



SUPPLEMENTARY MATERIAL TO

Spatial distribution of PAHs in riverbed sediments of the Danube River in Serbia: Anthropogenic and natural sources

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SECTION S-1 – SEDIMENT SAMPLING

Riverbed sediments were collected using a stainless-steel grab sampler from ten sampling sites in October 2012, during the period of low water levels. The sediment samples were collected at sites that are representative of a particular watercourse. This approach required *a priori* understanding of the hydrological and morphological characteristics of the site and its close proximity to the riverbank, as well as the identification of the impact of various pollutants. The following sites along the Danube's course through Serbia were included in this study: Apatin (D1), Labudnjača (D2), Neštin (D3), Begeč (D4), Ratno Ostrvo (D5), Šangaj (D6), Knićanin (D7), Belegiš (D8), Ritopek (D9) and Dubravica (D10) (Fig. S-1). Basic information on sampling points is shown in Table S-I.

At each sampling point, six to eight subsamples of 1.0 to 1.5 kg were collected at 0–10 cm depth from the riverbed in a radius of 10 m and were included into a pooled sample. The samples were kept in vacuum sealed buckets (20 L in volume) and were transferred in appropriate cool boxes to the processing laboratory. No chemicals were used for preservation. The temperature of the samples during transport was maintained at 4 °C. Pre-treatment of the samples was included a wet sediment sieving (sieve of 2 mm) in order to remove leaves, stones, and roots (with local water). The sediments were well homogenized, and 50 g subsamples were collected in glass jars for freeze drying.

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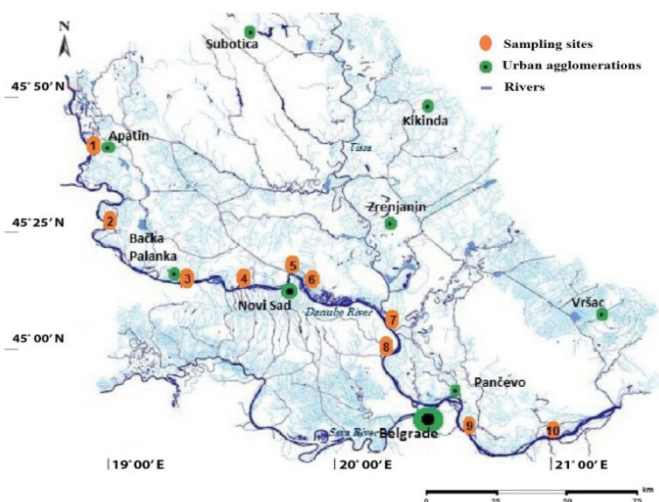


Fig. S-1. Geographic position of the sampling sites.

SECTION S-2 – SAMPLE PREPARATION AND ANALYSIS

Chemicals and reagent. The organic solvents used during the Danube sediment analysis, dichloromethane, chloroform, and n-hexane, were supplied by Sigma–Aldrich, Czech Republic. Silica gel 60 was obtained from Merck, Czech Republic. Standards of 29 PAHs, (d8-naphthalene, d10-phenanthrene, d12-perylene) were obtained from Sigma-Aldrich, Czech Republic. For the GC/MS equipment, nitrogen and helium were used as the electron capture detector (ECD) make-up gas, and as a carrier gas, respectively. Both were purchased from Messer Tatragas, Czech Republic. Terphenyl, the internal standard for instrumental analysis by GC/MS, was supplied by Sigma-Aldrich, Czech Republic.

Sediment analysis. About 7–8 g of the freeze-dried sediment samples of Danube river were spiked with 330 ng of surrogate recovery standards per sample (designated for PAH analysis). In addition to a set of 10 samples, blank and reference material were tested. Automated Soxhlet extraction of spiked sediment samples was performed using dichloromethane (DCM) (duration of 2 h; Büchi B-811, Switzerland). Activated copper was added for desulfurization. For PAH analysis, 20% of the prepared sample was separated and was cleaned using activated silica, while the remaining 80% was exploited for analysis of other pollutants that are not subject of this paper. After a thorough cleaning, the sample volume was reduced to about 1.0–1.5 ml and transferred to hexane using azeotrope principle on Kuderna-Danish evaporation unit to a final 1 ml of extract in hexane. Extracts were quantitatively transferred to 2 ml GC–MS vials and the volume was reduced to approximately 1 ml. For the analysis of 29 PAHs terphenyl (250 ng), an instrumental internal standard was added. Using a GC–MS

