Identification of the Sources of Polycyclic Aromatic Hydrocarbons in Sediments from the Romanian Black Sea Sector
(Daniela Ţigănuş, Valentina Coatu, Luminiţa Lazăr, Andra Oros, Alina Daiana Spînu)

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IDENTIFICATION OF THE SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS IN SEDIMENTS FROM THE ROMANIAN BLACK SEA SECTOR

Daniela Ţigănuş, Valentina Coatu, Luminiţa Lazăr,
Andra Oros, Alina Daiana Spînu

NIRDEP - National Institute for Marine Research and Development
"Grigore Antipa", 300 Mamaia Blvd., 900581 Constanța, Romania,
E-mail: dtiganus@alpha.rmri.ro

ABSTRACT

The objective of the study was to determine the concentration of total PAHs and to identify the potential sources of contamination in sediments from the Romanian Black Sea sector. Sixteen priority polycyclic hydrocarbons were identified and determined quantitatively in samples of sediment collected from marine areas (depths over 20 m) from the 12 stations located between Sulina and Mangalia, during 2011-2012. The total concentration of $\sum_{16}$ PAHs ranged from 82 to 6,983 (µg/kg) dry weight in sediments. The ratios of specific PAH compound including low molecular weight/high molecular weight, phenanthrene/anthracene, fluoranthene/(fluoranthene plus pyrene), anthracene/(anthracene plus phenanthrene), indeno(1,2,3-cd)pyrene/(indeno (1,2,3-cd) pyrene plus benzo(g, h, i)perylene) were calculated to evaluate the possible sources of PAHs emission into environment. These ratios reflect pyrolitic inputs in Romanian sediments.

KEYWORDS: Polycyclic aromatic hydrocarbon - PAHs, pollution sources, Black Sea, sediments

AIMS AND BACKGROUND

Polycyclic aromatic hydrocarbons are a group of compounds which include the largest known class of chemical carcinogens and mutagens. Therefore, they have been included in the priority list of the Water Framework Directive 2000/60/EC and also sixteen of them have been regulated by the US EPA as priority pollutants [1].

It is generally accepted that the sources of PAHs are categorized into two origins: pyrolytic (incomplete combustion of organic matters - combustion fossil fuel, vehicular engine combustion, smelting, waste incinerators, forest fires and coal combustion) and petrogenic (unburned petroleum and its product - gasoline, kerosene, diesel, lubricating oil and asphalt). The sources of emissions of these compounds are numerous. PAHs released into the environment are mainly anthropogenic and it is currently assumed that PAHs are mainly of pyrolytic origin. Hydrocarbons of anthropogenic origin are introduced into the aquatic environment as a result of activities related to oil exploration, maritime transport (operational discharges and tanker accidents), harbor activities and domestic and industrial effluents [2]. Most lipophilic anthropogenic organics tend to be associated to the suspended particles in the water column due to their low solubility and accumulate in the sediments. Sediments frequently contain higher concentrations of pollutants than those found in the water column. Lipophilic PAH levels in sediments are generally several orders of magnitude higher compared to their water phase concentrations. PAHs also accumulate in fish and shellfish, especially in mussels and may lead to serious human health hazards [3].

The objectives of the study can be summarized as follows: (i) analysis of the degree of contamination by PAHs in sediments from Romanian Black Sea sector in the period 2011-2012 (ii) determination of the possible sources of the PAHs emission into environment through the use of the ratios of LMW/HMW-Low Molecular Weight/High Molecular Weight), Phe/An - phenanthrene/anthracene, Fl/Fl+Py-fluoranthene/(fluoranthene plus pyrene), An/178 - anthracene/(anthracene plus phenanthrene), BaA/228-benzo(a)anthracene/benzo(a)anthracene plus chrysene) and IP/(IP+BghiP)-indeno(1,2,3-cd)pyrene/(indeno(1,2,3-cd) pyrene plus benzo(g, h, i)perylene).

MATERIAL AND METHODS

Superficial bottom sediments from the northern and southern marine areas (depths over 20 m) of the Romanian Black Sea sector were collected from the stations located between Sulina and Mangalia during 2011-2012. The sampling locations are shown in Fig. 1. Twelve sampling stations were selected in the Romanian Black Sea sector: northern stations (1-4) - Sulina, Mila 9, Sf. Gheorghe and Portița under the Danube’s direct influence, stations in open sea (9-12), two stations (5-7) - Casino Mamaia and Costinești in vicinity of tourist areas, one station (6) - East Constanța St. 3 under the influence of vessel traffic and another station (8) - Mangalia in the neighborhood of harbor activities.

