DETERMINATION OF 16 PRIORITY POLYCYCLIC AROMATIC HYDROCARBONS IN BOTTOM SEDIMENTS OF THE DANUBE ESTUARINE COAST BY GC/MS

K.K. TSYMBALYUK(1), Y.M. DEN'GA(1), N.A. BERLINSKY(1), V.P. ANTONOVICH(2)

(1) Ukrainian Scientific Center of Sea Ecology, Odessa,
kiorgg@yahoo.com; lawmd@te.net.ua; nberlinsky@ukr.net
(2) A.V. Bogatsky Physico- Chemical Institute of the National Academy of Sciences of Ukraine, Odessa,
antonovichvp@ukr.net

Abstract. 16 priority polycyclic hydrocarbons were identified and determined quantitatively by gas chromatography-mass spectrometry method in bottom sediments of estuarine coast of Danube. Concentration of the sum of 16 polyarenes in bottom sediments ranged from 329.6 to 1093.3 µg/kg. The total B(a)P_{eqv}, whose values at the stations of sampling ranged from 34.9 to 195.1 µg/kg, was determined to evaluate the toxicity.

Key words: Bottom sediments, polycyclic aromatic hydrocarbons, GC/MS, the estuarine coast of Danube

1. INTRODUCTION

According to the different international agreements, around 60 chemicals were included in the lists limiting their distribution and requiring the mandatory control of the content in the environment. A group of persistent organic pollutants (POPs) was nicknamed the "dirty dozen", in particular: aldrin, endrin, dieldrin, mirex, DDT, hexachlorobenzene, heptachlor, toxaphene, chlordane, polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofuranes. United Nations Organization/UN in 2003, within the project aimed at the assessment of the POP's threat to the environment and human health, recommended 16 priority polycyclic aromatic hydrocarbons (PAHs, polyarenes, Table 1) as well as six esters of phthalate acid for inclusion in the list of controlled chemicals.

PAHs are limited volatile persistent organic pollutants characterized by carcinogenic, mutagenic, teratogenic and hepatotoxic effects. They are able to be accumulated in lipid tissues of living organisms and to provoke various serious diseases.

The main anthropogenic sources of PAHs emission into the environment are industrial emissions (formed at the processing of organic raw materials at the high temperature), automotive transport (more than 150 PAHs were detected in automobile exhaust), aviation, shipping, mining and transportation of oil. As natural sources providing the PAHs background level, synthesis of these compounds by certain plants and microorganisms, forest fires, volcanic activity should be noted. The PAHs accumulation in the environment takes place simultaneously with their degradation. There are bacteria favoring PAHs metabolism in waters and soil; some PAHs are partially decomposed under UV radiation in atmosphere.

Given work is aimed at the determination of 16 priority PAHs in bottom sediments of the Danube estuarine coast. The GC/MS method, as the most efficient analytical tool, was used in this work to identify and to quantify individual PAHs in complex mixtures, after a preliminary separation and concentration of polyarenes from the sedimentary material.

2. METHODS AND MATERIALS

Superficial bottom sediments from the region of estuarine Danube coast were sampled in ten stations (Figure 1, Table 1) in May 2010, with a stainless steel Petersen bottom sampler with 0.25 m^2 sampling surface (Smedes, 1997).

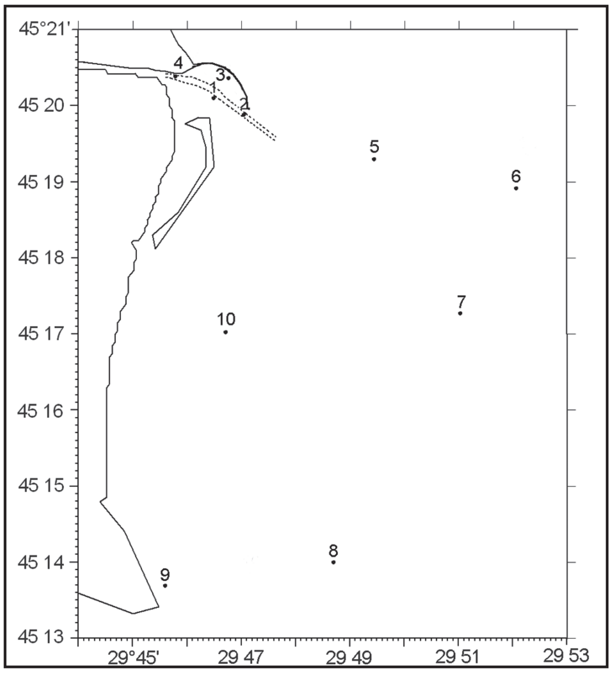

Fig. 1 Scheme of stations for the sampling of sediments at the estuarine Danube coast