systems (GC 7890/MS-MS Triple Quadrupole 7000B; Agilent) equipped with a J&W Scientific fused silica column DB-5MS (60 m×25 µm×0.25 µm), 29 PAHs (**Nap**, naphthalene; **Acy**, acenaphthylene; **Ace**, acenaphthene; **Fl**, fluorene; **Phe**, phenanthrene; **Ant**, anthracene; **Flu**, fluoranthene; **Pyr**, pyrene; **B[a]A**, benzo[*a*]anthracene; **Chr**, chrysene; **B[b]Flu**, benzo[*b*]fluoranthene; **B[k]Flu**, benzo[*k*]fluoranthene; **B[a]P**, benzo[*a*]pyrene; **IP**, indeno[1,2,3-*cd*]pyrene; **DB[ah]A**, dibenzo[*ah*]anthracene; **B[ghi]P**, benzo[*ghi*]perylene; **Bip**, biphenyl; **Ret**, retene; **B[b]Fl**, benzo[*b*]fluorene; **Bnt**, benzonaphthothiophene; **B[ghi]Flu**, benzo[*ghi*]fluoranthene; **Cp[cd]P**, cyclopenta[*cd*]pyrene; **Tph**, triphenylene; **B[j]Flu**, benzo[*j*]fluoranthene; **B[e]P**, benzo[*e*]pyrene; **Per**, perylene; **DB[ac]Ant**, dibenzo[*ac*]anthracene; **Anth**, anthanthrene; and **Cor**, coronene) were quantified in all examine sediment samples.

Measurement of TOC in the sediments. The total organic carbon (TOC) was determined to apply a vario TOC Cube Manufacturer, Elementary Analysen Systeme, Germany. In preparation for TOC analyses, the samples were weighed into aluminium cups and acidified using a few drops of 6 mol/L HCl solution (1:1). The acidified samples were dried for one hour at 70 °C before packing and placing them into autosampler for analysis. A certified reference material sediment sample was analyzed in order to test the various TOC cube performance at 950 °C combustion temperature. The samples were decomposed by temperature on carbon dioxide, which was purified through a halogen trap and determined by non-dispersive infrared (NDIR) photometer.

EXPERIMENTAL METHODOLOGY

The chart shown in Fig. S-2 illustrate the workflow of the present study. The experimental methodology included acquiring basic information, implementation of analytical methods, processing of data for statistical analysis, as well as consideration of the consequences that PAHs could cause in aquatic systems.

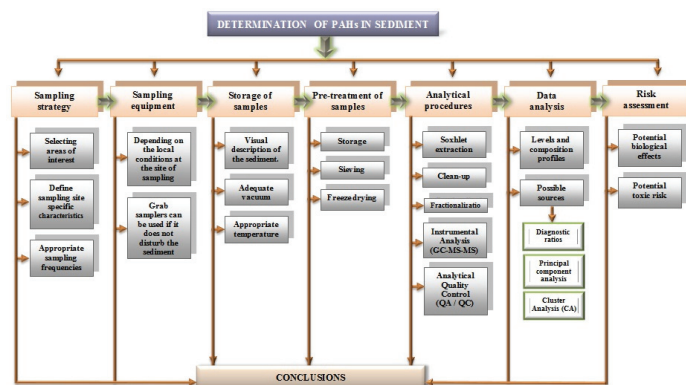


Fig. S-2. Flowchart of the methodology applied for determining PAHs in the sediment of the Danube river.

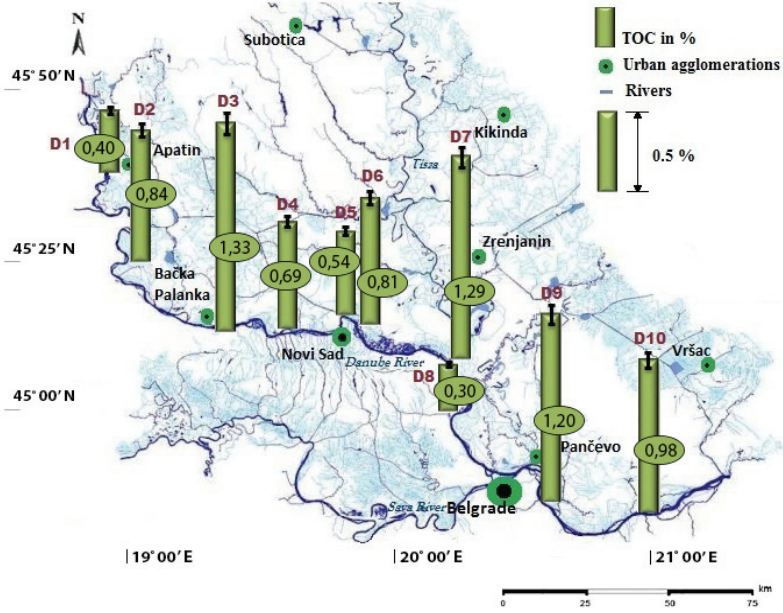


Fig. S-3. TOC content in sediments from the Danube, [%].