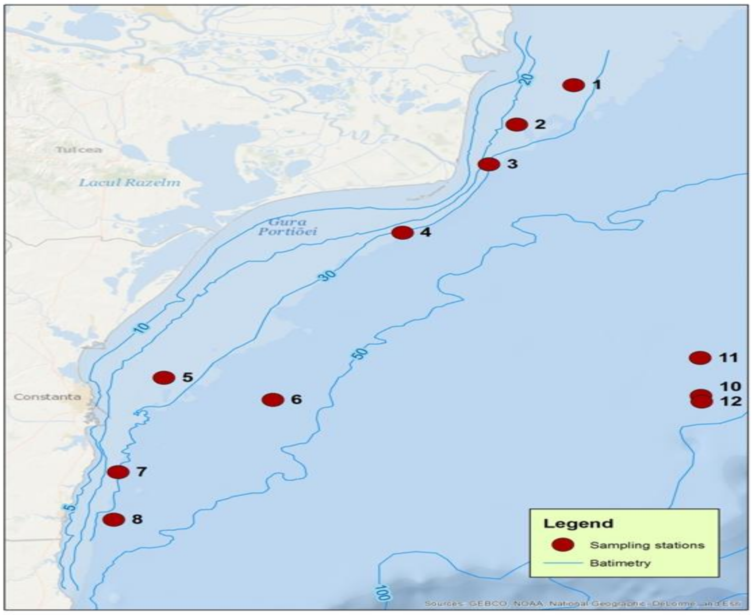

Fig. 1 - Sediment sampling locations in the Romanian Black Sea sector

A method developed by NIMRD based on the methods described in the IAEA-MEL/Marine Environmental Studies Laboratory, “Training manual on the measurement of organochlorine and petroleum hydrocarbons in environmental samples“, was used to determine the PAHs [4]. The solvents used were gas - chromatography grade manufactured by the Merck Company. A standard mixture manufactured by LGS Standards GmbH, containing 16 priority PAHs dissolved in toluene in individual concentrations of 100 μg/ml, was used to calibrate the GS-MS. Internal standard 9,10 dihydroanthracene was added to the samples for quantifying the overall recovery of the analytical procedures. The international standard sample of bottom sediments IAEA - 383 with certified PAHs concentrations was used to check the accuracy of the results.

The samples of bottom sediments were freeze-dried using a LABCONCO free zone 2.5 plus 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 85-98%. 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 (stream) of nitrogen to 1 ml and it was subjected to qualitative and quantitative analysis on GC/MS Perkin Elmer Clarus 500. The following analytical conditions were used: capillary column Elite 35 MS, stationary phase: Dimethylpolysiloxane (35% Diphenil), length 30 m, internal diameter 0,32 mm, film thickness 0,25 μm; carrier gas - helium, rate - 1 cm^3/min., injector split/splitless in split mode, split flow 15 cm^3/min., sample volume – 2 µl, injector temperature - 300°C, temperature program - initial temperature 100°C, 5 min., heating rate - 6°C*min-1, first isotherm - 250°C for 0 min., heating rate - 10°C*min.-10, second isotherm - 330°C for 10 min., ionization - E +70 eV, interface temperature - 330°C, temperature of source - 270°C,- data collection method - SIR. The PAHs were identified by retention times and characteristic ions of identified compounds

RESULTS AND DISCUSSION

PAH levels and composition

The results from the total polycyclic aromatic hydrocarbon analyses are summarized in Table 1, where $\sum_{16}$PAHs (µg/kg dry weight) is the sum of the sixteen determined PAHs, LMW/HMW - the ratio of low molecular weight PAHs (2-3 rings) to high-molecular weight PAHs (4-6 rings), CPAHs% - the carcinogenic PAHs percentage to the total PAHs, B(a)Peqv - the total equivalent of toxicity by benzo(a)pyrene (µg/kg) and Total PAHs index.

