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

MAJA BRBORIĆ[1], BRANISLAV VRANA[2], JELENA RADONIĆ[1#], MIRJANA VOJINOVIĆ MILORADOV[1] and MAJA TURK SEKULIĆ[1*#]

[1]*University of Novi Sad, Faculty of Technical Sciences, Department of Environmental Engineering and Occupational Safety and Health, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia and* [2]*Masaryk University, Faculty of Sciences, Research Center for Toxic Compounds in the Environment, RECETOX, Kotlářská 267/2, 611 37 Brno, Czech Republic*

Abstract: The top layer of riverbed sediments from 10 sites along the Danube River in Serbia were analysed for the presence of polycyclic aromatic hydrocarbons (PAHs). Potential sources of pollution were identified using different multivariate techniques. Total concentrations of 29 PAHs in sediment samples ranged from 128.27 to 676.85 μg/kg dry weight. Based on these concentrations and PAH patterns, a clear spatial distribution of examined localities was determined. The diagnostic ratios indicated a pyrolytic origin of PAHs, except for two sites where PAH contamination originated from petrogenic sources. The Varimax rotated principal component analysis revealed three main factors, namely coal, wood, and biomass combustion; vehicular emissions; and natural sources; accounting for 92.1 % of the variance in the original datasets. The sites with different pollution load, depending on main sources identified, were arranged by hierarchical cluster analysis. The results were compared with sediment quality guidelines, and the obtained values of equilibrium partitioning sediment benchmarks toxic unit and total toxic equivalent quantities of benzo-[*a*]pyrene suggested that Danube sediments do not pose an elevated ecotoxicological risk for benthic organisms.

Keywords: polycyclic aromatic hydrocarbons; contaminant pattern; aquatic sediment.

INTRODUCTION

Discharge of partially treated or untreated wastewater from human agglomerations, industry, and agriculture into surface water bodies cause significant pollution of the Danube. In Serbia, 44 human agglomerations are located in the

---

Danube basin, with a total of 1.44 million population equivalent (PE). Most agglomerations (70 %) are connected to neither sewage collecting systems nor wastewater treatment plants. A large portion of industrial wastewaters from 326 registered water polluters (flow: 461,315 m^3/day, suspended solids: 629 ton/day) is still being discharged without any or with insufficient pre-treatment into the Danube and its tributaries. The contribution of organic pollutants from 113 registered agricultural sources is also significant.[1] The situation in the Vojvodina region is further complicated by the previous armed conflicts. Burning or damaging of industrial objects (oil refineries, fuel storages, power plants), infrastructure (bridges, roads), and military objects in the Federal Republic of Yugoslavia during the NATO Operation Allied Force led to the release of a large amount of various hydrophobic organic compounds (HOCs) into the environment.[1]

Various HOCs, including polycyclic aromatic hydrocarbons (PAHs), pose a high risk for humans and the environment and represent a significant environmental problem.[2] Some PAHs remain and cycle in the aquatic environment and can be transported as dissolved or sorbed on sediments over vast distances, potentially damaging aquatic ecosystems and consequently impacting water status and human health.[3] Most of PAHs are released from well-known anthropogenic sources[4] rather than natural ones like volcanic activity and biosynthesis by bacteria and plants.[5] Considering that certain number of PAHs, as well as their environmental mixtures, are potent immunosuppressants and show strong mutagenicity and carcinogenicity,[2] there is a need to continuously collect the monitoring data on these contaminants and information on sources and relevant pathways.

Sorption of HOCs on sediments and suspended particles controls their environmental fate, transport, and effects in aquatic systems. Concentration levels of pollutants in aquatic sediments may give elementary information on the contamination status, sources, long-term dynamic changes responding strongly to the hydrological situation, and environmental risks incurred by contaminants in freshwater ecosystems.[6] River pollution is distinguished by complex processes of sedimentary deposition that include an archive of past and present anthropogenic activities.[5] Hydrophobic toxic contaminants tend to accumulate in sediments and, therefore, a contaminant buried in the sediment could be released in the aquatic eco-environment through different processes, posing a risk to biota and human population.

In Serbia, the available data on the sources and levels of PAHs are limited. The primary goal of the paper was to determine the level of riverbed sediment pollution with 29 PAHs and, in particular, to determine the composition of PAHs, their spatial distribution and contamination sources, as well as to assess environmental and human health risk. The findings of this study can serve as the basis for the remediation initiatives aimed at eradicating PAHs from Danube sediments.

EXPERIMENTAL

Details related to sediment sampling and preservation are given in Supplementary material to this paper (Section S-1).

Sediment analysis

Only a brief summary of the analytical procedure is shown here, while an itemized explanation of sample preparation and chemical analysis are presented in Section S-2 and Fig. S-2 (Supplementary material). Firstly, the collected sediment samples were freeze-dried and sieved. Hot automated Soxhlet extraction of spiked sediment samples with internal standards was performed using dichloromethane. Activated silica was used for sample clean-up. The sample volume was reduced using the azeotrope principle on Kuderna-Danish evaporation. Quantification was performed using gas chromatography–mass spectrometry (GC–MS).