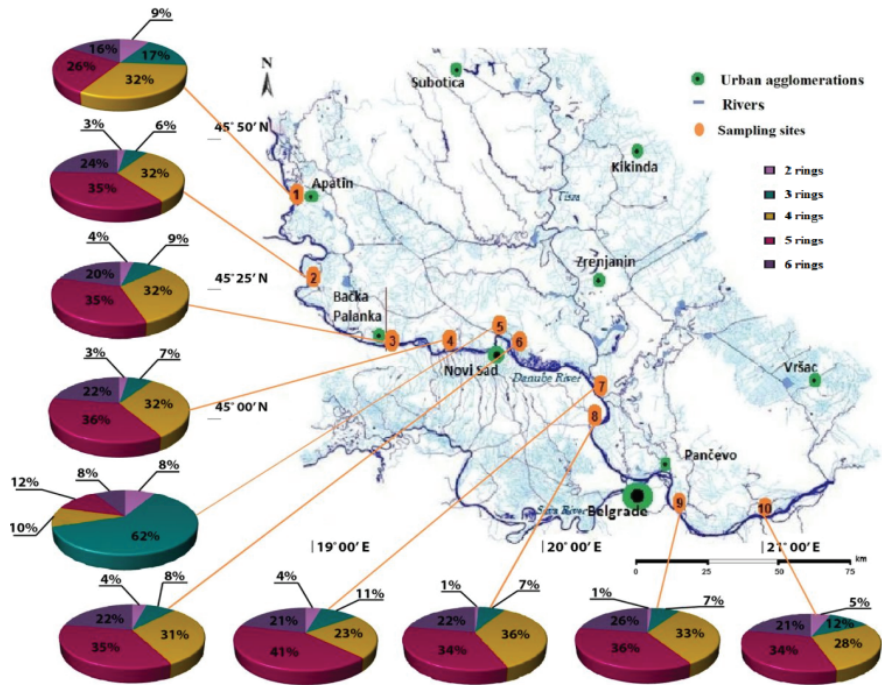


Fig. S-4. Distribution of PAHs in the Danube sediment according to the number of rings.

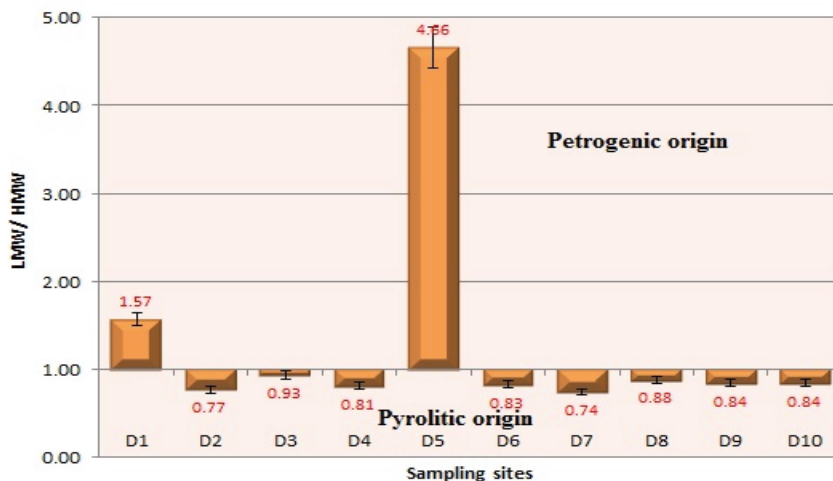


Fig. S-5. LMW/HMW ratio for 29 PAHs detected in the Danube sediments.