A method developed by us at the Ukrainian Scientific Centre of the Sea Ecology and using as solvents hexane, dichloromethane and acetone «for residue analysis» manufactured by Merck Company was used to determine the PAHs. A standard mixture manufactured by Supelco (Cat. № 4-9156) and containing 16 priority PAHs dissolved in an acetone/benzene mixture (v\v 1:1) in individual concentrations of 2000 µg/ml was used to calibrate the device. Deuterated pyrene (pyrene-d10) manufactured by Cambridge Isotope Laboratories (Andover, MA, USA) was used as internal standard. The international standard sample of bottom sediments IAEA – 383 with certified PAHs' concentrations was used to check the accuracy of the results. Results on the analysis of this sample (Table 2) indicate both a satisfactory convergence and accuracy of data obtained by our method.

Table 1. Coordinates of stations of selection

№ of station	Coordinates	Depth, m
1	45°20`N; 29°47`E	1.5
2	45°19`N; 29°46`E	4.5
3	45°20`N; 29°46`E	2.0
4	45°20`N; 29°45`E	6.0
5	45°19`N; 29°49`E	18.0
6	45°18`N; 29°52`E	22.5
7	45°17`N; 29°51`E	22.5
8	45°14`N; 29°48`E	20.0
9	45°13`N; 29°45`E	2.0
10	45°17`N; 29°46`E	9.5

Table 2. Results of determination of 16 PAHs in SRM IAEA - 383

Compound	Certified value, µg/kg	Confidence Interval level	Obtained value, µg/kg	Sr, %
Naphthalene	96	52–110	87	40
Acenaphthalene	47	31–59	51	34
Acenaphthene	16	13–21	19	41
Fluorene	27	24–34	31	42
Phenantren	160	140–190	144	17
Antracene	30	25–34	34	16
Fluoranten	290	260–350	323	8
Pyrene	280	210–350	300	8
Benzo(a)antracene	105	83–130	120	16
Chrysene	170	120–220	187	9
Benzo(b)fluoranthene	150	96–190	173	16
Benzo(k)fluoranthene	73	48–76	59	24
Benzo(a)pyrene	120	77–140	112	23
Indeno(1,2,3-cd) pyrene	150	130–160	134	17
Dibenzo(a,h)antracene	20	18–41	27	25
Benzo(g,h,i)perylene	90	69–230	204	14

Samples of bottom sediments were freeze-dried using a CHRIST ALPHA 1 – 4 freeze-dryer. A solution of internal standard was added to dried samples weighing 2 g; the samples were subsequently extracted in a Soxhlet apparatus for 8 hours with 50 ml of dichloromethane: methanol (v \ v 3:1) mixture. The recovery factor calculated on the basis of the internal standard was 80 - 95%. Concentrations of PAHs in bottom sediments, as a rule, are 2-3 orders of magnitude lower compared with aliphatic hydrocarbons; therefore the extract was fractionated on a column filled with a sequence consisting in silica gel, aluminum oxide and sodium sulfate. The first fraction, containing mainly aliphatic hydrocarbons, was eluted with 60 mL of hexane. The second fraction, containing mainly polycyclic aromatic hydrocarbons, was eluted with 30 mL of hexane-dichloromethane (9:1v\v). The fraction containing PAHs was evaporated under a weak (flow) of nitrogen to 1 ml and it was subjected to qualitative and quantitative analysis on GC/MS Agilent 7890/5975C. The following analytical conditions were used: capillary column – HP- 5MS (30 m, 0.32mm 25 microns), rate of the carrier gas – 1,2 cm^3/min, input method – PTV, sample volume – 1µl, injector temperature – 300°C, temperature program – initial temperature 70°C, 1 min, heating rate – 10°C*min^{-1}, first isotherm – 190°C for 1 min, heating rate – 4°C*min^{-1}, second isotherm – 280°C for 20 min, ionization – E +70 eV, – data collection method – SIM, interface temperature – 285°C, temperature of source – 230°C, temperature of Quadrupole – 150°C.

