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Unveiling the impact of lipophilic brominated flame retardants in Danube River sediments: a deep dive into pollution sources and distribution

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Abstract
Aquatic sediments serve as both a major sink and a potential secondary source of polybrominated diphenyl ethers (PBDEs), a class of persistent, bioaccumulative, and toxic pollutants. This study assessed ten PBDE congeners in Danube River sediments (Serbia), addressing their occurrence, spatial distribution, and potential sources. Σ10PBDE concentrations ranged from 0.52 μg/kg dry weight (War Island) to 31.21 μg/kg dry weight (Neštin), with BDE-209 as the predominant congener. Localized dominance of hepta- and penta-BDEs at Neštin and Šangaj suggested site-specific contamination patterns. Comparative analysis indicated levels comparable to or exceeding those in other industrialized regions, implicating anthropogenic activities as key contributors. Source apportionment using Principal Component Analysis identified three major pathways: (1) transport and debromination of higher-brominated congeners, (2) direct anthropogenic discharge, and (3) improper waste management. Hierarchical cluster analysis and Kohonen's self-organizing maps pinpointed Neštin as a pollution hotspot. This study provides the most comprehensive dataset on PBDE contamination in Serbian Danube sediments, establishing a critical baseline for future monitoring and regulatory actions.

Keywords: *PBDEs, aquatic sediment, Danube River*

Introduction

The Danube River plays a vital role in Serbia's environmental and socio-economic systems, supporting biodiversity, agriculture, industry, and human settlements (Milić et al., 2019; ICPDR, 2021; Grzywna et al., 2023). However, intensive anthropogenic pressure-primarily from untreated wastewater discharges, industrial activities, and diffuse pollution has resulted in the degradation of water and sediment quality (Brboric et al., 2019; Rusina et al., 2019; Chițescu et al., 2021). The unmitigated discharge of pollutants threatens the delicate balance of aquatic ecosystems, jeopardizes the health of aquatic organisms, and endangers the well-being of communities dependent on the river for various purposes. Addressing the pollution of the Danube is imperative not only for the preservation of its ecological integrity but also for ensuring the continued health and sustainability of the interconnected natural and human systems it sustains (Rus et al., 2025). As a major sink for hydrophobic contaminants, aquatic sediments can also serve as secondary sources of pollution through remobilization processes triggered by hydrological events, sediment disturbance, or river management interventions (Pandey et al., 2021; Lakshminarasimma et al., 2024). Among the most concerning groups of sediment-associated pollutants are lipophilic and persistent organic compounds, particularly brominated flame retardants (BFRs). These compounds, widely incorporated into consumer and industrial goods to meet fire safety standards, are

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known for their environmental persistence, bioaccumulation potential, and toxicity (Meng et al., 2025; Yang et al., 2025). Specific polybrominated diphenyl ethers (PBDEs)—notably tetra to hexa congeners and Deca-BDE—have been listed as Persistent Organic Pollutants (POPs) under the Stockholm Convention due to their long-range environmental transport and ecological risks. Other BFRs, while not yet regulated globally, are categorized as emerging contaminants requiring urgent monitoring (NORMAN, 2017). Despite the introduction of strict bans and phase-out measures in many developed countries (US EPA, 2023; Environment Agency, 2021; Government of Canada, 2016), PBDEs continue to be detected in aquatic environments worldwide. Their persistence is attributed to the recycling and continued use of legacy products, as well as unregulated releases in developing regions (Nzangya et al., 2021; Olaniyan et al., 2023). In Serbia, BFRs are currently not subject to specific environmental regulations, and data on their occurrence in sediments remain extremely limited. This study addresses this gap by providing a comprehensive assessment of PBDE contamination in bottom sediments along the Serbian stretch of the Danube River. Through the application of advanced analytical and statistical methods, it explores the spatial distribution, compositional profiles, and potential sources of ten target PBDE congeners. Furthermore, it evaluates the role of organic matter in PBDE partitioning and evaluates the role of organic matter in PBDE partitioning. The outcomes of this research establish a baseline for long-term environmental monitoring and contribute essential knowledge for the development of effective pollution control strategies.