Data analysis

In order to determine potential sources of PAHs in Danube sediments, we applied the diagnostic ratios method, principal component analysis (PCA), and hierarchical cluster analysis (HCA). Although the diagnostic ratios method is the most commonly used technique, it does not provide information on the quantitative contribution of different PAH sources.[8] Thus, it was supplemented by PCA, which is a helpful tool for the assessment of potential emission sources and their distribution. In this paper, PCA was applied to the parameters pertaining to different sites, in order to extract components relative to different factors, as well as to clarify the variance in the observed data. According to Liu *et al.*,[9] factor loadings were classified as "strong" (matches the absolute values of > 0.75), "moderate" (0.75–0.50) and "weak" (0.50––0.30). HCA allowed for the grouping of sampling localities depending on the probable sources of PAHs in Danube sediments based on the factors identified through PCA. This approach allowed for a precise point of pollution to be identified as a possible transport route of contamination. Statistical analysis was implemented using the IBM SPSS Statistics 22 software (IBM Corporation, Armonk, New York, USA).

RESULTS AND DISCUSSION

Quantification and composition profiles of PAHs

PAH concentration levels in the sediment samples collected at the studied localities and the TOC content are given in Table S-II (Supplementary material). The total amount of 29 PAHs in sediments ranged from 128.27 (D10) to 676.85 µg/kg dry weight (d.w.), D9, Fig. 1, with an average value of 347.61 µg/kg d.w. and median ± *SD* of 298.46±179.06 µg/kg d.w. The highest level was recorded at site D9, located 10 km downstream of Pančevo. During the 1999 NATO operations in Serbia, the bombing and destruction of the petrochemical plant "HIP Petrohemija", the chemical fertilizer plant "HIP-Azotara", and the oil refinery "NIS Petrol" resulted in complete or incomplete combustion.

After the air strikes, the United Nations Environment Programme (UNEP) defined Pančevo as one of the key ecological hot spots in the Western Balkans. This military campaign led to the discharge of toxic pollutants into the air, soil, surface water, and consequently groundwater.[10] According to UNEP,[10] the recorded sediment concentrations of 16 PAHs in the vicinity of Pančevo ranged

between 67 and 460 μg/kg d.w. Thirteen years later, within the presented study, for the same group of PAHs, we detected the increased values of 520 μg/kg d.w. In our case, the values increasing from 460 μg/kg to 520 μg/kg d.w., without any new registered anthropogenic influences, are a typical example of the pseudo--persistence of PAHs. This confirms the fact that the input rate is significantly higher than the output rate. The increase of 60 μg/kg can be attributed to the natural sources of PAHs with great certainty. Observing the categorization of 26 PAHs in sediments, proposed by Baumard *et al.*[10] (low: 0 to 100 μg/kg, moderate: 100 to 1000 μg/kg, high: 1000 to 5000 μg/kg, and very high: > 5000 μg/kg), PAH levels at all our sampling points can be classified as moderate.
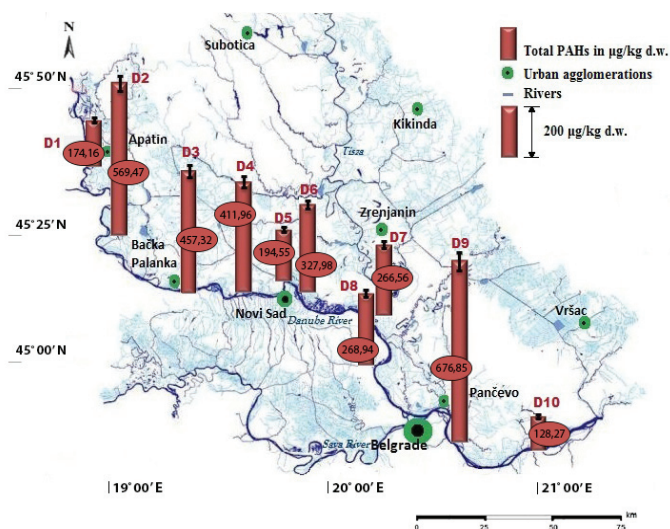

Fig. 1. Concentration levels of PAHs in the Danube riverbed sediment samples.

A differentiation of PAH concentration levels in bottom sediments collected from various waterways in Serbia and abroad is shown in Table I. The concentration levels of PAHs obtained in our study were only slightly higher than those observed within the Yangzi River Delta and Guan River Estuary, in China[11,13] and considerably lower than those in the majority of other analysed aquatic sediments shown in Table I. The PAH concentration levels detected in our study were similar to those registered in Serbian sediment samples reported by Crn-ković *et al.*[14] and Sakan *et al.*[14]

When interpreting these findings, it should be noted that, in most of the aforementioned studies, the levels of only 16 EPA PAHs were assessed. The comparison of sedimentary PAH data with maximum allowed concentrations, that the Serbian and Canadian legislation prescribed for sediments, have showed

that these pollutants are not expected to be a fundamental cause for eco-chemical and ecological degradation in examined sediments in Serbia.