The PAHs were identified by retention times and characteristic ions of identified compounds (Table 3).

3. RESULTS AND DISCUSSION

16 polyarenes were identified and determined quantitatively in bottom sediments of the Danube estuarine coast. The results are given in Table 4, where Σ*PAH* is the sum of the 16 determined PAHs, Σcarc. PAHs/ΣPAHs*100 - the carcinogenic PAHs percentage to the total PAHs, LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings), B(a)P$_{eqv}$ - the total equivalent of toxicity by benzo(a) pyrene.

Table 3. Characteristic PAHs ions

Compound	Quantitation ion (m/z)	Confirmation ions (m/z)
Naphthalene	128	127; 129
Acenaphthylene	152	153; 151
Acenaphthene	154	153; 152
Fluorene	166	165; 167
Phenanthrene	178	176; 179
Anthracene	178	89; 179
Fluoranthen	202	203; 101
Pyrene	202	203; 101
Benzo(a)anthracene	228	229; 114
Chrysene	228	229; 114
Benzo(b)fluoranthene	252	253; 126
Benzo(k)fluoranthene	252	253; 125
Benzo(a)pyrene	252	253; 126
Indeno(1,2,3-cd)pyrene	276	138; 227
Dibenzo(a,h)anthracene	278	139; 279
Benzo(g,h,i)perylene	276	138; 277

According to Traven *et al.* (Traven *et al.* 2008), the marine bottom sediments can be classified into three categories, depending on the total content of PAHs: slightly polluted (ΣPAHs<250 µg/kg), polluted (ΣPAHs from 250 to 500 µg/kg), highly polluted (ΣPAHs >500 µg/kg). Our results (Table 4) allow the classification of the Danube estuarine coast bottom sediments as polluted and highly polluted.

Samples 1-4 contain the highest number of PAHs. The sum of 16 PAHs at these stations ranged from 0.8 to 1.1 mg/kg. The following individual compounds are dominant at all stations): phenanthrene (from 28.3 to 165.7 µg/kg), fluoranthene (from 36.7 to 154.8 µg/kg), pyrene (from 37.0 to 128.5 µg/kg), benzo(a)anthracene (from 11.4 to 100.3 µg/kg), chrysene (from 16.9 to 87.0 µg/kg), benzo(b)fluoranthene

Table 4. The results of GC/MS determination of PAHs in bottom sediments of estuarine coast of Danube

№ of station	Σ PAHs, µg/kg	Σ carc PAHs / Σ PAHs·100	LMW/HMW	B(a)P$_{eqv}$, µg/kg	Total PAHs index
1	809.8	46	0.32	124.7	6.28
2	1093.3	50	0.23	195.1	6.15
3	1078.2	46	0.31	156.8	6.13
4	789.6	47	0.31	121.4	6.14
5	352.7	47	0.31	48.7	6.14
6	462.5	46	0.30	65.5	6.56
7	341.1	40	0.52	37.9	4.72
8	622.5	52	0.25	103.5	8.58
9	329.6	40	0.52	34.9	4.92
10	557.8	43	0.28	69.6	5.47

(from 27.8 to 75.5 µg/kg), benzo(k)fluoranthene (from 20.7 to 76.8 µg/kg), benzo(a)pyrene (from 22.6 to 131.6 µg/kg). Maximum contaminant levels (MCLs) of polyarenes in marine bottom sediments are not regulated in Ukraine. However, according to the norms accepted in the European Union (The Netherlands), MCL in bottom sediments for benzo(a)pyrene is 25 µg/kg, for chrysene is 20 µg/kg and for fluoranthene - 15 µg/kg.

The degree of the PAHs anthropogenesis is usually estimated as the ratio of so-called „technogenic" PAHs to the „natural" ones. High molecular PAHs with a large number of aromatic rings are considered as technogenic ones, and low molecular PAHs with the 2-3 aromatic rings are natural ones. Polycyclic aromatic hydrocarbons formed under natural conditions are characterized by the dominance of relatively low molecular PAHs, whereas the concentration of macromolecular compounds is low. As shown in Fig. 2, PAHs with 4-6 aromatic rings are dominant in the bottom sediments from the estuarine Danube area.