Table 1 - The results of PAH determination in bottom sediments of the Romanian Black Sea sector, 2011-2012

Station no.	***$\sum_{16}$PAHs (µg/kg)***	***LMW / HMW***	***CPAHs %***	***B(a)Peqv, (µg/kg)***	**Total PAHs index**
1	341-1525	0.1-2.8	13-52	9.4-171	6-9
2	513-3639	0.1-2.2	2-37	8.3-115	7-13
3	452-1552	0.4-0.9	1-35	0.6-173	6-14
4	1015-4284	1.0-29	1-39	4.7-305	4-12
5	141-1327	1.0-8.0	3-13	1.4-19	4-14
6	532-6519	0.4-1.7	5-25	25-26	9-16
7	281-6983	0.1-31	nd*- 3	nd-14	11-13
8	209-1822	0.7-11	2-46	1.3-233	1-16
9,10-12	82-498	0.4-1.7	33-61	12.9-36	2-10
11	107	0.5	22	8.2	16
range	82-6983	0.1-31	1-61	0.6-305	1-16

*not detected

The sum of the 16 PAHs in the sediments of the Romanian Black Sea sector varied from 82 to 6983 (µg/kg), with the mean value of 1462 (µg/kg). Extremely high PAH levels were found in southern sector stations (6, 7) in 2012. In particular, the following individual compounds are dominant at all stations: fluoranthene (410 ± 73 µg/kg), pyrene (408 ± 81 µg/kg), phenanthrene (295 ± 43 µg/kg) and anthracene (129 ± 14 µg/kg). During 2011-2012 period, it is remarkable that the fluoranthene and pyrene concentrations had significantly high contributions to the $\sum_{16}$PAHs (about 82%) in the sediment samples from station 2 -

Mila 9 located in the northern part of the Romanian Black Sea sector and indicating the presence of local sources.

According to Traven *et al.*, 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) [5].

Our results allow the classification of sediments as polluted (17%) and highly polluted (65%) and comparable to areas in the northern Black Sea and north western Black Sea (Table 2)[3, 6, 7, 8 and 9]). Generally, PAH concentrations in the 2012 samples were higher than those in the samples from 2011 (Fig. 1).

Table 2 - Comparison of PAH concentrations in sediments (μg/kg dry weight) from different areas

Survey Area	Total PAHs	References
Abyssal, Black Sea	200–1200 (Σ28 PAHs)	Wakeham (1996)
Bosphorus, Black Sea, Turkey	13.8–531 (Σ17 PAHs)	Readman et al. (2002)
Sochi, Black Sea, Russia	61.2–368 (Σ17 PAHs)	Readman et al. (2002)
Odessa, Black Sea, Ukraine	66.9–635 (Σ17 PAHs)	Readman et al. (2002)
Coastline, Black Sea, Ukraine	7.2–126 (Σ17 PAHs)	Readman et al. (2002)
Danube Coastline, Black Sea, Ukraine	30.5–608 (Σ17 PAHs)	Readman et al. (2002)
Istanbul Strait, Turkey	2.1-3152 (Σ16 PAHs)	Karacık(2008)
Istanbul Strait,Turkey	0.4–1703 (Σ8PAHs)	Taşkın (2010)
Southern Black Sea Shelf, Turkey	13–2342 (Σ15 PAHs)	Balkıs(2011)
The estuarine coast of Danube, Ukraine	329-1093 (Σ16 PAHs)	Tsymbalyuk (2010)
Romanian Black Sea sector, open sea	82 -6983 (Σ16 PAHs)	This study (2013)

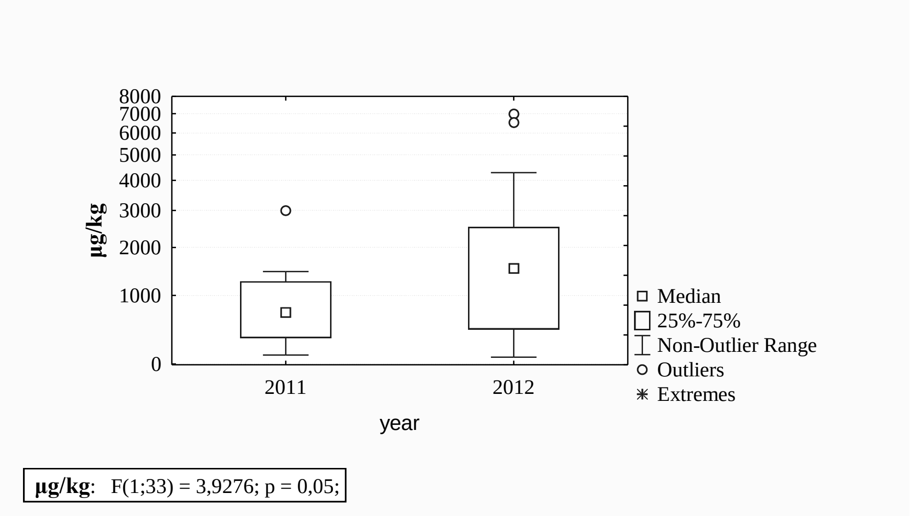

Fig. 1 - Box plot of $\sum_{16}$PAHs (μg/kg) in sediments of the Romanian Black Sea sector grouped by year, 2011 against 2012

The carcinogenicity of PAHs is due to the production of active metabolites which can covalently bind to DNA. That is considered a common mode of action for all carcinogenic PAHs [10]. The amount of potentially carcinogenic PAHs in sediments of the Romanian Black Sea sector varied from 100 to 800 μg/kg, representing 1% and 61% of the total concentration of polycyclic aromatic hydrocarbons. The high percentage ranges of carcinogenic PAHs were recorded in northern stations.

