

*In memory of prof. dr. Simion Gocan*

**THE OCCURRENCE AND SOURCE EVALUATION OF  
POLYCYCLIC AROMATIC HYDROCARBONS IN URBAN  
ATMOSPHERE USING MOSS AS BIOMONITOR  
AND GC-MS ANALYSIS**

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**ABSTRACT:** Polycyclic aromatic hydrocarbons (PAHs) make part of the persistent organic pollutants (POPs) class. The most harmful PAHs are generated during the incomplete combustion of organic material. European legislation has recommended the use of bioindicators to estimate the impact of PAHs on the ecosystem. The aim of this work was to investigate the occurrence of atmospheric PAHs in 14 urban areas in Romania, using moss as bioindicator. The pollution emission sources, applying diagnostic ratios, were also evaluated. The PAHs were analyzed by gas chromatography-mass spectrometry (GS-MS). This study showed the presence of PAHs in moss samples in concentrations which are dependent on the sampling zone, ranging from 2.7 to 394 ng g<sup>-1</sup>. The cities with the highest amounts of total PAHs were found within the Carpathian arch, while the cities situated in the western part of the country had the lowest amounts. The FLA/(FLA+PYR) and FL/(FL+PYR) ratios indicate both the diesel emission source, fossil fuels combustion source and wood or coal combustion source which may come either from traffic, industrial activities, centralized heating systems, or from other type of industry.

**Keywords:** *Polycyclic aromatic hydrocarbons, urban atmosphere, bioindicators, moss, GC-MS*

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## 1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) make part of the persistent organic pollutants (POPs) class, having a structure composed of multiple aromatic rings. In 1976, the U. S. Environmental Protection Agency (U.S. EPA) reduced the group of hundreds of PAHs to only 16 relevant compounds [1-2], issuing a list with "16 priority PAHs" to be representative for all. Furthermore the 16 EPA PAHs became crucial subjects for environmental examinations.

The most harmful PAHs are generated during the incomplete combustion of organic material, predominantly due to anthropogenic activities [3]. In highly populated and heavily urbanized or industrialized areas, the most significant emission sources are residential heating, coal gasification, asphalt production, and motor vehicle exhaust [4]. In order to identify and assess the source of PAHs, numerous studies have been published most of them based on different diagnostic ratios [5-8].

Environmental assessment of PAHs in ambient air is essential, because many of them exhibit carcinogenic and mutagenic properties [9]. PAHs are omnipresent in two phases in the lower atmosphere with different concentration levels: in vaporous phase and in solid phase as sorbet onto aerosols [10]. After their atmospheric transportation and dry or wet deposition, processes that are strongly dependent on the relationship between vapor pressure and molecular weight [11], PAHs tend to accumulate in vegetation. Biomonitoring of PAHs on plants gained popularity in the last four decades [12-15]. The application of moss as passive accumulator for PAHs is widespread in so-called air quality assessment studies, because of (i) their strong presence at the local scale, (ii) their relative cost-effective methodology (compared to physical-chemical approach) [15], (iii) their morphological and physiological properties [16-17].

In addition, European legislation has recommended the use of bioindicators to estimate the impact of PAHs on the ecosystem [18]. Directive 2004/107/EC of the European Parliament granted the Member State the employment of any other sampling methods to assess spatial deposition of PAHs, with which they can demonstrate that the results are equivalent to those obtained with reference methods, such as: ISO standard 12884:2000.

The aim of this work was to investigate the occurrence of atmospheric PAHs in 14 urban areas in Romania, using moss as bioindicator and to establish the pollution emission sources, applying different diagnostic ratios.

## 2. RESULTS AND DISCUSSION

### 2.1. Analytical performance of the analysis method

Precision, linearity, limit of detection (LOD) and limit of quantification (LOQ) (Table 1) were the variables taken into account to study the performance of the GC-MS method used for the analysis of PAHs in moss samples.