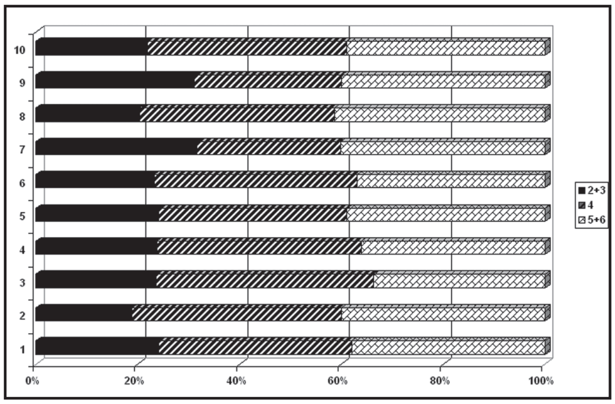

Fig. 2 Percentage composition of ΣPAH in the bottom sediments of Danube estuarine according to the number of aromatic rings (the number of stations is located along the vertical axis; the PAHs percentage of on the number of rings is located along horizontal axis.

LMW/HMW indexes (low molecular weight PAHs (2-3rings)/high molecular weight PAHs (4-6 rings)) were calculated for all samples. The values of LMW/HMW<1 indicate a PAHs pollution of pyrolytic origin (Magi *et al.*, 2002). As shown in Table 4, the value of LMW/HMW index at all stations of sampling is less than 1 and it ranges from 0.23 to 0.52. Noteworthy, the lowest value of the LMW/HMW index was found at stations 2 and 8, and the highest 0.52 - at stations 7 and 9, also having the lowest ΣPAH concentrations. The data indicate that the most significant technogenic PAHs pollution was at stations 2 and 8.

International Agency for Research on Cancer (IARC) has classified seven PAHs, namely: (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a, h)anthracene) as probable (2A) and possible (2B) carcinogens (International agency for research on Cancer, 1987). Figure 3 shows the distribution of total concentrations of 16 PAHs, seven carcinogenic PAHs and total toxicity equivalent by benzo(a)pyrene (B(a) P_{eqv} mkg/kg), in each station. The amount of carcinogenic PAHs in samples of sediments 1-10 varies from 130.5 to 551.9 µg/kg, representing 40% and 52% of the total concentration of polyarenes.

The largest percentage of carcinogenic PAHs was in bottom sediments from stations 2 and 8 (50% and 52%, respectively). Benzo(a)pyrene is the only PAH out of the sixteen determined polyarenes for which toxicological data for the calculation of the carcinogenicity factor are available (Peters *et al.*, 1999). Therefore, to assess PAHs' total toxicity, the total $B(a)P_{eqv}$ was calculated as total equivalent concentration as benzo(a)pyrene), using the toxicity equivalent (TE) for each PAH (Table 5) (Tsai *et al.*, 2004), and the formula:

$$\text{Total } B(a)P_{eqv} = \sum_i C_i \times TE_i$$

where:

C_i – concentration of the respective PAHs µg/kg

TE_i – the toxicity equivalent of the corresponding PAHs

Table 5. PAH toxicity equivalent

Compound	TE	Compound	TE
Acenaphthalene	0.001	Benzo(a)antracene	0.100
Acenaphthene	0.001	Chrysene	0.010
Fluorene	0.001	Benzo(b)fluranthene	0.100
Phenantren	0.001	Benzo(k) fluranthene	0.100
Antracene	0.010	Benzo(a)pyrene	1.000
Fluoranten	0.001	Indeno(1,2,3-cd) pyrene	0.100
Pyrene	0.001	Dibenzo(a,h)antracene	1.000
Benzo(g,h,i) perylene	0.0100		

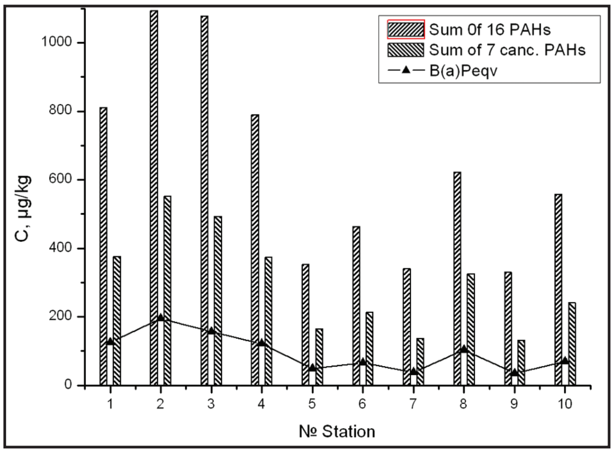

Fig. 3 Levels of bottom sediments pollutions from the Danube estuary, the sum of 16 PAHs, by 7 carcinogenic PAHs and total $B(a)P_{eqv}$.