Materials and methods

Sediment sampling and preservation

Sediment sampling was carried out in October 2022 at ten representative locations along the Serbian section of the Danube River (Fig. 1). The sites were selected to reflect a range of hydrological and anthropogenic conditions. Detailed information about the site characteristics has been published previously (Brborić et al., 2019). At each site, six to eight subsamples were collected using a grab sampler within a 10-meter radius, from a sediment depth of 0–10 cm. The subsamples were combined into composite samples of 700–1000 g and transported under cooled conditions (~4 °C) to the laboratory. Upon arrival, samples were sieved through a 2 mm mesh to remove coarse material, and the fraction below 63 μm was retained for analysis. Samples were then freeze-dried, ground, homogenized, and stored at −20 °C until chemical analysis.

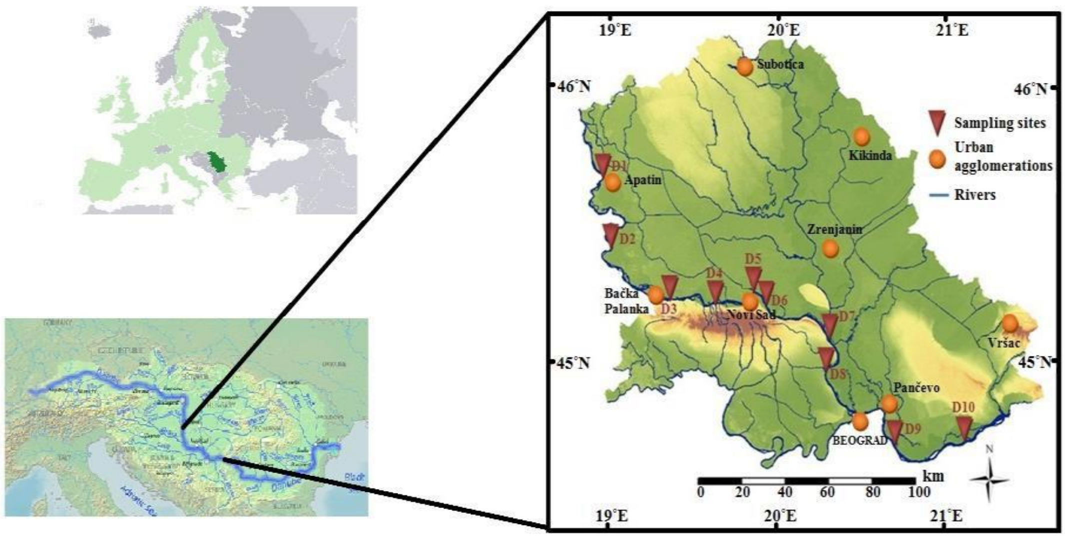

Figure 1
Area of the collected Danube sediments and individual sampling point

Sediment analysis

Sediment samples underwent extraction using toluene in a Soxhlet extractor (a 60-minute warm Soxhlet process followed by 30 minutes of solvent purification) with toluene in a B-811 extraction component (Büchi, Switzerland). Prior to extraction, surrogate recovery standards 13C BDEs (28, 47, 66, 85, 99, 100, 153, 154, 183, 209) were introduced into the examined sediment samples. The resulting extract was purified utilizing activated silica and eluted with a mixture comprising 40 ml DCM/n-hexane in a 1:1 ratio. Fractionation for each sample occurred in microco-

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lumns (6 mm), where the bottom layer contained 50 mg of silica gel, the middle layer comprised 70 mg of charcoal/silica gel (in a ratio of 1:40), and the upper layer consisted of 50 mg of silica gel. Preceding the fractionation, the columns were washed with 5 ml of toluene, rinsed with a 5 ml DCM/cyclohexane mixture (30%), and ultimately eluted using a mixture of 9 ml DCM/cyclohexane. All samples were concentrated using a nitrogen flow in a concentrator (TurboVap II, Caliper LifeSciences, USA) and transferred into vials. Prepared standards were added to all applied samples, bringing them to a final volume of 50 μl. PBDEs were analyzed using a 7890A gas chromatography-mass spectrometry system equipped with an Agilent J&W Scientific fused silica column DB-5MS (60 m × 25 μm × 0.25 μm), coupled with an AutoSpec Premier MS (Waters, Micromass, UK).