**Table 1.** Analytical performances of GC-MS method

Compound	Linear curve equation (range 0.04-2 $\mu\text{g mL}^{-1}$ )	R <sup>2</sup>	Slope	SD	LOD ( $\mu\text{g mL}^{-1}$ )	LOQ ( $\mu\text{g mL}^{-1}$ )	RSD %
Naphthalene	y= 104860236x + 14714750	0.994	104860236	208708	0.006	0.020	3.26
Acenaphthylene	y= 86128700x + 12316361	0.992	86128700	213042	0.007	0.025	4.35
Acenaphthene	y= 29620687x + 4318189	0.992	29620687	76969	0.008	0.026	4.07
Fluorene	y= 53467933x + 7941405	0.991	53467933	90371	0.005	0.017	2.93
Phenanthrene	y= 57418106x + 8784001	0.992	57418106	164470	0.009	0.029	3.49
Anthracene	y= 65827841x + 11237350	0.981	65827841	146115	0.007	0.022	3.54
Fluoranthene	y= 52675476x + 9097934	0.985	52675476	114724	0.007	0.022	3.31
Pyrene	y= 52161938x + 9634681	0.982	52161938	105882	0.006	0.020	3.00
Benz[a]anthracene	y= 30467866x + 5076543	0.976	30467866	48552	0.005	0.016	2.36
Chrysene	y= 30795452x + 7611809	0.957	30795452	59244	0.006	0.019	2.37
Benzo[b]fluoranthene	y= 22969230x + 3977709	0.973	22969230	61071	0.008	0.027	3.43
Benzo[k]fluoranthene	y= 23772068x + 5908712	0.951	23772068	41801	0.005	0.018	2.11
Benzo[a]pyrene	y= 21785791x + 4918591	0.954	21785791	33623	0.005	0.015	1.84
Indeno(1.2.3-cd)pyrene	y= 14855868x + 3799708	0.946	14855868	71739	0.014	0.048	5.06
Dibenz[a,h]anthracene	y= 18949082x + 6355776	0.929	18949082	152213	0.024	0.080	6.62
Benzo[ghi]perylene	y= 16697664x + 5366355	0.928	16697664	79739	0.014	0.048	4.12

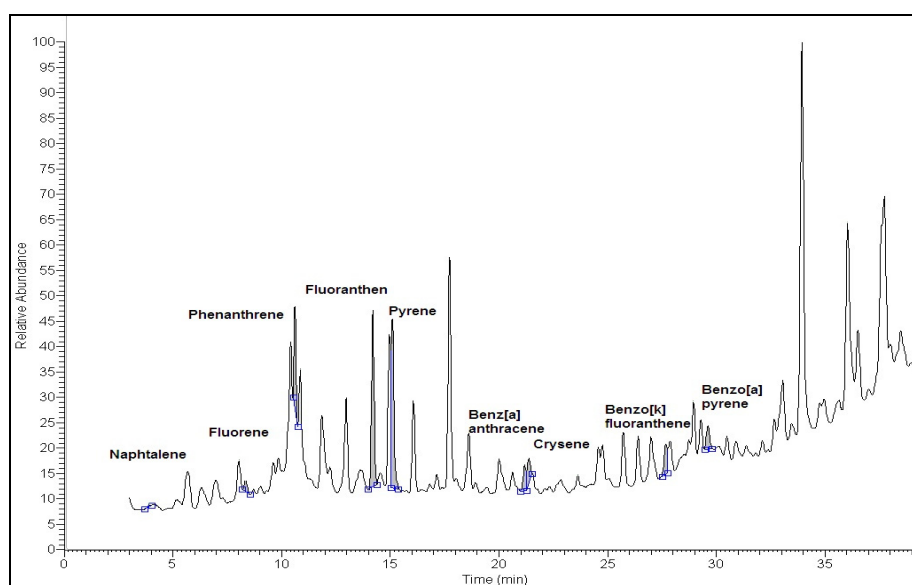
R<sup>2</sup> - coefficient of determination; SD - standard deviation; LOD - limit of detection, LOQ - limit of quantification, RSD - relative standard deviation for (n = 6);

Intra-day precision (repeatability) was expressed by means of six replicates (n=6) of a 16 PAHs standard mixture in concentration of  $0.04 \mu\text{g mL}^{-1}$ . The obtained results were situated under 15%, ranging between 1.84% and 6.62%, which prove a good repeatability of the method.