TABLE I. Comparisons of the total PAHs concentrations (µg/kg d.w.) in sediments obtained in previously conducted research; n.d. – not detectable (below detection limits)

Sampling sites	Country	Date of sampling	Number of samples	Number of PAHs compounds	ΣPAHs – Range µg/kg	Ref.
This study	Serbia	2012	10	29	128.3 – 676.8	–
Mansfeld region, Saxony-Anhalt	Germany	1998	3	15	$5.1\times10^3 – 10.4\times10^3$	4
Danube	Serbia	2001	6	16	307 – 1,452	15
Upper Danube river	Germany	2002	10	16	n.d. – $26.32\ 10^3$	16
Yangzi River Delta	China	2005	38	15	91.3 – 614.4	11
Danube and Sava	Serbia	2005	2	16	D: 213.1 – 575.4 S:416.2–595.3	14
Danube, Sava, Tisa	Serbia	2008	3	6	D: < n.d. – 689 S: 240–395 T: 86.1–728	14
Mithi River	India	2009-11	3	17	1206.0–4735.0	18
Danube estuarine coast	Ukraine	2010	10	16	329.6 – 1,093.3	19
Cauca River	Colombia	2010-11	8	12	n.d. – 3,739.0	3
Guan River Estuary	China	2011	13	21	90 – 218	13

The correlation between total organic carbon (TOC) content and PAH concentrations in Danube sediments was examined. Statistical correlation analysis of all obtained results showed a non-significant linear correlation between TOC and PAH concentration levels in this study ($r = 0.60$, $p < 0.05$). This was confirmed with the Shapiro–Wilk test of normality and its significance of 0.639. Pearson correlation coefficient analysis demonstrated that only several individual compounds at selected locations were more significantly positively associated with TOC values. Locality D7 showed a positive correlation of two PAHs with TOC: Ret ($r = 0.75$, $p\ 0.05$) and Per ($r = 0.72$, $p < 0.05$). Moreover, within our study, the measured TOC range was low and very narrow (between 0.3 and 1.3 %, Fig. S-3, Supplementary material). In such a situation, a very strong correlation between PAHs and TOC would be more surprising than expected. These conclusions were confirmed with PCA scatter plot before and after the normalization to TOC values (Fig. S-8, Supplementary material).

PAH composition profiles in Danube sediments were dominated by 5-ring PAHs, as shown in Fig. S-4 (Supplementary material). In the total PAH amount, the 2- and 3-ring PAHs contributed 15 %, while the remaining 85 % comprised of 4-, 5- and 6-ring PAHs. These findings indicate a greater presence of PAHs introduced into Danube sediments by pyrolytic processes sources, that were subsequently introduced in each environmental matrix through dry and wet atmo-

spheric deposition and soil surface and river runoff. IP shows the highest concentrations in all analysed samples (8.60–80.58 μg/kg d.w.), followed by Flu and Pyr (6.99-77.82 and 4.96–56.24 μg/kg d.w., respectively,Table S-II, Supplementary material). Only site D5 is characterized by the predominance of 3-ring PAHs, contributing more than 60 % to the total PAH amount. At the same point, significantly greater Fl concentration was observed (88.37 μg/kg d.w.), almost 60 times higher than at site D8. These results show that this is probably caused by heavy traffic and vicinity of the Novi Sad oil refinery.

Possible PAHs sources

Diagnostic ratios. To elucidate the sediment contamination origin, the High Molecular Weight/Low Molecular Weight (LMW/HMW) ratios were used, where LMW PAHs = Nap + Acy + Ace + Fl + Phe + Ant + Flu + Pyr + B[a]A + Chr + Bip + Ret + B[b]Fl + Bnt + Tph and HMW PAHs = B[b]Flu + B[k]Flu + B[a]P + IP + DB[ah]A + B[ghi]P + B[ghi]Flu + Cp[cd]P + B[j]Flu + B[e]P + Per + DB[ac]Ant + Ant + Cor. As displayed in Fig. S-5 (Supplementary material), apart from localities D1 and D5 (with the LMW/HMW ratio of 1.57 and 4.66, respectively), all LMW/HMW ratios were lower than 1. These findings indicate that PAHs at sampling sites D2–D4 and D6–D10 are pyrolytic, whereas those at sites D1 and D5 are petrogenic in origin. In addition to intensive road and water traffic at both sites, Danube Goods-Transportation Centre has an impact on site D1, while oil refinery and traffic hold-ups at the Port of Novi Sad contributed to the pollution at D5. Tsymbalyuk *et al.*[19] also showed that PAHs with 4 to 6 aromatic rings clearly predominated in riverbed sediments from the estuarine Danube area.

Flu/Pyr and Phe/Ant ratios are calculated by another method for assessing the contamination sources. It is generally agreed that low Phe/Ant values (< 10) and high Flu/Pyr values (> 1) are characteristic of pyrolytic pollution.[5] Fig. S-6 (Supplementary material) displays the distribution of Phe/Ant and Flu/Pyr ratios for the studied sampling points. The resulting plot reveals that for the analysed sediments Phe/Ant is < 10 and Flu/Pyr is > 1, indicating predominantly pyrogenic origin of these PAHs. However, diagnostic Phe/Ant and Flu/Pyr ratios are usually unreliable and may be lower than those used as emission sources indicators.[19]

In addition, the PAH sources in sediments can also be identified using the following concentration ratios: Fl/Fl+Pyr, Ant/178 (Ant/Ant+Phen), IP/IP + B[ghi]P and B[a]A/228 (B[a]A/B[a]A+Chr). In this study, the values of these ratios indicate the dominance of pyrolytic emission sources, with the highest values of ratios associated with site D4 (Table S-III, Supplementary material). In certain cases, the PAH ratios are incompatible, because the origins of PAH emissions can be different, random, and irregular.[19] To compensate for these discre-

pancies, the total PAH index was calculated using Eq. (1).[19] Various sources reported single indexes, normalized by the lower limit of four considered ratios (petrogenic source in Table S-III) were used.

$$TotalPAHs_{index} = \frac{\frac{Fl}{(Fl+Pyr)}}{0.4} + \frac{\frac{Ant}{178}}{0.1} + \frac{\frac{B[a]A}{228}}{0.2} + \frac{\frac{IP}{(IP+B[ghi]P)}}{0.2}$$ (1)

When the total PAHs index > 4, high-temperature processes are investigated as sources of PAHs, while values < 4 indicate a predominance of oil sources.[19] The values obtained in this study are within the 5.66–7.27 range (Table S-III), confirming that high-temperature processes are dominant sources of PAHs.