Quality assurance and quality control

To ensure accuracy, reliability, and reproducibility of the results, a comprehensive QA/QC strategy was implemented throughout sample preparation, instrumental analysis, and data validation. Quality control procedures included surrogate standard recovery, instrument calibration, procedural blanks, and duplicate analysis. Instrumental performance was assessed using six-point calibration curves for each target PBDE congener, with correlation coefficients (R^2) > 0.993, confirming excellent linearity. Limits of detection (LOD) and quantification (LOQ), defined by signal-to-noise ratios of 3:1 and 10:1, ranged from 0.5–1.5 μg/kg and 1.5–4.0 μg/kg dry weight, respectively. Analytical reproducibility was verified by duplicate analysis of sample D7, with relative differences within ±15%, while recovery efficiencies ranged between 78% and 112%, confirming satisfactory extraction and low variability. Recovery values for spiked solvent samples and internal standards indicated no significant signal suppression or enhancement. Procedural blanks and storage blanks showed no detectable target analytes above LOQs, excluding contamination risks. This multi-tiered QA/QC approach ensured the robustness, reliability, and accuracy of PBDE concentration data reported in this study.

Data analysis

To identify compositional trends and potential contamination sources, multivariate statistical analyses were applied. Principal Component Analysis (PCA) with vari-max rotation was used to reduce dimensionality and uncover latent structure within PBDE congener profiles. Hierarchical Cluster Analysis (HCA) and k-means clustering grouped sampling locations based on similarities in contamination levels. Additionally, Kohonen's self-organizing maps (KSOM) (Kohonen, 2012), a neural network–based unsupervised learning technique, were employed to enhance pattern visualization and support classification. Given the limited number of sampling sites (n = 10) and variables (10 PBDE congeners), the statistical evaluation should be interpreted as exploratory rather than confirmatory. This study prioritizes the integration of complementary techniques to enhance pattern recognition and guide hypothesis generation. While statistical power is inherently limited in small datasets, the convergent use of PCA, HCA, and KSOM increases confidence in the observed clustering patterns and source attributions. All analyses were performed using IBM SPSS Statistics 22 and MATLAB 2.0.

Results and discussion

Spatial distribution of PBDEs

The investigation examined the concentrations and distribution of ten PBDE congeners in bottom sediments from ten locations along the Serbian section of the Danube River. All congeners were detected, with Σ10PBDE concentrations ranging from 0.52 μg/kg dry weight (D5) to 31.21 μg/kg dry weight (D3), with a mean of 8.09 μg/kg and a median of 3.14 μg/kg (Fig. 2; Table A.2). BDE-209 was the most abundant congener, detected at all sites in concentrations ranging from 0.49 to 23.37 μg/kg (average: 4.22 μg/kg), accounting for 30.8% to 99.3% of the total PBDE burden. Its concentration at site D10 was the highest, exceeding other

