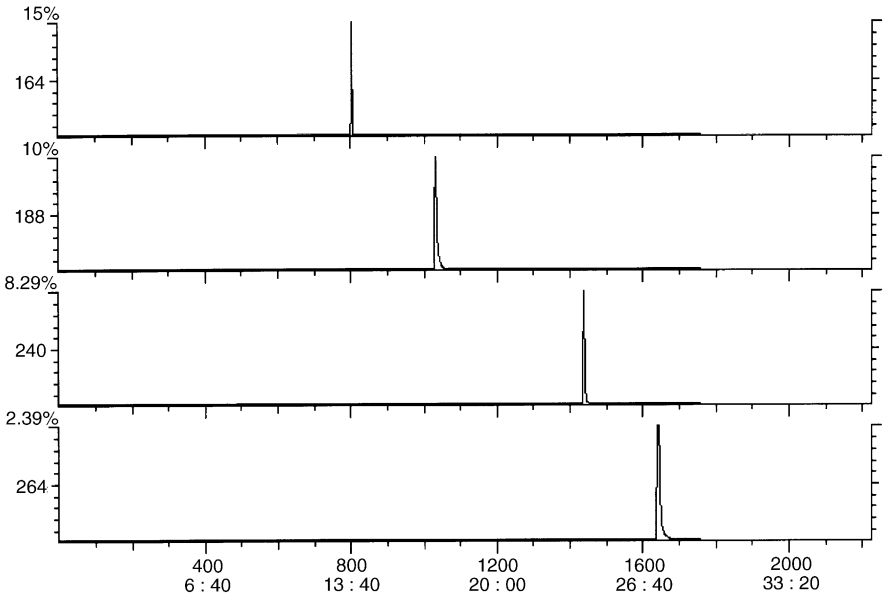

Fig. 3. Selected ion chromatograms of the four internal standards.

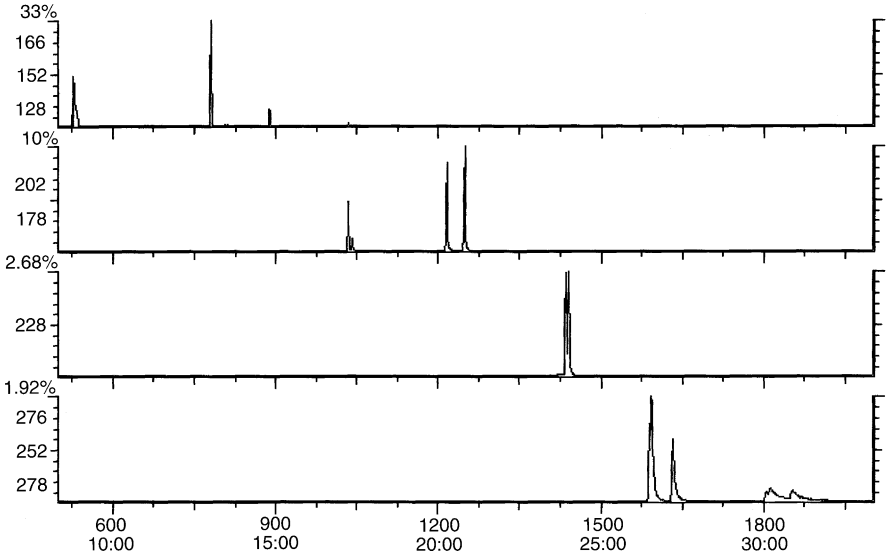

Fig. 4. Selected ion chromatogram of the 16 EPA priority PAH.

while Fig. 8 shows the comparison of the three samples. The high-molecular-mass PAHs such as benzo[ghi]perylene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene were not detected in the water sample. This can be attributed to their lower water solubility. In general, fish samples contain highest concentration of the 16 target compounds. PAHs are lipophylic, hence tend to accumulate more in fish than in sediment. This work shows that fish is the best biomarker for the level of PAH contamination in marine samples.