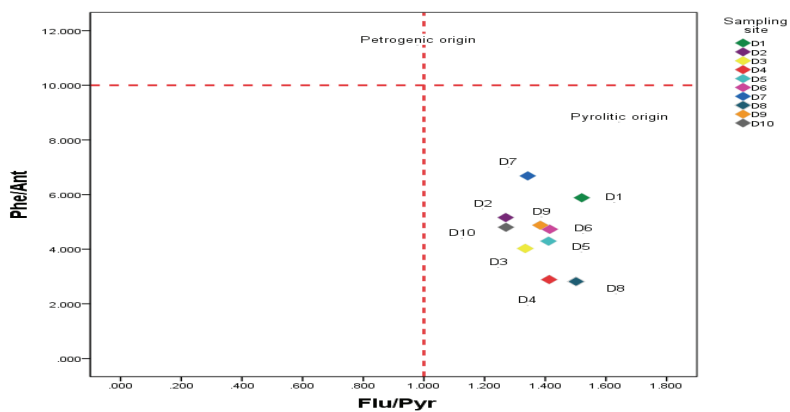


Fig. S-6. The plot of the Phe/Ant values compared with the Flu/Pyr values.

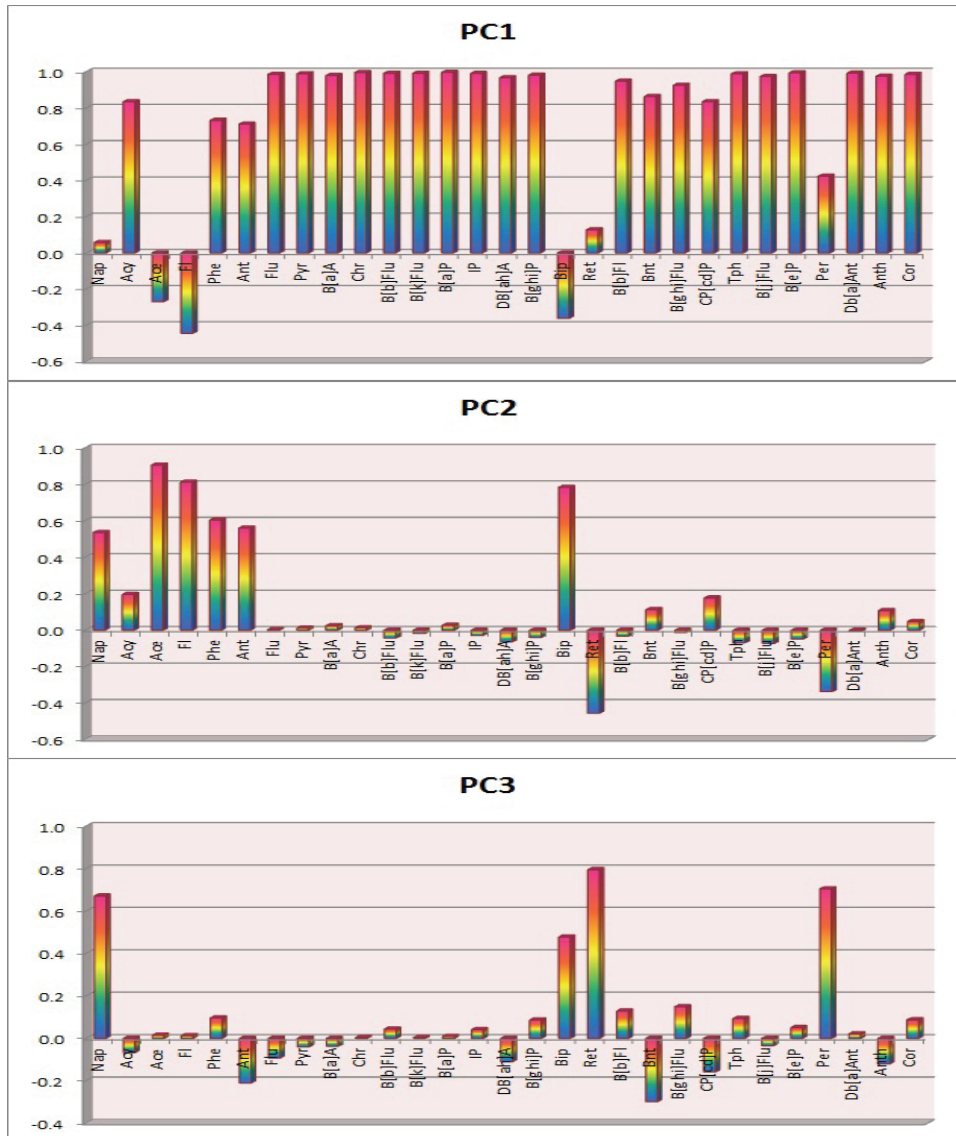


Fig. S-7. PCA obtained by Varimax rotation for chosen PAHs in Danube riverbed sediments.