Calibration curve method was used to quantify the target compounds in real samples. In Table 1 one may observe a good linearity for all target PAHs,  $R^2$  values ranging from 0.928 to 0.994. LOD and LOQ of each of the PAHs were determined using the standard deviation and the slope of each calibration curve. LODs and LOQs were situated in the range of 0.005 and  $0.024 \mu\text{g mL}^{-1}$ , and between 0.016 and  $0.08 \mu\text{g mL}^{-1}$ , respectively.

## 2.2. Analysis of moss samples

The presence of multiple PAHs in moss samples collected from various central parks from some of Romania's largest cities is demonstrated in all analyzed samples. In Figure 1 it is shown an integrated chromatogram of an extract of mosses collected from Copou Park, Iași city, where the presence of PAHs can be observed.



**Figure 1.** SIM Chromatogram of tested PAHs in moss sample (Iași city)

The content of PAHs in the analyzed moss samples collected from the selected sampling areas is shown in Table 3.

**Table 3.** The occurrence of PAHs in analyzed moss samples

Compound	Abb.	Sample code/Concentration (ng g <sup>-1</sup> )													
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
Naphthalene	NP	0.87	nd	0.06	16.42	nd	3.9	1.95	nd	0.76	2.44	0	0	nd	nd
Acenaphthylene	ACY	1.38	nd	nd	97.43	8.85	nd	3.59	2.28	3.5	1.81	4.12	4.62	nd	2.79
Acenaphthene	ACE	nd	nd	nd	34.24	2.71	nd	1.92	nd	1.54	nd	nd	nd	nd	2.35
Fluorene	FL	1.1	2.36	1.26	5.95	5.31	nd	nd	nd	8.81	nd	nd	10.09	nd	2.68
Phenanthrene	PHE	1.57	0.98	6.69	12.84	21.15	17.17	9.33	1.79	2.54	4.92	4.14	1.87	nd	10.14
Anthracene	ANT	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.59	50.46
Fluoranthene	FLA	2.73	1.39	19.41	33.47	28.74	16.26	13.27	8.47	5.98	8.28	2.66	6.38	nd	3.15
Pyrene	PYR	0.91	nd	18.83	nd	14.6	11.06	11.57	4.85	4.74	11.02	2.62	5.22	1.13	5.78
Benz[a]anthracene	BaA	0.67	0.18	7.2	5.7	1.33	0.3	0.84	15.85	1.08	0.44	0.68	0.54	nd	0.98
Chrysene	CHR	nd	0.02	5.11	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Benzo[b]fluoranthene	BbF	nd	nd	nd	4.93	nd	nd	nd	nd	nd	nd	1.81	nd	nd	nd
Benzo[k]fluoranthene	BkF	nd	nd	12.89	23.75	nd	nd	nd	nd	nd	nd	nd	nd	nd	88.01
Benzo[a]pyrene	BaP	2.4	6.56	10.81	8.65	54.77	10.39	9.76	8.43	15.73	4.04	8.14	5.71	nd	2.53
Indeno(1,2,3-cd)pyrene	IcdP	344.9	156.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	206.3
Dibenz[a,h]anthracene	DahA	nd	4.94	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	16
Benzo[ghi]perylene	BghiP	2.49	nd	nd	nd	nd	nd	nd	3.59	nd	nd	nd	nd	nd	2.81
Total PAHs		359	173.3	82.3	243.4	137.5	59.1	52.2	45.3	44.7	33	24.2	34.4	2.7	394

nd- not detected

The results of the analyzed moss samples showed that, the cities with highest amounts of ΣPAHs were found within the Carpathian arch. The highest amount of ΣPAHs, 394 ng g<sup>-1</sup> was found in Cluj-Napoca city (S14), followed by Alba Iulia city (S1), with 359 ng g<sup>-1</sup> and Braşov city (S4), with 243 ng g<sup>-1</sup>, Sibiu city (S2), with 173.3 ng g<sup>-1</sup>, Bistriţa city (S5), with 137.5 ng g<sup>-1</sup>, and Târgu Mureş city with 45.3 ng g<sup>-1</sup>