Principal component analysis

PCA with Varimax rotation was applied to ascertain the quantitative contribution of different PAH sources and provide the most convincing results. Three principal components explaining 92.1 % of the variance are presented in Fig. S-7 (Supplementary material).

PC1 explained approximately 70.8 % of the variance, with high loadings of Acy, Phe, Ant, Flu, Pyr, B[a]A, Chr, B[b]Flu, B[k]Flu, B[a]P, IP, DB[ah]A, B[ghi]P, B[b]Fl, Bnt, B[ghi]Flu, Cp[cd]P, Tph, B[j]Flu, B[e]P, DB[a]Ant, Anth and Cor. According to Irwin *et al.*,[21] Acy can be released to the environment through petroleum refining effluents, coal tar distillation, municipal wastewater treatment, and waste incineration processes. On the other hand, IP can be formed during combustion or raised-temperature processes that include different hydrocarbon mixtures. Ant, Chr, B[a]A, B[b]Flu, B[b]Flu and B[a]P are considered specific markers for coal combustion, while the typical indicators of wood combustion include Phe, Ant, Pyr, B[a]A and Chr.[21] Based on the PAH emissions released from the ignition of coal dust and crumb rubber at 1000 °C (combustion temperature in a coal power plant), Phe, Flu and Pyr were determined as predominant PAHs in the emission from furnaces.[23] High loading factor for B[k]Flu, B[a]P, B[ghi]P, DB[ah]A and Chr was attributed to coal combustion, while high loading factor for Ant, Chr, B[b]Flu and B[k]Flu suggested stationary PAH emission sources.[24] Schauer *et al.*[24] measured the PAH emission rates from household combustion of different types of wood (eucalyptus, oak and pine). Their results showed presence of ACE, ACY, FL, PHE, ANT, RET, FLU, PYR, B[ghi]Flu, Cp[cd]P, CHR, TPH, B[b]Flu, B[k]Flu, B[j]Flu, B[e]P, B[a]P, Per, IP and Anth in all samples of emitted smoke. Gryglewicz *et al.*[25] examined sulfur-containing polycyclic aromatic compounds in supercritical coal and observed the presence of Bnt in coal flame. According to Ravindra *et al.*,[26] the increased B[e]P levels in sediments are the consequence of fossil fuel power plants. Therefore, it is reasonable to assign PC1 as sources of coal, wood and biomass com-

bustion, as a result of heating, industrial, and agricultural activities. A significant number of point sources originating from the industrial and individual production of smoked meat, as a significant part of a diet in this part of Serbia, also increase the emission levels.

PC2 explained around 12.0 % of the variance with high loadings of Ace, Fl and Bip. It also has a moderate loading for Nap, Phe and Ant. Higher levels of Ace and Fl were suggested to indicate vehicular combustion, while the high abundance of Nap can be characteristic of exhaust from diesel engines[21]. According to Irwin *et al.*,[21] Bip is detected in various petroleum products. Moreover, volatilization or spills of petroleum products across the waterway transport contribute to the increased emissions of Ace, Fl and Nap.[28] Ravindra *et al.*[26] found that engines with diesel exhaust emissions result in Phe and Ant dominance in pollutant profiles. The Danube is characterized by intensive shipping activities, so PC2 is selected to represent vehicular emission sources.

PC3 explained about 9.3 % of the variance with high loadings of Ret and Per and moderate loading for Nap. Gocht *et al.*[28] claimed that there are strong indications of natural processes that produce Per at low temperatures. Zakaria *et al.*[28] indicated that perylene can be produced during early diagenesis through *in situ* conversion of pigment (perylenequinone) or other types of organic matter. The results reported by Wen *et al.*[29] suggest that the phytoplankton could be a possible source of Ret in sediments and Nap and Phe are PAHs arising from "natural" sources (biological and diffuse).[31] Thus, factor PC3 was attributed to natural sources.

After comparing the PCA scatter plots of PAH concentrations before and after the normalization to TOC, it is evident that there are no significant differences in sediment concentrations across the sites. PCA scatter plots before and after the normalization to TOC values confirmed a non-significant correlation between PAH concentration values and TOC. Furthermore, these plots confirm a clear separation of D5. Site D5 was clearly separated from other sites in terms of PAH composition along PC1 and PC2 (Fig. S-8a, Supplementary material). PC1 was positively loaded with HMW PAHs, while PC2 was loaded with LMW PAHs, indicating recent sediment pollution, as water solubility of PAHs with two and three aromatic rings is much greater, relative to those of higher molecular mass (Fig. S-8b, Supplementary material).