Benzo(a)pyrene is the only PAH out of the sixteen determined PAHs for which toxicological data for the calculation of the carcinogenicity factor are available [11]. The EPA (1993) approach uses benzo(a)pyrene (BaP) as the index chemical (i.e., having a relative potency of 1.0) and includes TE (toxicity equivalent) values for seven carcinogenic PAHs [10]. These PAHs include benzo(a)anthracene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, indeno(1,2,3-cd) pyrene and TE for those PAHs are 0.1, 0.1, 0.1, 1, 0.01, 0.1 and 0.1, respectively. Therefore, to assess PAHs' total toxicity, the total B(a)Peqv was calculated as total equivalent concentration as benzo(a)pyrene), using the toxicity equivalent (TE) for each PAH according to the following equation:

$$\text{Total B(a)Peqv} = \Sigma i\ Ci\ \text{X}\ TEi\ (\mu g/kg)$$

where:

Ci - concentration of the respective PAHs (μg/kg);
TEi - the toxicity equivalent of the corresponding PAHs

The calculated total B(a)Peqv at sampling stations was within the range 0.6 to 305 (μg/kg). The high values of Total B(a)Peqv (115-232 μg/kg) and $\sum$16PAHs (2995-1456 μg/kg) were recorded in the northern and southern stations Mila 9 and Mangalia and indicated the presence of local pollution sources.

Identification of PAH sources using molecular indices

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 [9, 12, 13]. The abundance ratio of two- and three ring hydrocarbons (LMW) to four- to six-ring hydrocarbon (HMW) PAHs is the commonly used ratio to help in distinguishing the petrogenic and pyrolytic sources. Values below 1 are considered as combustion sources and values above 1 are considered for petrogenic contributions [14]. As shown in Table 1, the value of LMW/HMW index at all stations of sampling ranges from 0.1 to 31. The data indicate that 54% of samples are of combustion origin, the remainder is the result of petrogenic influence. In these cases, the sources of PAHs emission may be different, random and irregular. To compensate, we calculated the Total PAHs index that represented a normalized sum of the ratios of polycyclic aromatic hydrocarbons (Fl/(Fl+Py), An/178, IP/(IP +BghiP) and BaA/228.

Table 3 shows that the Phe/An ratio in this study is in the range of nd - 45, with the highest value in northern station 2 (Mila 9). The value >10 is generally considered indicative of a predominance of petrogenic sources, whereas ratio values < 10 are characteristic of pyrolytic sources. The Phen/Anth concentration ratios indicate that the PAHs in this study area were derived from pyrolytic (>80%) rather than petrogenic sources (Table 4).

Table 3 - Values of the PAH ratios for stations of the Romanian Black Sea sector

No. of station	*Phe / An*	*Fl / Fl+Py*	*An/ 178*	*BaA/ 228*	**IP/ (IP+BghiP)**
1	1.6-8.6	0.4-0.7	0.1-0.4	0.3-0.7	nd - 1.0
2	0.2-45	0.5-0.9	0.1-0.8	0.5-0.6	nd - 0.7
3	2.9-43	0.4-0.8	0.1-0.9	0.4-0.6	nd - 0.7
4	0.5-12	0.3-0.7	0.1-0.7	0.4-0.5	nd - 0.7
5	0.2-28	0.5-1.0	0.1-0.8	0.5-0.6	nd - 0.4
6	0.4	0.5-1.0	0.7-1.0	0.2-0.4	nd - 0.3
7	0.5	0.4-1.0	0.7-1.0	0.6	nd
8	0.2-44	0.1-0.6	0.1-0.9	0.2-0.6	nd - 0.7
9,10-12	3.3	0.8	0.2-1.0	0.4	nd - 0.4
11	nd	0.3	0.1	nd	1.0
range	**nd-45**	**0.1-1.0**	**0.1-1.0**	**nd-0.7**	**nd - 0.7**