The cities situated in the western part of the country had the lowest amounts, below  $40 \text{ ng g}^{-1}$ , top of which, with  $2.7 \text{ ng g}^{-1}$  was Timișoara city (S13), followed by Arad city (S11), with  $24.2 \text{ ng g}^{-1}$ , and Oradea city (S12), with  $34.4 \text{ ng g}^{-1}$ , although Ploiești city, situated in the southern part of Romania had a slighter smaller amount, of  $33 \text{ ng g}^{-1}$ .

The cities situated in the eastern part of the country have slightly higher concentrations of PAHs in moss samples than those situated in the southern part, with  $52.2 \text{ ng g}^{-1}$  in Bacău city (S7) and  $82.7 \text{ ng g}^{-1}$  in Iași city (S3). The south of the country had values between  $33 \text{ ng g}^{-1}$  in Ploiești city (S10),  $59 \text{ ng g}^{-1}$  in Pitești city (S6) and  $44.7 \text{ ng g}^{-1}$  in Bucharest (S9), the country's capital.

### 2.3. PAHs sources identification using isomeric ratios

To establish the source of the analyzed PAHs, several isomeric ratios were used such as:  $\Sigma$ sum of Low Molecular Weight PAHs (two-three ring PAHs) and  $\Sigma$ sum of High Molecular Weight PAHs (four-six rings PAHs) ratio ( $\Sigma_{\text{LMW}}/\Sigma_{\text{HMW}}$ ); sum of PAH resulted from combustion (FLA, PYR, BaA, CHR, BkF, BbF, BaP, IcdP and BghiP) and sum of total PAHs ( $\Sigma_{\text{COMB}}/\Sigma_{\text{PAHs}}$ ), the FL/(FL+PYR) and FLA/(FLA+PYR) ratios [19]. As one may observe in Table 3, the  $\Sigma_{\text{LMW}}/\Sigma_{\text{HMW}}$  ratio indicates that there is a pyrogenic source in all the samples, except Brașov city (S4) the obtained values being situated under 1 value. The  $\Sigma_{\text{COMB}}/\Sigma_{\text{PAHs}}$  ratio indicates a combustion source ( $\sim 1$ ) in most of the cities, except Brașov city (S4), Oradea city (S12) and Timișoara city (S13) of which the values are far away from 1. The FL/(FL+PYR) ratio indicates a petrol emission in Alba Iulia city (S1), București city (S9) and Oradea city (S12) (values under 0.5), and a diesel emission in Iași city (S3), Bistrița city (S5) and Cluj-Napoca city (S14) (values over 0.5). Finally, the FLA/(FLA+PYR) ratio, indicates petrogenic source in Cluj-Napoca city (S14) (value under 0.4), fossil fuel combustion source in Ploiești city (S10) (value between 0.4 and 0.5), and grass, wood or coal combustion source (value over 0.5) in all the other cities that could have this ratio calculated.

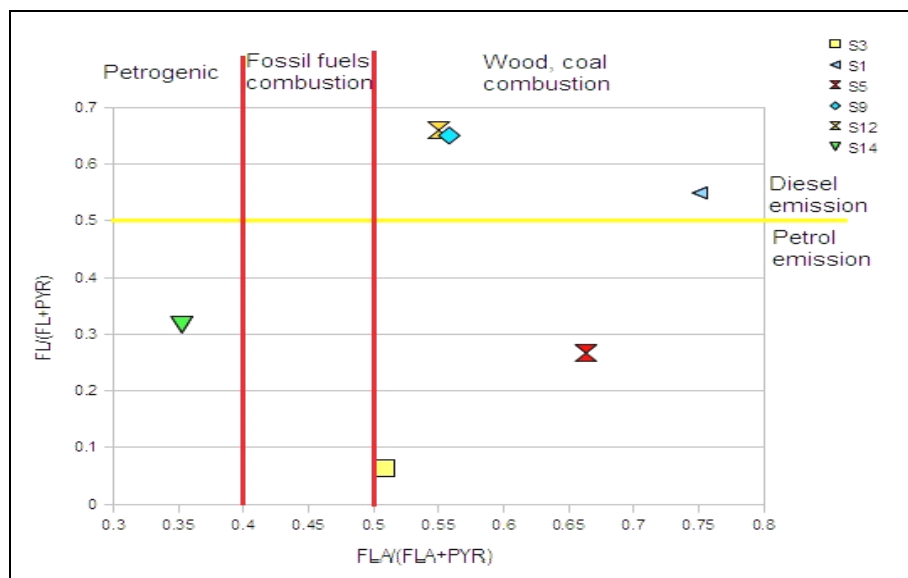
**Table 3.** Available PAH diagnostic ratios according to sampling locations