Hierarchical cluster analysis (HCA)

The results of hierarchical cluster analysis are presented graphically, as the cluster tree. The dendrogram of the HCA of sampling sites compared to the first factor PC1 is presented in Fig. S-9a (Supplementary material). The first group includes sampling sites D3 and D4. The vicinity of Beočin Cement Plant probably has a great influence on the elevated concentrations of the above-men-

tioned PAHs at these sites. The second group includes sites D6, D7 and D8, where a moderate value of PAHs was recorded, most likely due to the vicinity of rural areas where the combustion of coal and wood is the main source of heating, as well as biomass burning on agricultural fields. The proximity of "NIS (Petroleum Industry of Serbia) Gazprom Neft" Oil Refinery, oil and gas production, and processing facilities, along with transport of petroleum products, as well as the vicinity of the cogeneration system, contribute substantially to the increased PAH concentrations at site D6. The third group includes sites D1, D5 and D10. The production processes that include smoking of meat delicacies in the meat factory "Neoplanta" (10 km from the sampling point) have an impact on the presence of PAHs at site D5. A significant contribution to PAHs recorded at D1 arises from individual domestic production and processing of smoked meat. For site D10, the confluence of the Great Morava into the Danube leads to the dilution and lowering of PAH concentrations.[31] The fourth group represents sites D2 and D9, which are mostly contaminated with PAHs emitted from coal, fuel, and wood combustion. These locations are distinguished by the highest concentration levels of PAHs, except Nap, Ace and Fl, most likely due to close proximity to areas with intensive agricultural and industrial activities. It is worth mentioning that there are no official data on the quantity of smoked and dried meat from individual households in Serbia, or on the quantities of burnt grass and crops. Intensive agricultural processes involving wheat and corn are the key activities in the studied area, and the burning of these residues could be an important source of PAHs.

The dendrogram which corresponds to the second factor PC2 is shown in Fig. S-9b (Supplementary material). This dendrogram separates into two clusters all the sites at which we detected elevated concentrations of PAHs originating from vehicular (mobile) emissions. One cluster includes only site D5, whereas the second one groups all other sites. The highest concentration of Ace, Fl and Bip was measured at sampling site D5 as a result of the proximity of Novi Sad (the second largest city in Serbia with 341,625 inhabitants in 2014). Novi Sad has one of the largest ports in the region, as well as intensive commercial and tourist activities, due to which the vehicular emissions are much higher than those observed at other sites.

The dendrogram of selected sites compared to the third factor PC3 is presented in Fig. S-9c (Supplementary material). Since factor PC3 denotes the natural sources of PAHs, four clusters have been singled out, whereby the highest concentrations of Ret and Per were recorded at D7 in the vicinity of the Danube-Tisa confluence. The Tisa River is characterized by diverse desmid flora, such as *Chlorophyta* and *Zygnematophyceae*, bacteria, and various plants that can produce certain PAHs through biosynthesis.[33] The cluster with medium loading included D2, D3, D4, D6 and D9. However, for these sites, no data on sources of

natural PAHs exist; thus, it can be assumed that the thermal waters of the Pannonian Basin are one of their sources, as these were found to contain light PAHs in deeper layers (below 1,300 m). Moreover, PAHs could occur as a result of naturally accumulated oil and gas in this basin, which is probably the case for sites with detectable (D8 and D10) and low detectable load (D1 and D5).

Pollution impact of PAHs

Biological effects. In order to estimate the potential of PAHs from Danube sediments to produce harmful effects on all environmental matrices, wildlife, and human health, concentration levels were compared to the international Sediment Quality Guidelines (SQGs). Two sets of SQGs, effects range low–effects range median (ERL/ERM) and threshold effect level–probable effect level (TEL/PEL) values, have been used in this research to estimate the environmental toxicity of chosen PAHs in Danube sediments. Moreover, these sets can be applied to recognize three ranges of contaminant concentrations, *i.e.*, 1) low range – probably without the appearance of harmful biological effects (below the TEL or ERL values); 2) middle range – possible occurrence of harmful biological effects (between the TEL or ERL and PEL or ERM values); 3) upper range – probably harmful biological effects will occur (values beyond PEL or ERM values). ERL//ERM and TEL/PEL have been established to have a certain predictive capability, while taking into account their chemical bioavailability. Comparison of pollutant levels with SQGs (Table S-IV, Supplementary material) confirmed that the concentration levels of all PAHs were less than ERM and PEL values at all 10 studied sites. Fluorene concentrations in riverbed sediment at D5 (88 µg/kg) were in the middle range (ERL/TEL and ERM/PEL) and were below ERL/TEL at the remaining nine sites. Considering that site D5 is characterized by high contamination and low content of TOC, this finding is expected. Only light PAHs were recorded at elevated levels and no adverse biological effects are anticipated at this site. In addition, at D9 (677 µg/kg), the total concentration of all quantified PAHs exceeded the TEL value, while remaining below the ERL limit. In the analysed sediment samples, no value exceeds the ERM/PEL limits. These results are contrary to those shown in an investigation performed in Taiwan, where the ΣPAHs were between ERL and ERM values in five sediment samples (6.3 %), and below ERL at the remaining 15 samples (93.7 %).[34]

PAHs are pseudo-persistent in the environment and are characterized by liposoluble and carcinogenic properties. The bioavailability of most pollutants in sediments, according to the US EPA,[35] could be estimated using equilibrium partitioning sediment benchmarks (EPSBs). For EPSBs toxic unit (EPSBTU) development of PAHs in sediments, the normalized value of individual PAHs compared with its final chronic value (FCV) was applied. After calculating ΣEPSBTU for all examined PAHs, it is necessary to apply a factor of uncertainty.