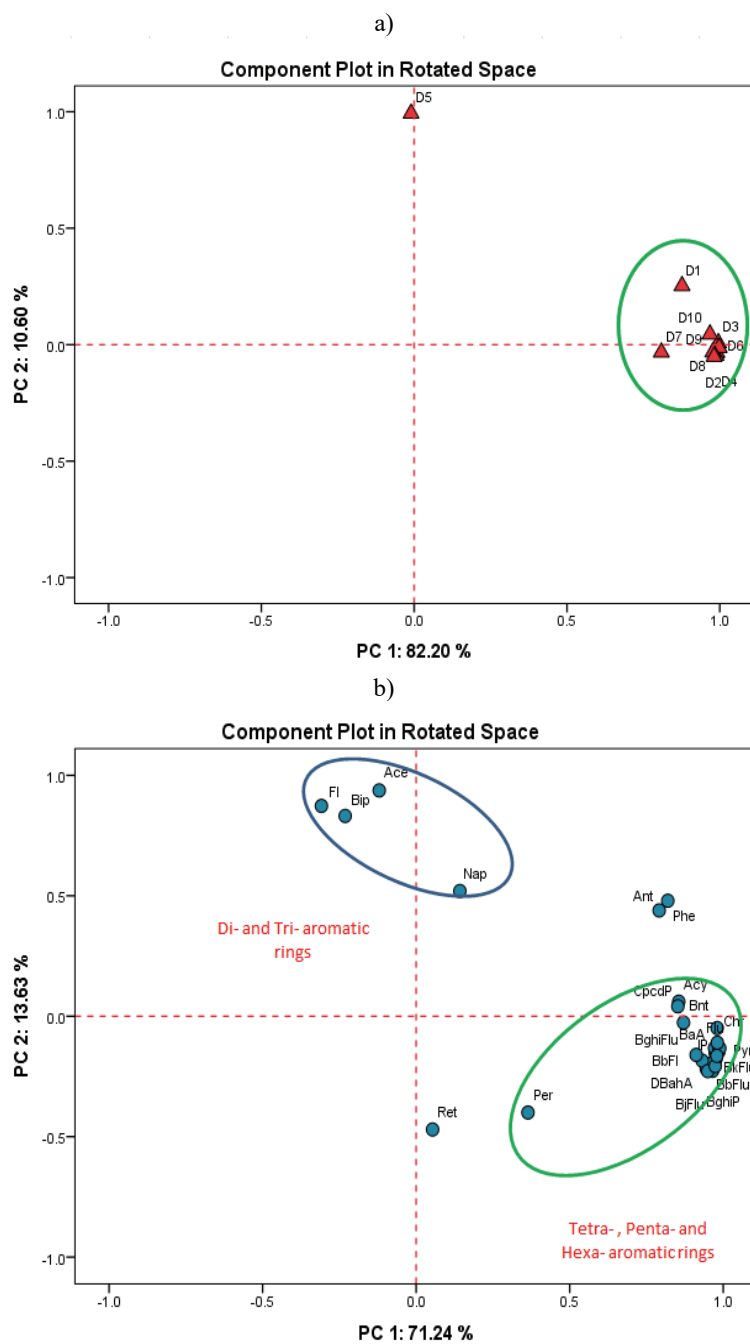


Fig. S-8. PCA Scores plot for: a) PAH profiles at studied sites and b) PAHs (normalized data).