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
$\Sigma_{\text{LMW}}/\Sigma_{\text{HMW}}$	0.01	0.01	0.02	1.72	0.14	0.07	0.17	0.05	0.49	0.15	0.21	0.75	NA	0.02
$\Sigma_{\text{COMB}}/\Sigma_{\text{PAHs}}$	0.99	0.95	0.9	0.31	0.72	0.64	0.68	0.91	0.62	0.72	0.66	0.52	0.42	0.79
FL/(FL+PYR)	0.55	NA	0.06	NA	0.27	NA	NA	NA	0.65	NA	NA	0.66	NA	0.32
FLA/(FLA+PYR)	0.75	NA	0.51	NA	0.66	0.6	0.53	0.64	0.56	0.43	0.5	0.55	NA	0.35

NA- not available

If the sources according to the FLA/(FLA+PYR) and FL/(FL+PYR) ratios are cross-examined (Figure 2), one may observe that mixed sources of

PAH are present in all studied areas. Thus, in Cluj-Napoca (S14) there is a petrogenic and petrol emission source, which is probably due to vehicle emissions. In Alba Iulia city (S1), București city (S9) and Oradea city (S12) the sources are both from diesel emission and from wood or coal combustion. These ratios indicate both heavy traffic (the diesel emission source) and industrial activities (wood or coal combustion source – either from the centralized heating systems or from other type of industries). In Iași city (S3) and Bistrița city (S5) the sources are both from petrol and from wood or coal combustion. Bistrița city seems to be more inclined towards industry, while Iași city tends to have a more likely fossil fuels combustion source.



**Figure 2.** Cross examination of the FLA/ (FLA+PYR) against FL/ (FL+PYR) ratios in the selected locations

### 3. CONCLUSIONS

This study showed the presence of PAHs in moss samples in concentrations which are dependent on the sampling zone.

The cities with the highest amounts of  $\Sigma$ PAHs were found within the Carpathian arch, while the cities situated in the western part of the country had the lowest amounts. The cities located in the eastern part of the country have slightly higher concentrations of PAHs in moss samples than those situated in the southern part.

The  $\sum_{LMW}/\sum_{HMW}$  ratio indicates that there is a pyrogenic source in all the samples, and the  $\sum_{COMB}/\sum_{PAHs}$  ratio confirms the combustion source ( $\sim 1$ ) in most of the cities.

The FLA/(FLA+PYR) and FL/(FL+PYR) ratios indicate both heavy traffic (the diesel emission source and fossil fuels combustion source) and industrial activities (wood or coal combustion source), which may come either from the centralized heating systems, or from other type of industries.

## 4. EXPERIMENTAL SECTION

### 4.1. Reagents and solutions

For the qualitative and quantitative analysis an EPA PAH standard mixture containing 16 compounds in concentration of  $2 \mu\text{g mL}^{-1}$  of each compound purchased by Supelco was used. The PAHs determined were: naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno(1,2,3-cd)pyrene, dibenz[ah]anthracene and benzo[ghi]perylene. Dichloromethane and n-hexane in purity of 99.99% were purchase from Merck, Germany and helium in purity of 99.9999% from Linde Gas, Romania.