PAH uncertainty factors were set to 4.14. Values of the adjusted ΣEPSBTU of PAHs detected in the Danube sediment ranged from 0.06 to 0.42. Since the obtained values are below 1, it is assumed that the sediment will not be toxic to aquatic life.

The reported results are consistent with the fact that PAH concentration levels determined in Danube sediments cannot be associated with a significant risk to humans and wildlife.

Potential toxic risk. The estimation of sediment toxicity collected from 10 sites along the Danube was accomplished based on the summed concentrations of seven proven/probably carcinogenic individual PAHs (PAHs^{carc}), containing B[a]A, Chr, B[b]Flu, B[k]Flu, B[a]P, DB[ah]A and IP[34]. The summary concentration values of the seven PAHs^{carc} (PAHs$_7^{carc}$) in this paper were in the range of 26.54 to 277.54 μg/kg, with an average concentration of 135.31 μg/kg. In addition, the sediments from D9 contained slightly higher levels of PAHs^{carc} than those at other sites, while the sediment collected at site D5 had the lowest values. The obtained values were significantly lower than those determined for PAHs$_7^{carc}$ of superficial sediments from the region of estuarine Danube coast (PAHs$_7^{carc}$: 130.5−551.9 μg/kg[19]) and Mithi River, India (PAHs$_7^{carc}$ for three sampling stations: 1,513±238, 1,369±242 and 1,018±130 μg/kg[18]). Considering all probably carcinogenic PAHs, B[a]P is the unique determined polyarene for which sufficient published toxicological data exist, in order to allow the calculation of carcinogenic potency factor. Therefore, the probable toxicity of Danube sediments was estimated by calculating the total toxic Benzo[a]pyrene equivalent (TEQ^{carc}) for all PAHs^{carc}, using the toxic equivalency factor (TEF^{carc}) relative to B[a]P for each PAH (Table S-V, Supplementary material) and using the following equation:[8]

$$\text{TotalTEQ}^{carc} = \sum C_i \text{TEF}_i^{carc} \qquad (2)$$

with: C_i representing the values of particular PAHs^{carc} concentration (μg/kg) and total TEQ^{carc} in the Danube ranged from 6.49 to 74.94 ng TEQ/g, with an average concentration of 35.20 ng TEQ/g. In comparison, obtained values of TEQ$_7^{carc}$ were slightly higher than those pertaining to another region examined in the previous investigations, such as Mvudi and Nzhelele Rivers (TEQ$_7^{carc}$: 24.86 ng TEQ/g and 18.40 ng TEQ/g, respectively[35]), and lower then total TEQ^{carc} values in sediments of Danube estuary (TEQ$_7^{carc}$: 34.9 to 195.1 ng TEQ/g[19]), and Mithi River in India (TEQ$_7^{carc}$ for three sampling stations: 322.6, 255.28 and 188.21 ng TEQ/g[18]). The contribution of individual carcinogenic PAHs to the total TEQ^{carc} in the Danube varied based on the following chronology: B[a]P (67.66 %), IP (11.29 %), B[b]Flu (7.95 %), DB[ah]A (5.39 %), B[a]A (4.40 %), B[k]Flu (2.84 %), and Chr (0.47 %). The low and considerable values of TEQ$_7^{carc}$ concentrations indicate low carcinogenicity.

CONCLUSION

Presence, origin and ecological risk of twenty-nine PAHs detected and quantified in riverbed layer sediment collected from 10 sites along the Danube in Serbia were investigated. Total 29 PAH concentrations in sediment samples were relatively low in comparison with other relevant studies around the world, but still provide highly valuable information on the current status of aquatic systems. Different statistical tools were used to provide a more precise picture of PAH sources. The origin of PAHs, as determined by the diagnostic ratios method, revealed the prevalence of pyrolytic origin at all studied sites, with the exception of D1 and D5, where the petrogenic origin was noted. Due to the high presence of "light" PAHs in sediment, the site D5 was distinguished as the area with significant recent contamination. Analysis of the results using PCA confirmed the dominant presence of anthropogenic sources (combustion and vehicular emissions). In addition to the expected potential anthropogenic sources of PAH pollution, PCA also revealed the presence of natural sources in this area. Cluster analysis proves that the sediment sample collected near Pančevo (D9), situated downstream of Belgrade and industrial agglomeration Pančevo, was characterized by the most significant pollution. The outcome of possible toxicity, as well as the evaluations of biological effects, show that Danube riverbed sediments have a relatively low level of hazardous pollution. The only exception is site D9, which has a medium level of toxic pollution.

The findings contribute to the establishment of a surveillance monitoring programme that would cover the Serbian part of Danube, with special focus on the impact of extensive areas under heavy agricultural activities. For that purpose and in order to measure the freely dissolved concentrations, the continuous sampling (with time weighted average concentration) should be performed, extremely low levels of organic pollutants should be detected and passive samplers should be used in future investigations.