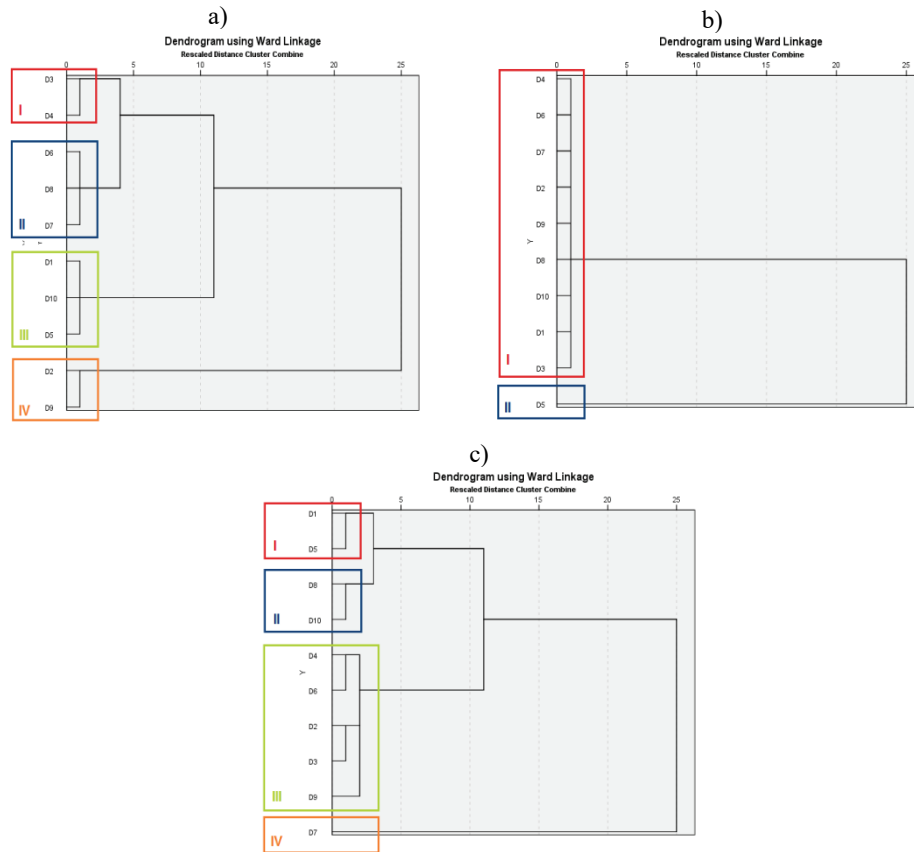


Fig. S-9. HCA-dendrograms of 29 PAHs identified in sediments from 10 sites along the Danube associated with the previously defined PC1, PC2, and PC3.

TABLE S-I. The characteristics of sampling sites

Sample No.	Latitude	Longitude	Elevation above sea level, m	Location characteristic
Apatin	D1 45°40'12.59"N	18°58'10.78"E	81	Urban zone- a residential area encircled by the industrial section, regional routes and "Danube river Goods-Transport Center".
Labudnjača	D2 45°25'12.63"N	19°01'54.25"E	77	Rural/ industrial zone- area in the vicinity of the chemical industry.
Neštin	D3 45°13'50.25"N	19°26'16.35"E	107	Rural zone- an agricultural area encircled by local routes and residential buildings.
Begeč	D4 45°13'54.20"N	19°39'55.64"E	76	Rural/ industrial zone- a residential zone located in the vicinity of Beočin Cement Plants.
Ratno ostrvo	D5 45°15'48.02"N	19°53'00.87"E	75	Urban/industrial zone- an area close to refinery and thermal power and heating plant.
Šangaj	D6 45°14'08.33"N	19°55'35.65"E	72	Urban zone- an area surrounded by the industrial section, local and regional routes, and agricultural fields.
Knićanin	D7 45°07'40.34"N	20°17'08.81"E	71	Rural/ industrial zone- the confluence of the Tisza into the Danube.
Belegiš	D8 45°00'34.70"N	20°20'28.59"E	68	Rural/ industrial zone- area with developed agriculture.
Ritopek	D9 44°44'39.99"N	20°38'32.66"E	134	Industrial zone- area in the vicinity of the chemical industry and refinery.
Dubravica	D10 44°42'49.35"N	21°02'35.85"E	68	Rural/ industrial zone - area in the vicinity of plants for production of metal alloys (iron and steelmaking) and the confluence of the Velika Morava River into the Danube.