PAHs have received a considerable attention in recent years because of their carcinogenic properties. The average background value for uncooked fish ranges from 0.01 μg/kg to 1 μg/kg for individual PAHs [21]. Benzo[a]pyrene has been chosen as a general indicator of total PAHs in a given sample. The Joint FAO/WHO Expert Committee on Food

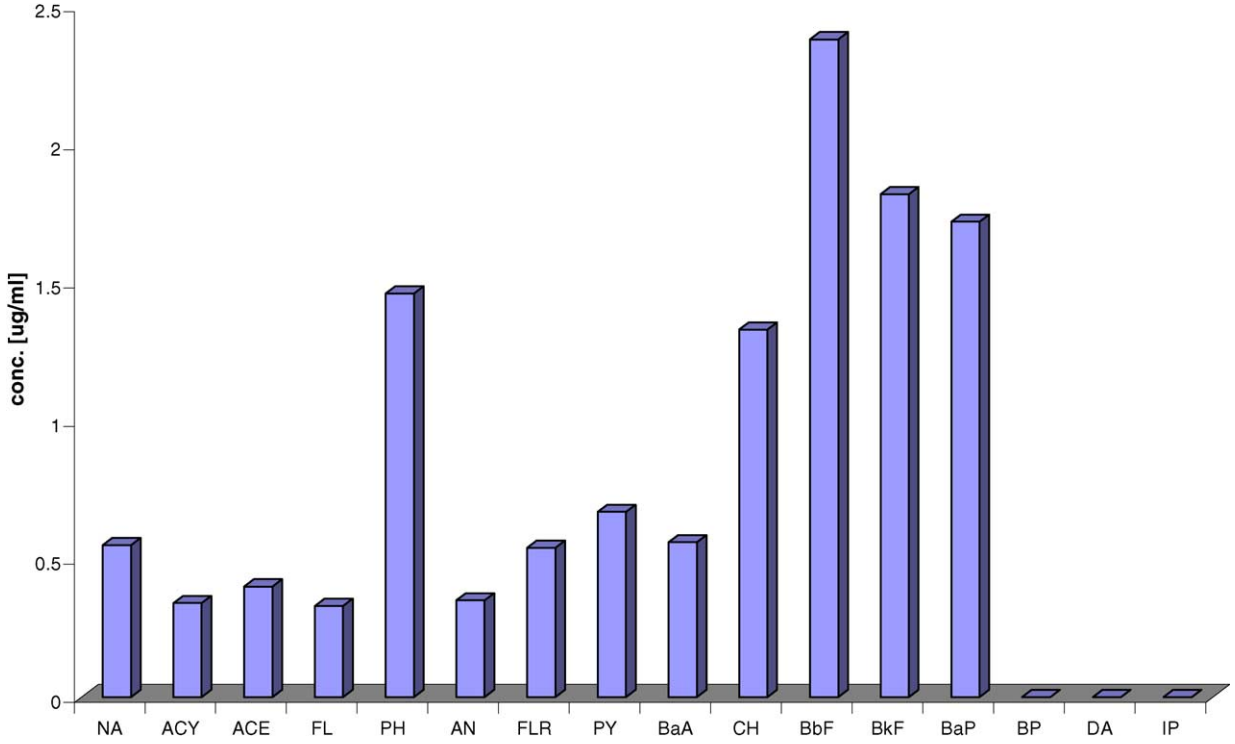

Fig. 5. The distribution of PAH in the water sample.