SUPPLEMENTARY MATERIAL

Additional data are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

ПРОСТОРНА ДИСТРИБУЦИЈА ПОЛИЦИКЛИЧНИХ АРОМАТИЧНИХ УГЉОВОДОНИКА У СЕДИМЕНТУ РЕКЕ ДУНАВА У СРБИЈИ: АНТРОПОГЕНИ И ПРИРОДНИ ИЗВОРИ

МАЈА БРБОРИЋ[1], БРАНИСЛАВ ВРАНА[2], ЈЕЛЕНА РАДОНИЋ[1], МИРЈАНА ВОЈИНОВИЋ МИЛОРАДОВ[1] и МАЈА ТУРК СЕКУЛИЋ[1]

¹Универзитет у Новом Саду, Факултет техничких наука, Департман за инжењерство заштите животне средине и заштите на раду, Трг Доситеја Обрадовића 6, Нови Сад и ²Masaryk University, Faculty of Sciences, Research Center for Toxic Compounds in the Environment, RECETOX, Kotlářská 267/2, 611 37 Brno, Czech Republic

У раду је анализирано присуство полицикличних ароматичних угљоводоника (РАН) у површинском слоју седимената прикупљених на 10 локација дуж реке Дунав на територији Србије. Потенцијални извори загађења су идентификовани коришћењем различитих мулти-варијатних техника. Укупне концентрације 29 РАН у узорцима седимената кретале су се у распону од 128,27 до 676,85 µg/kg. На основу добијених концентрација и РАН образаца, утврђена је јасна просторна дистрибуција на испитиваним локалитетима. Дијагностички односи указују на присуство пиролитичких РАН, осим на два места где је РАН контаминација настала из петрогених извора. Варимакс ротирана анализа главних компоненти је дефинисала три главна извора (сагоревање угља, дрва и биомасе; емисије из возила; природни извори) који чине 92,1 % варијансе у оригиналним скуповима података. Локације са различитим оптерећењима контаминације у зависности од идентификованих главних извора су уређене помоћу кластер анализе. Резултати су упоређени са смерницама за квалитет седимента, а добијене вредности токсичних јединица током примене равнотежног партиционисања расподеле седимента и укупне токсичне еквивалентне количине бензо[*a*]пирена указују да дунавски седименти не представљају екотоксиколошки ризик за бентосне организме.

(Примљено 6. јуна, ревидирано 14. јуна, прихваћено 17. јуна 2019)

REFERENCES

1. B. Dalmacija, *Strategy for water supply and water protection in AP Vojvodina*, University of Novi Sad, Novi Sad, 2009.
2. J. Klánová, J. Kohoutek, R. Kostrhounová, I. Holoubek, *Environ. Int.* **33** (2007) 719 (https://doi.org/10.1016/j.envint.2007.02.004)
3. J. Radonić, N. Jovčić Gavanski, M. Ilić, S. Popov, S. Batić Očovaj, M. Vojinović Miloradov, M. Turk-Sekulić, *Stoch. Env. Res. Risk, A* **31** (2017) 2201 (https://link.springer.com/article/10.1007/s00477-016-1372-x)
4. R. Sarria-Villa, W. Ocampo-Duque, M. Páez, M. Schuhmacher, *Sci. Total. Environ.* **540** (2016) 455 (https://www.sciencedirect.com/science/article/pii/S0048969715303624)
5. B. Vrana, A. Paschke, P. Popp, *J. Environ. Monitor.* **3** (2001) 602 (https://www.ncbi.nlm.nih.gov/pubmed/11785633)
6. G. Perra, K. Pozo, C. Guerranti, D. Lazzeri, V. Volpi, S. Corsolini, S. Focardi, *Mar. Pollut. Bull.* **62** (2011) 874 (https://doi.org/10.1016/j.marpolbul.2011.01.023)
7. R. Prokeš, B. Vrana, K. Komprdová, J. Klánová, *J. Soil Sediments* **14** (2014) 1738 (https://link.springer.com/article/10.1007/s11368-014-0931-3)
8. J. Li, H. Dong, D. Zhang, B. Han, C. Zhu, S. Liu, X. Liu, Q. Ma, X. Li, *Mar. Pollut. Bull.* **96** (2015) 485 (https://doi.org/10.1016/j.marpolbul.2015.05.002)
9. C.W. Liu, K.H. Lin, Y.M. Kuo, *Sci. Total. Environ.* **313** (2003) 77 (https://doi.org/10.1016/S0048-9697(02)00683-6)

10. UNEP Final Report: *The Kosovo Conflict: Consequences for the Environment and Human Settlements*, 1999 (https://postconflict.unep.ch/publications/finalreport.pdf)

11. P. Baumard, H. Budzinski, P. Garrigues, *Environ. Toxicol. Chem.* **17** (1998) 765 (https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5620170501)

12. Y. Chen, L. Zhu, R. Zhou, *J. Hazard. Mater.* **141** (2007) 148 (https://www.ncbi.nlm.nih.gov/pubmed/16901627)

13. X. He, Y. Pang, X. Song, B. Chen, Z. Feng, Y. Ma, *Mar. Pollut. Bull.* **80** (2014) 52 (https://doi.org/10.1016/j.marpolbul.2014.01.051)