The calculated total $B(a)P_{eqv}$ at sampling stations was within the range 34.9 to 195.1 µg/kg. The largest B(a) P_{eqv} was found at stations 1-4 (121.4 to 195.1 µg/kg). Noteworthy, the P_{eqv} high value at the station 8, where it was 103.5 µg/kg.

Possible sources of the PAHs emission into environment can be found through the use of indices, which are the ratio of concentrations of some PAHs in the sample (Yunker *et al.*, 2002, Soclo *et al.*, 2000, Mannino *et al.*, 2008).

It is assumed that the ratio Fl/Fl+Py<0,4 indicates pollution by polyarenes of petroleum origin. The values of this ratio within the range from 0,4 to 0,5 are typical for the pollution by combustion products of liquid fuel and oil, >0.5 indicates pollutions that will occur as a result of combustion of kerosene, coal, creosote, etc. (Yunker *et al.*, 2002). The values of the ration Fl/Fl+Py (fluoranthene/fluoranthene + pyrene) varies from 0.47 to 0.64 (Figure 4), with the highest value in station 8.

Ratio An/178 (Anthracene/Anthracene plus Phenanthrene) may also characterize the nature of the PAHs formation in the environment. Ratio An/178 <0,1 indicates the PAHs formation as a result of low temperature processes (oil) while An/178>0,1 indicates the dominance of combustion processes for PAHs formation. For our samples the An/178 ratios range from 0.09 to 0.32 (Figure 4), with the highest value also in station 8. The ratio BaA/228 (Benzo(a)Anthracene/Benzo(a)Anthracene+Chrisen) <0.2 indicates a petroleum source for PAHs; values from 0,2 to 0,35 points to a mixed pollution source (oil or combustion) while values >0.35 indicates pollution by polyarenes formed as a result of pyrolytic processes (Yunker *et al.*, 2002). In our case the values of the BaA/228 ratio (Figure 4) vary from 0,4 to 0,6, again with the highest value in station 8.

The ratio IP/(IP + BghiP) (Indeno(1,2,3-cd)pyrene Indeno(1,2,3-cd)pyrene plus Benzo(g, h, i)perylene) <0.2 indicates a petroleum origin of PAHS, values from 0.2 to 0.5 points to liquid fossil fuel combustion and values >0.5 suggest grass, wood and coal combustion (Yunker *et al.*, 2002). In the estuarine Danube area the values of IP/(IP + BghiP) vary from 0.37 to 0.6. As usual, the highest values were recorded in station 8.

In some cases, the ratios of polycyclic aromatic hydrocarbons (Fl/(Fl+Py), An/178, IP/(IP +BghiP) and BaA/228) are not compatible, since sources of PAHs emission may be different, random and irregular. To compensate we calculated the total PAHs index (Yunker *et al.*, 2002, Mannino *et al.*, 2008) representing a normalized sum of the previously discussed indices, using the formula:

$$\text{Total PAHs index} = \frac{\frac{\text{Fl}}{(\text{Fl+Py})}}{0.4} + \frac{\frac{\text{An}}{178}}{0.1} + \frac{\frac{\text{BaA}}{228}}{0.2} + \frac{\frac{\text{Ip}}{(\text{Ip+BghiP})}}{0.2}$$

Typically, high-temperature processes in case of total index PAHs> 4 are considered as sources of PAHs, and values <4 indicate mainly the oil sources. The values obtained for the total index of PAHs for the stations of Danube estuary coast are within the range from 4.72 to 8.58 (Table 4), which confirms the anthropogenic origin of PAHs as a result of high temperature processes.