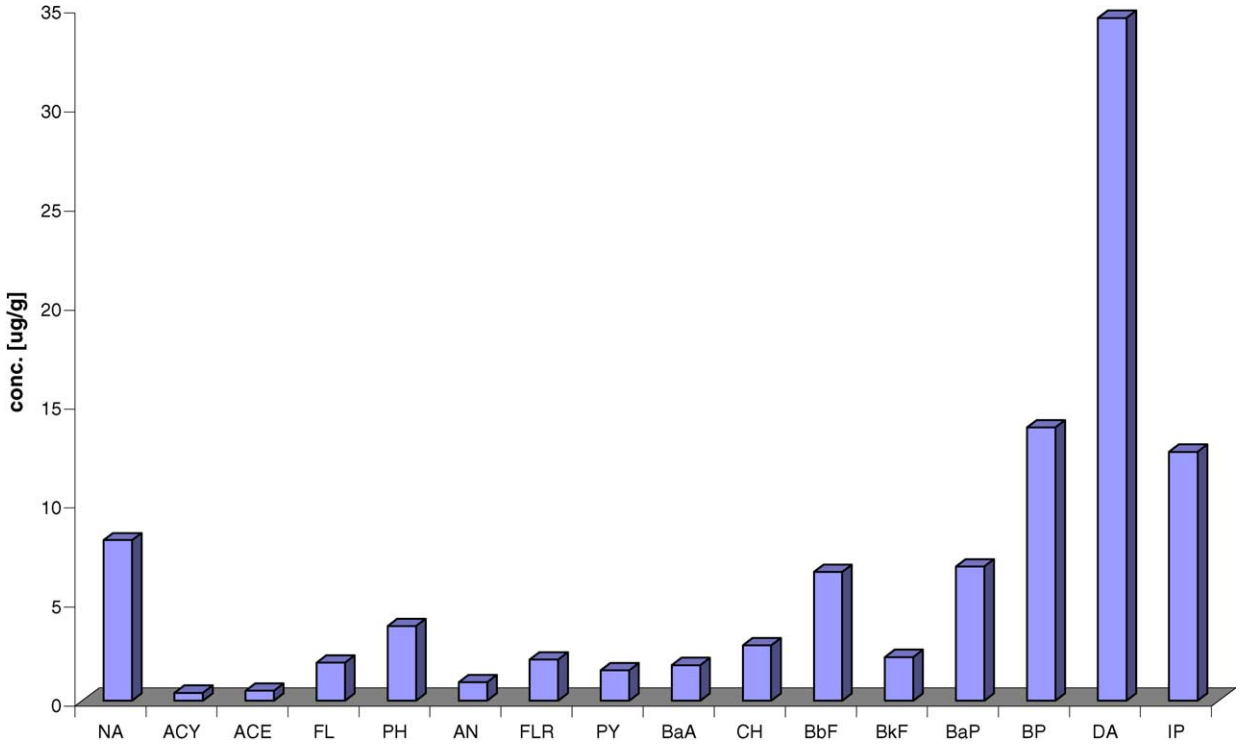

Fig. 6. The distribution of PAH in the fish sample.

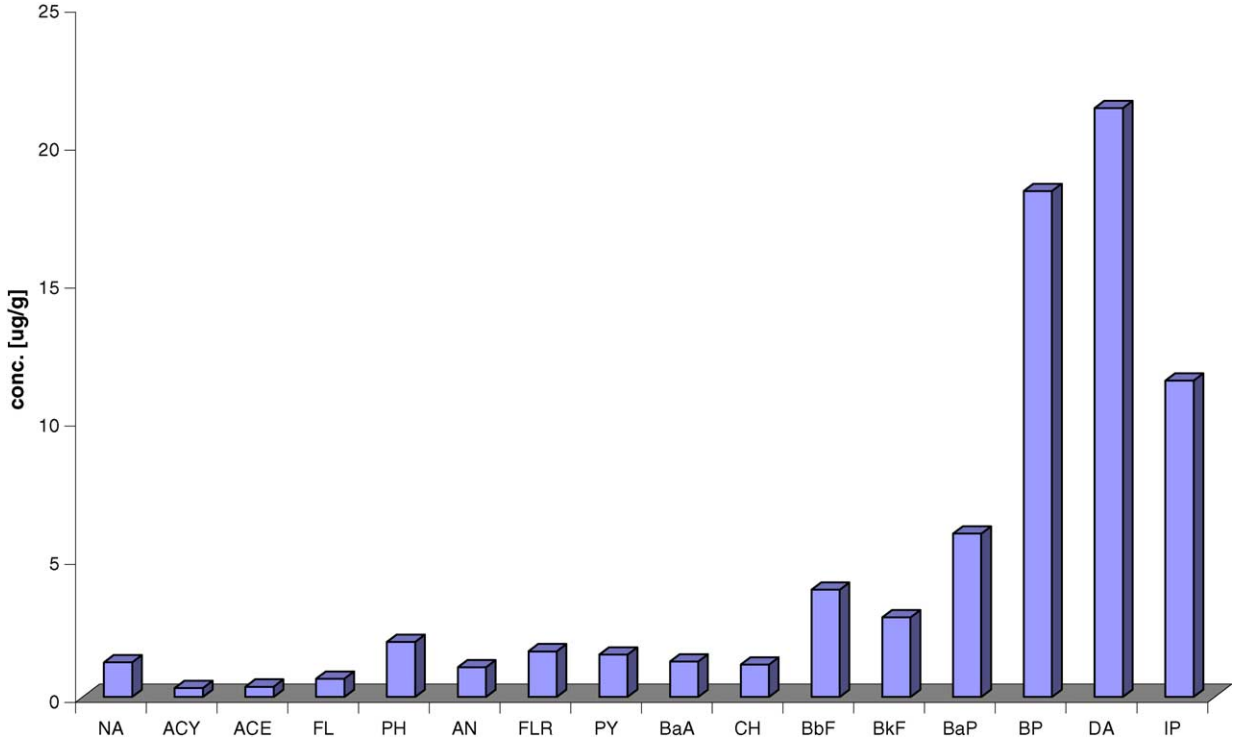

Fig. 7. The distribution of PAH in the sediment sample.