### 4.2. Instrumentation and chromatographic conditions

The PAHs were analyzed by gas chromatography-mass spectrometry using a gas chromatograph model Thermo Electron Corporation (Focus GC) equipped with a DSQII mas spectrometer and a TriPlus Autosampler. The separation of target compounds was performed on DB-5 MS column (25 m x 0.25 mm x 0.25  $\mu\text{m}$ ) using helium as carrier gas at a constant flow of 1,2 mL  $\text{min}^{-1}$ . The MS ion source was heated at 200°C, and the detection of the target compounds was made on selected ion monitoring (SIM) mode.

The separation of the PAHs was performed with two gradient of temperature as follows: from 120°C, up to 220°C with 10°C  $\text{min}^{-1}$ , and from 220°C to 300°C with 3°C  $\text{min}^{-1}$ . Injection volume was 1  $\mu\text{L}$  in splitless mode.

The quantification of the target compounds in real samples was made by the calibration curve method. For this purpose five standard solutions in concentration of 0.04, 0.05, 0.07, 0.1 and 0.2  $\mu\text{g mL}^{-1}$  were prepared by dilution of the standard mixture (2  $\mu\text{g mL}^{-1}$ ). The calibration curves were built using the chromatographic peak area and the concentration of each of the PAHs.



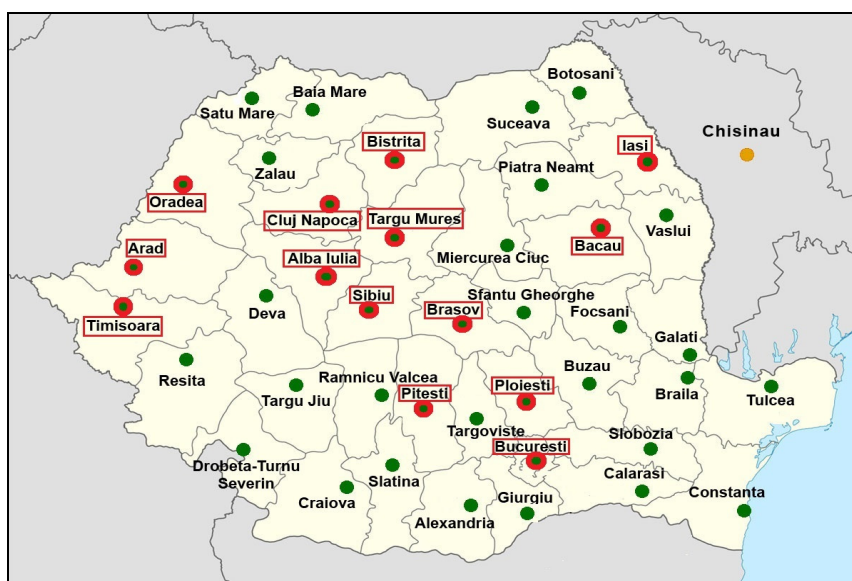
### 4.3 Ultrasound assisted extraction procedure

From each sample, 3 g of milled moss were weighed in a Berzelius beaker then added thirty milliliter of a mixture of n-hexane:dichloromethane (1:1 v/v). The samples were placed in ultrasonic bath for 15 minutes and then the solvent was decanted and filtered through a PVDF sample filter with pore size of 0.45  $\mu\text{m}$  (Merck Milipore). The resulted extract was evaporated to dryness with a rota-evaporator and the residue was dissolved with 2 milliliter of n-hexane:dichloromethane (1:1 v/v) mixture and kept in the freezer until the analysis.

### 4.4 Sampling points

The study was carried out in 14 of the largest cities in Romania (Figure 3). All of the samples were collected at distances of more than 5 m from roads and houses, from 1 - 2 meters high. The samples were all collected from central parks areas.

The moss samples were collected with a stainless steel scissors in sterilized polyethylene bags and kept at 8°C until analysis.



**Figure 3.** The map of moss sampling points (Alba Iulia city (S1), Sibiu city (S2), Iași city (S3), Brașov city (S4), Bistrița city (S5), Pitești city (S6), Bacău city (S7), Târgu Mureș city (S8), Bucharest (S9), Ploiești city (S10), Arad city (S11), Oradea city (S12), Timișoara city (S13), Cluj-Napoca city (S14))

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