Table 4 - Interpretation of measurement data regarding the main sources of PAH emission found in sediments from the Romanian Black Sea sector

PAH ratio		*1-6* [%]*	*7-10** [%]*	**source**
Fl /Fl+Py	>0.4	**72**	**93**	**combustion**
	<0.4	28	7	petrogenic
Phe /An	<10	**86**	**84**	**combustion**
	>10	14	16	petrogenic
An/178	>0.1	**72**	**84**	**combustion**
	<0.1	28	16	petrogenic
BaA/228	>0.2	**81**	**84**	**combustion**
	<0.2	19	16	petrogenic
IP(IP+BghiP	>0.2	**78**	**71**	**combustion**
	<0.2	22	29	petrogenic
TOTAL		**78**	**83**	**combustion**

* Northern stations; ** Southern stations

The ratio Fl/Fl+Py <0.4 indicates pollution by PAHs 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. The values of the ratio Fl/Fl+Py (fluoranthene/fluoranthene + pyrene) vary from 0.1 to 1.0 with the highest values in southern stations 5, 6 and 7. More than 93% of the samples are of combustion origin; the remainder is the result of petrogenic influence (Tables 3, 4).

An/178 ratios <0.1 are usually taken as an indication of petroleum, while ratios >0.1 indicate combustion. The results in Table 3 show that the An/178 was found to be in the range of 0.1 to 1.0. For the samples from the southern stations, 84% are due to combustion and 16% to petrogenic influence (Table 4).

The ratio BaA/228 <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 PAHs formed as a result of pyrolytic processes. In our case, the values of the BaA/228 ratio (Table 3) vary from nd to 0.7. More than 84% of the southern sediments are of combustion origin (Table 4).

The ratio IP/(IP + BghiP) <0.2 indicates a petroleum origin of PAHs, values from 0.2 to 0.5 point to liquid fossil fuel combustion and values >0.5 suggest grass, wood and coal combustion. In the Romanian Black Sea sector, the values of IP/(IP + BghiP) vary from nd to 1.0. More than 78% of the nortern sediments are of combustion origin (Table 4).

The total PAHs index [12], representing a normalized sum of the previously discussed indices, was calculated using the formula:

Total PAHs index = $Fl/(Fl+Py) / 0.4 + An/178 / 0.1 + BaA/228 / 0.2 + BaA/228 / 0.2$

Source: K.K. TSYMBALYUK , Geo-Eco-Marina 17/2011, pp.71 [9]

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 in sediments from Romanian Black Sea sector (Table 1) are within the range from 1-16, with a mean of 9 which confirms the anthropogenic origin of PAHs as a result of high temperature processes. In some stations, (Fl/(Fl+Py), An/178, IP/(IP +BghiP) and BaA/228) ratios are not compatible with LMW/HMW ratio because sources of PAHs emission may be different, random and irregular. During 2011-2012, the measurement data (Table 4) show that most of the contamination comes from the combustion source for the sediments from the southern stations (83%). Finally, the total index shows that the pollution by PAHs formed as a result of pyrolytic processes in sourthern sediments was higher than that in northern stations (Fig. 2).

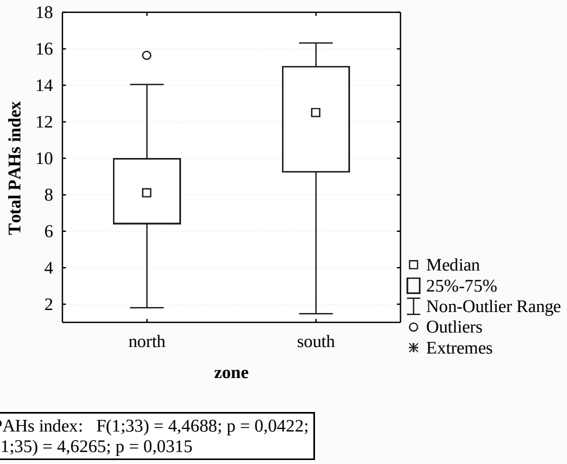

Fig. 2 - Box plot of Total PAHs index in sediments of the Romanian Black Sea sector grouped by northern against southern stations, 2011-2012

CONCLUSIONS

The analysis of the degree of contamination by PAHs in sediments from the Romanian Black Sea sector during 2011-2012 allows the classification of sediments as polluted (17%) and highly polluted (65%).

The ratio of PAH species concentration applied to identify sources of the anthropogenic pollutants indicated that the source is pyrolytic as a result of high temperature processes, meaning that inputs to the marine environment are dominated by the atmospheric deposition of contaminated particulates.

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