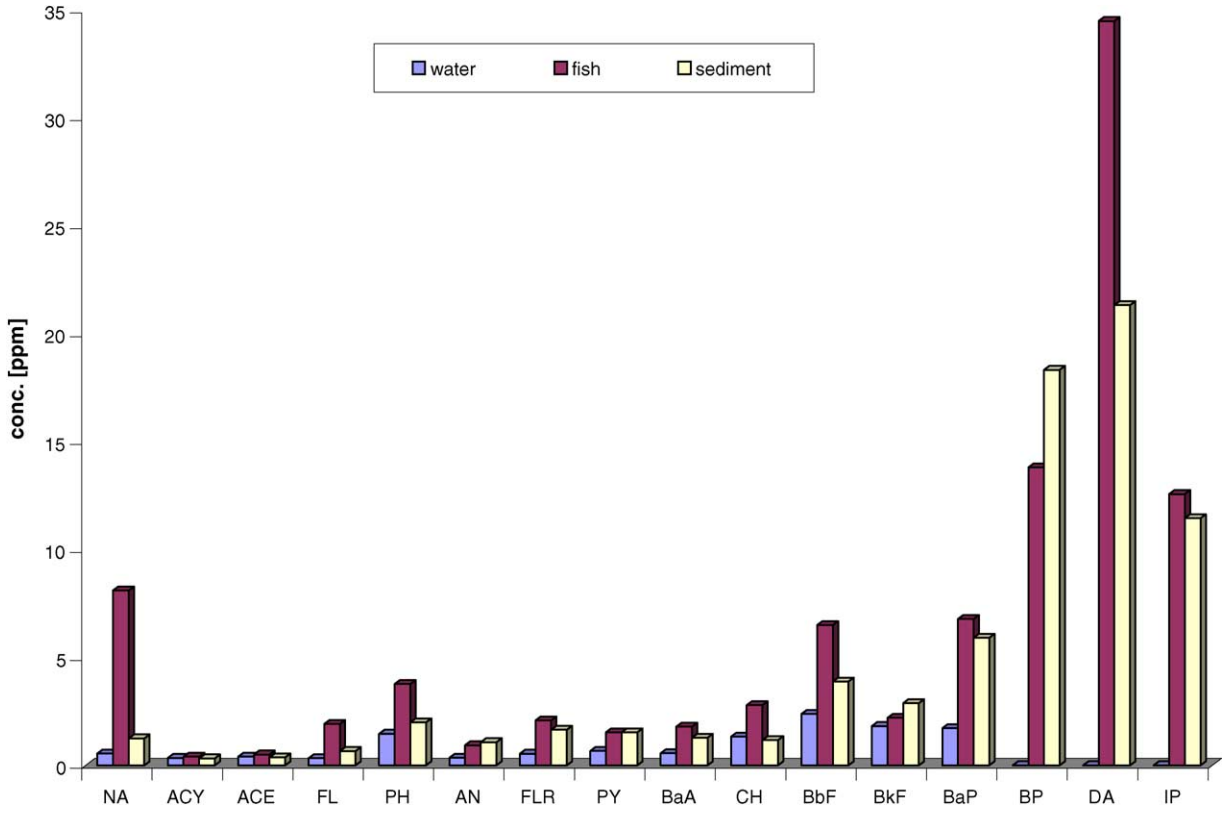

Fig. 8. The comparison of PAH distribution in the three samples.

Additives has adopted a specification, which requires that the concentration of benzo[a]pyrene should not exceed a limit of 10 μg/kg [22]. This value is much below the average of 6.78 μg/g (6780 μg/kg) obtained in the fish samples, indicating high level of contamination. It is known that the level of PAHs increases after some method of preparation such as grilling, roasting, frying and baking [23]. According to the World Health Organization study in 1997, the concentration of individual PAHs in surface and coastal waters is generally in the neighborhood of 0.05 μg/l and concentration above this point indicates some contamination; also, a study carried out by the World Health Organization in 1993 revealed that benzo[a]pyrene concentration of 0.7 μg/l corresponds to an excess lifetime cancer risk of 10^{-5}. According to studies done in the USA, in four major cities, the total PAHs in drinking water ranged between 4.7 μg/l and 600 μg/l [24] as against 12440 μg/l obtained in this study.