14. D.M. Crnković, N.S. Crnković, A.J. Filipović, L.V. Rajaković, A.A. Perić-Grujić, M.D. Ristić, *J. Environ. Sci. Health* **43** (2008) 1353 (https://www.ncbi.nlm.nih.gov/pubmed/18780212)

15. S. Sakan, B. Ostojić, D. Đordevic, *J. Geochem. Explor.* **180** (2017) 91(https://doi.org/10.1016/j.gexplo.2017.06.008)

16. B. Škrbić, J. Cvejanov, N. Durišić-Mladenović, *J. Environ. Sci. Health, A* **40** (2007) 29 (https://www.tandfonline.com/doi/abs/10.1081/ESE-200033512?journalCode=lesa20)

17. S. Keiter, S. Grund, B. Van Bavel, J. Hagberg, M. Engwall, U. Kammann, M. Klempt, W. Manz, H. Olsman, T. Braunbeck, H. Hollert H, *Anal. Bioanal. Chem.* **390** (2008) 2009 (https://www.ncbi.nlm.nih.gov/pubmed/17938895)

18. P. Singare, *Mar. Pollut. Bull.* **101** (2015) 232 (https://doi.org/10.1016/j.marpolbul.2015.09.057)

19. K. Tsymbalyuk, Y. Den'ga, N. Berlinsky, V. Antonovich, *GeoEcoMar* **17** (2011) 67 (https://www.researchgate.net/publication/284696971_Determination_of_16_priority_polycyclic_aromatic_hydrocarbons_in_bottom_sediments_of_the_Danube_estuarine_coast_by_GCMS)

20. O. B. Said, H. Louati, A. Soltani, H. Preud, C. Cravo-Laureau, P. Got, O. Pringault, P. Aissa, R. Duran, *Environ. Sci. Pollut. R.* **22** (2015) 15319 (https://link.springer.com/article/10.1007/s11356-015-4105-7)

21. R.J. Irwin, M.V. Mouwerik, L. Stevens, M.D. Seese, W. Basham, *Environmental contaminants encyclopedia- Indeno 1, 2, 3-cd pyrene entry,* National park service water resources divisions, Water operations branch, Fort Collins, CO, 1997

22. K. Hussain, M. Rahman, A. Prakash, R. R. *Sustain. Cities Soc.* **19** (2015) 17 (https://doi.org/10.1016/j.scs.2015.07.010)

23. Y. Liu, L. Chen, Q. H. Huang, W. Y. Li, Y. J. Tang, J. F. Zhao, *Sci. Total. Environ.* **407** (2009) 2931 (https://doi.org/10.1016/j.scitotenv.2008.12.046)

24. G. C. Fang, K. F. Chang, C. Lu, H. Bai H, *Chemosphere* **55** (2004) 787 (https://doi.org/10.1016/j.chemosphere.2003.12.012)

25. J. J Schauer, M. J. Kleeman, G. R. Cass, B. R. T. Simoneit, *Environ. Sci. Tech.* **35** (2001) 1716 (https://pubs.acs.org/doi/abs/10.1021/es001331e)

26. G. Gryglewicz, P. Rutkowski, J. Yperman, *Fuel. Process. Technol.* **77**–**78** (2002)167 (https://doi.org/10.1016/S0378-3820(02)00046-2)

27. K. Ravindra, R. Sokhia, R.V. Griekenb, *Atmos. Environ.* **42** (2008) 2895 (https://doi.org/10.1016/j.atmosenv.2007.12.010)

28. M.P. Zakaria, H. Takada, S. Tsutsumi, K. Ohno, J. Yamada, E. Kouno, *Envir. Sci. Tech.* **36** (2002) 1907 (https://pubs.acs.org/doi/abs/10.1021/es011278%2B)

29. T. Gocht, J. Barth, M. Epp, M. Jochmann, M. Blessinga, T. Schmidt, P. Grathwohl, *Appl. Geochem.* **22** (2007) 2652 (https://www.sciencedirect.com/science/article/pii/S0883292707001904)

30. Z. Wen, W. Ruiyong, M. Radke, W. Qingyu, S. Guoying, L. Zhili, *Org. Geochem.* **31** (2000) 757 (https://doi.org/10.1016/S0146-6380(00)00064-4)
31. A. Ene, O. Bogdevich, A. Sion, *Sci. Total. Environ.* **439** (2012) 76 (https://doi.org/10.1016/j.scitotenv.2012.09.004)
32. R. Loos, G. M. Bernd, L. Giovanni, R. Erika, C. Serafino, B. Giovanni, *Environ. Pollut.* **157** (2009) 561 (https://doi.org/10.1016/j.envpol.2008.09.020)
33. M. Stamenković, M. Cvijan, *Botan. Serb.* **33** (2009) 89 (http://agris.fao.org/agris-search/search.do?recordID=RS2009001449)
34. C. F. Chen, C. W. Chen, C. D. Dong, C. M. Kao, *Sci. Total. Environ.* **463**–**464** (2013) 1174 (https://doi.org/10.1016/j.scitotenv.2012.06.101)
35. US EPA: *Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: PAH Mixtures, Office of Research and Development*, EPA/600/R-02/013 (2010) (https://www.epa.gov)
36. J. Edokpayi, J. Odiyo, O. Popoola, T. Msagati, *Int. J. Env. Res. Public Health* **13** (2016) 387 (https://www.ncbi.nlm.nih.gov/pubmed/27043597).