TABLE S-II. PAHs concentrations in the sediment of Danube River, Serbia

Sample No.	PAHs concentrations, µg/kg d.w.									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Nap	11.82	11.10	14.93	7.98	11.25	10.26	8.78	2.25	6.92	5.00
Acy	0.88	1.54	1.29	1.68	0.65	0.75	0.97	0.46	3.80	0.69
Ace	0.98	1.03	1.73	0.71	6.06	0.77	0.53	0.69	1.62	0.40
Fl	6.00	3.81	5.01	2.49	88.37	2.66	2.86	1.42	3.35	1.87
Phe	18.35	22.55	23.23	15.94	19.43	14.91	12.64	11.17	31.96	7.69
Ant	3.12	4.37	5.77	5.52	4.52	3.15	1.89	3.97	6.55	1.60
Flu	23.02	62.94	49.52	44.84	6.99	36.75	20.90	37.98	77.83	11.27
Pyr	15.14	49.57	37.12	31.73	4.95	25.98	15.57	25.29	56.24	8.87
B[a]A	7.00	24.37	24.18	23.19	2.47	14.92	8.28	12.73	32.32	5.47
Chr	7.27	28.01	23.92	21.77	2.89	15.24	10.39	12.79	34.82	6.60
B[b]Flu	11.21	48.90	39.31	35.07	5.86	28.93	20.37	23.74	54.29	12.21
B[k]Flu	4.12	17.04	14.27	13.55	2.18	9.94	6.63	8.71	19.58	3.97
B[a]P	9.45	42.72	33.91	32.97	4.20	22.65	15.17	19.27	51.67	6.15
IP	15.16	74.16	50.68	51.40	8.59	40.01	29.43	31.89	80.58	15.60
DB[ah]A	0.72	3.02	2.35	2.00	0.35	2.05	1.36	2.27	4.28	0.58
B[ghi]P	10.49	51.96	32.98	31.67	6.31	26.57	22.51	20.61	54.67	11.51
Bip	3.33	2.67	3.07	2.11	4.76	2.21	2.67	0.87	1.92	1.90
Ret	1.35	2.97	3.69	2.43	0.89	4.13	8.84	0.72	3.27	2.85
B[b]Fl	1.15	4.40	4.13	3.51	0.51	2.74	1.78	1.95	4.24	1.02
Bnt	0.19	2.86	1.68	1.80	0.17	0.75	0.35	2.16	7.76	0.43
B[ghi]Flu	1.46	7.24	4.68	3.83	0.69	3.25	2.20	2.17	5.57	1.52
CP[cd]P	0.61	5.75	3.19	4.72	0.37	1.74	1.28	0.71	20.46	0.70
Tph	1.95	8.18	5.91	5.68	0.94	4.55	3.99	3.46	9.20	2.25
B[j]Flu	3.83	16.52	12.27	12.80	1.96	9.61	5.99	9.27	16.63	3.78
B[e]P	8.40	35.58	27.50	26.74	4.20	20.25	16.05	16.65	40.78	8.89
Per	4.12	17.34	16.66	11.75	2.78	12.77	38.46	6.70	21.01	3.95
DB[ac]Ant	1.28	7.23	5.00	5.43	0.88	3.99	2.82	2.99	8.50	1.51
Anth	1.00	6.45	5.72	5.07	0.77	3.55	1.73	4.03	10.75	0.00
Cor	0.76	5.20	3.60	3.55	0.57	2.90	2.14	2.06	6.27	0.00
ΣPAHs	174.16	569.47	457.32	411.96	194.55	327.98	266.56	268.94	676.85	128.27
Range	0.19- 23.02	1.03- 74.16	1.29- 50.68	0.71- 51.40	0.17- 88.37	0.75- 40.01	0.35- 38.46	0.46- 37.98	1.62- 80.58	nd- 15.60
Mean	2.31	1.65	2.97	3.60	4.20	8.43	18.67	12.33	3.60	3.55
SD	6.09	20.84	15.27	14.47	16.23	11.41	9.67	10.32	23.57	4.28
TOC, %	0.40	0.84	1.33	0.69	0.54	0.81	1.29	0.30	1.20	0.98

TABLE S-III. Different ratios and determine the source of PAHs in sediment

RATIOS		Range	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
LMW/H	Pyrol.	<1	-	0.77	0.93	0.81	-	0.83	0.74	0.88	0.84	0.84
MW	Petrog.	>1	1.57	-	-	-	4.66	-	-	-	-	-
Phe/Ant	Pyrol.	<10	5.88	5.16	4.03	2.89	4.29	4.73	6.68	2.82	4.88	4.8
	Petrog.	>10	-	-	-	-	-	-	-	-	-	-
Flu/Pyr	Pyrol.	>1	1.52	1.27	1.33	1.41	1.41	1.41	1.34	1.5	1.38	1.27
	Petrog.	<1	-	-	-	-	-	-	-	-	-	-
Fl/Fl+Py	Pyrol.	>0.5	0.6	0.56	0.57	0.59	0.59	0.59	0.57	0.6	0.58	0.56
r	Combustion products of liquid fuel & oil	0.5-0.4	-	-	-	-	-	-	-	-	-	-
	Petrog.	<0.4	-	-	-	-	-	-	-	-	-	-
	Pyrol.	<0.1	0.15	0.16	0.20	0.26	0.19	0.17	0.13	0.26	0.17	0.17
Ant/178	Petrog.	>0.1	-	-	-	-	-	-	-	-	-	-
B(a)A / 228	Pyrol.	>0.35	0.49	0.47	0.50	0.52	0.46	0.49	0.44	0.50	0.48	0.45
	Mixed	0.2-0.35	-	-	-	-	-	-	-	-	-	-
	Petrog.	<0.2	-	-	-	-	-	-	-	-	-	-
IP/IP + B(ghi)P	Pyrol. (grass, wood and coal combustion)	>0.5	0.59	0.59	0.61	0.62	0.58	0.60	0.57	0.61	0.60	0.58
	Fossil fuel combustion	0.2-0.5	-	-	-	-	-	-	-	-	-	-
	Petrog.	<0.2	-	-	-	-	-	-	-	-	-	-
Total PAHs index			6.01	6.06	6.55	7.23	6.33	6.31	5.66	7.26	6.23	6.09
Origin of PAHs in this study			Pyrolytic/ petro- genic	Pyrolytic	Pyrolytic	Pyrolytic	Pyrolytic/ petro- genic	Pyrolytic	Pyrolytic	Pyrolytic	Pyrolytic	Pyrolytic