Table 3
PAH concentration in water, sediment and fish samples

Compound	Water (μg/ml)	Fish (μg/g)	Sediment (μg/g)	R.S.D. (%)
Naphthalene	0.55	8.1	1.25	4.76
Acenaphthylene	0.34	0.42	0.32	1.70
Acenaphthene	0.4	0.53	0.37	0.89
Flourene	0.33	1.92	0.66	6.20
Phenanthrene	1.46	3.77	1.99	7.17
Anthracene	0.35	0.93	1.07	4.92
Flouranthene	0.54	2.08	1.65	2.48
Pyrene	0.67	1.53	1.53	4.40
Benz[a]anthracene	0.56	1.79	1.28	5.36
Chrysene	1.32	2.79	1.17	4.26
Benzo[b]flouranthene	2.38	6.5	3.88	1.69
Benzo[k]flouranthene	1.82	2.21	2.88	2.71
Benzo[a]pyrene	1.72	6.78	5.91	2.11
Dibenz[a,h]anthracene	0	13.8	18.32	10.16
Benzo[ghi]perylene	0	34.48	21.32	15.79
Indeno[1,2,3-cd]pyrene	0	12.57	11.45	4.77

References

[1] M.J. Nieva-Cano, S. Rubio-Barroso, M.J. Santos-Delgado, Analyst 126 (2001) 1326.
[2] N. Grova, C. Feidt, C. Crepineau, C. Laurent, P.E. Lafargue, A. Hachimi, G. Rychen, J. Agric. Food Chem. 50 (2002) 4640.
[3] S.O. Baek, R.A. Field, M.E. Goldstone, P.W. Kirk, J.N. Lester, R.A. Perry, Water, Air Soil Pollut. 60 (1991) 279.
[4] M. Lorber, D. Cleverly, J. Schuam, L. Phillips, G. Schweer, T. Leighton, Environ. Sci. Technol. 156 (1994) 39.
[5] H.H. Yang, W.J. Lee, S.J. Chen, S.O. Lai, J. Hazard. Mater. 60 (1998) 159.
[6] S.C.U. Nwachukwu, J. Environ. Biol. 21 (2000) 241.
[7] S.C.U. Nwachukwu, P. James, T.R. Gurney, J. Environ. Biol. 22 (2001) 29.
[8] Internal Agency for Research on Cancer, IARC Monographs on Evaluation of Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental, and Experinmental Data, IARC, Lyon, 1983.

[9] M. Koyano, S. Mineki, Y. Tsunoda, O. Endo, S. Goto, J. Health Sci. 47 (2001) 452.
[10] D.O. Alonge, J. Sci. Food Agric. 43 (1988) 167.
[11] N. Dungel, J. Am. Med. Assoc. 178 (1961) 93.
[12] X. Liu, T. Korenaga, J. Health Sci. 47 (2001) 446.
[13] H. Ohshima, M. Friesen, C. Malaveille, I. Brovet, A. Hautefeutlle, H. Bartsch, Food Chem. 27 (1989) 193.
[14] R. Pancirov, R. Brown, Envir. Sci. Technol. 11 (1977) 989.
[15] J.M. Teal, K.A. Farrington, J.J. Burns, B.W. Stegeman, B.W. Tripp, C. Phinnley, Mar. Pollut. Bull. 24 (1992) 607.
[16] R.A. Roeder, M.J. Garber, G.T. Schelling, J. Anim. Sci. 76 (1998) 142.
[17] M.S. McLachlan, Chemosphere 34 (1997) 1263.
[18] US EPA, Method 3541, Organic Extraction and Sample Preparation, 1994.
[19] G. Wang, S.A. Lee, M. Lewis, B. Kamath, R.K. Archer, J. Agric. Food Chem. 47 (1999) 1062.
[20] US EPA, Method TO-13A, Compendium of Methods for Toxic Air Pollutants, 1999.
[21] European Union Health and Consumer Product Directorate General, Polycyclic Aromatic Hydrocarbons—Occurrence in Food, Dietary Exposures and Health Effect, 2002.
[22] P. Simko, J. Chromatogr. B 770 (2002) 3.
[23] Non-Heterocyclic Polycyclic Aromatic Hydrocarbons, International Programme on Chemical Safety, Environmental Health Criteria 202, World Health Organization, Geneva, 1997.
[24] ATSDR, Toxicological Profile for Polycyclic Aromatic Hydrocarbons, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA, 1995.