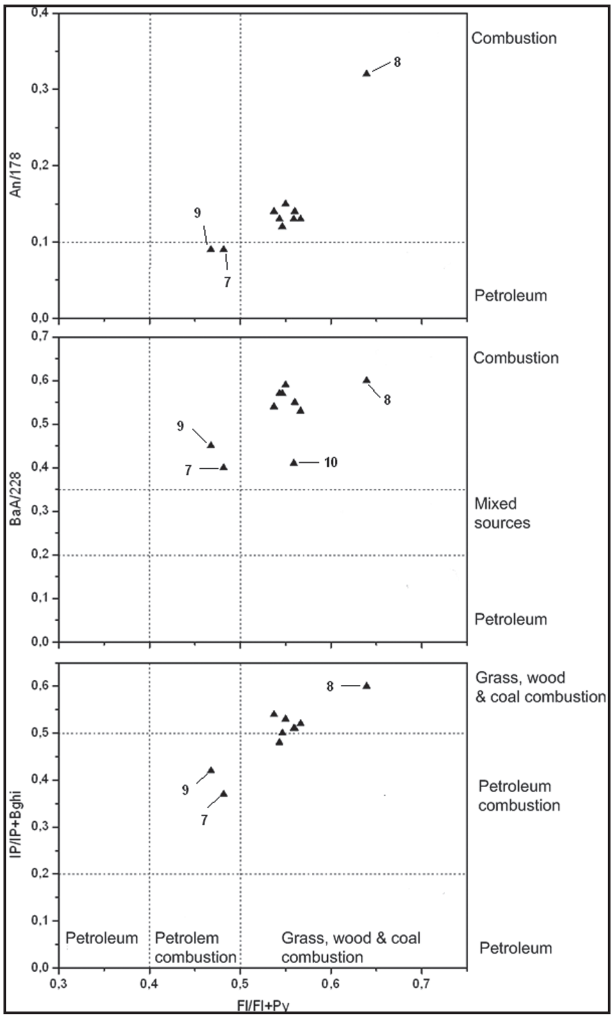

Fig. 4 Values of the ratio Fl/Fl +Py, BaA/228, IP / IP+ BghiP, An/178 for stations of Danube Seaside

An interesting fact is that the total value of the PAH index for stations 1-6 and 10 varies slightly (5.47-6.28), for stations 7 and 9, the value is somewhat lower (4.72-4.92), but the index rises sharply at 8.58 for station 8. Taking into account the substantial differences between indices LMW/HMW, Fl/Fl +Py, An/178, IP/IP +BghiP, BaA/228 and the total index of PAHs for the station 8, one can suggest a different source of pollution than for the remaining stations.

Lower concentrations of the sum of 16 polyarenes and slight differences of indices at stations 7 and 9 probably can be explained by a different granulometric composition of the sediments in the case of station 9 and by the greater distance of station 7 from the pollution sources.

The results of research can be indicators of anthropogenic pressure to the shallow waters in the Danube area exerted by industrial and municipal waste emissions and economic activity of the ports and dumping.

REFERENCES

International agency for research on Cancer, 1987. IARC monographers on the evaluation of the carcinogenic risk of chemicals to humans, IARC, Lyons (Suppl. 7)

Magi E., Bianco R., Ianni C., Di Carro M., 2002. Distribution of polycyclic aromatic hydrocarbons in the sediments of the Adriatic sea Environmental pollution, vol. 119, p. 91-98

Mannino M.R., Orecchio S., 2008. Polycyclic aromatic hydrocarbons (PAHs) in indoor dust matter of Palermo (Italy) area: extraction, GC–MS analysis, distribution and sources // Atmos. Environ. vol. 42, p.1801–1817

Peters C.A., Knightes C.D., Brown D.G., 1999. Long-term composition dynamics of PAH-containing NAPLs and implications for risk assessment Environ. Sci. Technol. - vol. 33, p. 4499-4507

Smedes F., 1997. Determination of chlorbiphenyls in sediments – analytical methods Trend. Analyt. Chem. vol. 16, № 9, p. 503 – 517.

Soclo H.H., Garrigues P., Ewald M., 2000. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas Mar. Pollut. Bull. vol. 40, p. 387– 396

Traven L., Zaja R., Loncar J., Smital T., Micovic V., 2008. CYP1A induction potential and the concentration of priority pollutants in marine sediment samples – In vitro evaluation using the PLHC-1 fish hepatoma cell line Toxicology in vitro, vol. 22, Issue 6, p. 1648-1656

Tsai P.-J., Shih T.-S., Chen H.-L., Lee W.-J., Lai C.-H., Liou S.-H., 2004. Assessing and predicting the exposures of polycyclic aromatic hydrocarbons (PAHs) and their carcinogenic potencies from vehicle engine exhausts to highway toll station workers Atmos. Environ. vol. 38, p. 333–343

Yunker M.B., Macdonald R.W., Vingarzan R., Mitchell R.H., Goyette D., Sylvestre S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition Org. Geochem. vol. 33, p. 489–515