TABLE S-IV. Corresponding SQGs values for PAHs and relative percentage of samples amongst ranges of Sediment Quality Guidelines for Danube stations

Compound	SQG ^a ERL ^b -ERM ^c , ng/g d.w.	No. of stations ^h			SQG ^a TEL ^d - PEL ^e , ng/g d.w.	No. of stations ^h		
		<ERL	ERL-ERM	>ERM		<TEL	TEL-PEL	>PEL
Nap	340–2100	D3	-	-	35–391	D3	-	-
Acy	44–640	D9	-	-	6–128	D9	-	-
Acc	16–500	D5	-	-	7–89	D5	-	-
Fl	35–640	D1	D5	-	21–144	D1	D5	-
Phe	225–1380	D9	-	-	42–515	D9	-	-
Ant	85–960	D9	-	-	47–245	D9	-	-
Flu	600–3600	D9	-	-	111–2355	D9	-	-
Pyr	350–2200	D9	-	-	53–875	D9	-	-
B[a]A	230–1600	D9	-	-	75–693	D9	-	-
Chr	400–2800	D9	-	-	57–862	D9	-	-
B[b + k]Fl	-	-	-	-	-	-	-	-
B[a]P	400–2500	D9	-	-	32–782	D9	-	-
IP	-	-	-	-	-	-	-	-
DB[ah]A	63–260	D9	-	-	6–135	D9	-	-
B[ghi]P	-	-	-	-	-	-	-	-
∑ ₁₆ PAHs	4000–35000	D9	-	-	655–6676	D2	D9	-
LMW PAHs ^f	552-3160	D9	-	-	-	-	-	-
HMW PAHs ^g	1700- 9600	D9	-	-	-	-	-	-

^a SQG values taken from MacDonald *et al.*¹

^b ERL = effects range-low value

^c ERM = effects range-median value

^d TEL = threshold effects level

^e PEL = probable effects level

^f LMW PAHs = the sum of the concentrations of low-molecular-weight PAHs

^g HMW PAHs = the sum of the concentrations of high-molecular-weight PAHs

^h Sampling point that has the highest value, ie. value that is closest to prescribed values of SQG sets.

TABLE S-V. PAH compounds and their toxic equivalent factors (TEFs)²⁻⁴

PAH compound	TEFs used in this study	PAH compound	TEFs used in this study
Naphthalene (Nap)	0.001	Benzo(b)fluoranthene (B[b]Flu)	0.1
Acenaphthylene (Acy)	0.001	Benzo(k)fluoranthene (B[k]Flu)	0.1
Acenaphthene (Ace)	0.001	Benzo(a)pyrene (B[a]P)	1
Fluorene (Fl)	0.001	Indeno pyrene (IP)	0.1
Phenanthrene (Phe)	0.001	Dibenzo(a,h)anthracene (DB[ah]A)	1
Anthracene (Ant)	0.01	Benzo(ghi)perylene (B[ghi]P)	0.01
Fluoranthene (Flu)	0.001	Cyclopenta(cd)pyrene (Cp[cd]P)	0.1
Pyrene (Pyr)	0.001	Benzo(e)pyrene (B[e]P)	0.01
Benzo(a)anthracene (B[a]A)	0.1	Perylene (Per)	0.001
Chrysene (Chr)	0.01	Coronene (Cor)	0.001

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