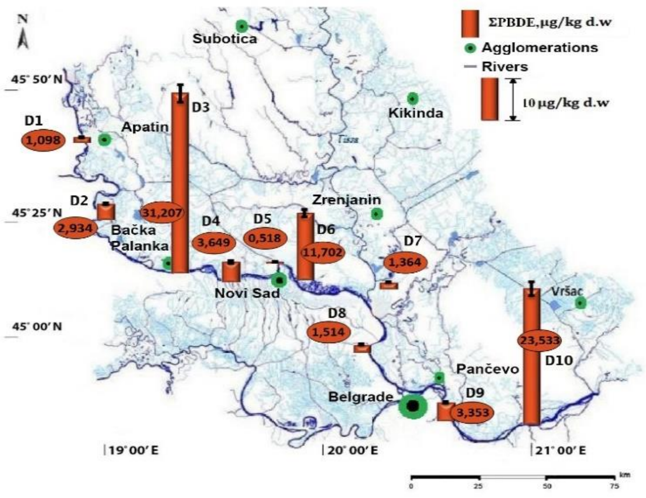

Figure 2. *Total concentration levels of PBDEs in Danube River sediments*

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sites by a factor of 3 to 48. BDE-100, one of the lower-brominated congeners, reached a maximum value of 23.64 μg/kg at site D3. The congener profile (Fig. 3) indicates a predominant influence from technical Deca-BDE mixtures, particularly BDE-209, with secondary contributions from Penta- and Hepta-BDE formulations. Interestingly, some industrial sites (D1, D5, D9) showed relatively low PBDE concentrations, suggesting that in addition to direct anthropogenic sources,. diffuse mechanisms such as atmospheric transport, photodegradation, and surface runoff influence contaminant dispersion. Notably, the majority of the detected congeners - BDE 47, 66, 85, 99, 100, 153, 154, 183, and 209 - are classified as emerging contaminants due to their persistence, bioaccumulative potential, and increasing detection frequency in aquatic environments. Their widespread presence highlights the continued need for regulatory oversight and environmental monitoring.

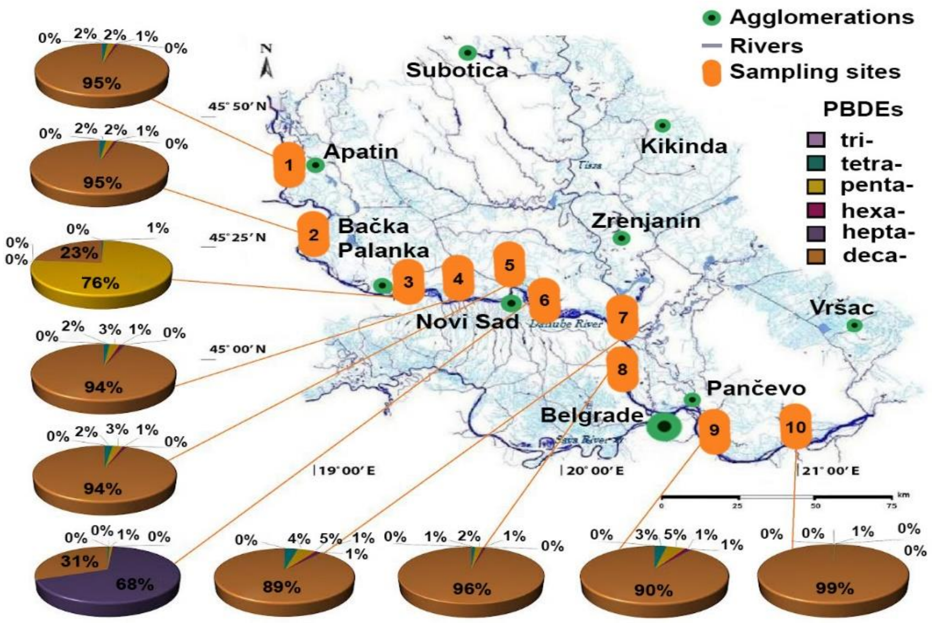

Figure 3
The composition of PBDEs in Danube River sediments

Total organic carbon (TOC) levels in sediments varied from 0.3% (D8) to 1.3% (D3). A moderate but statistically significant correlation was observed between TOC and ΣPBDEs ($r = 0.52$, $p < 0.01$), indicating the importance of organic matter in controlling PBDE distribution. This was particularly evident for BDE-28 ($r = 0.83$, $p < 0.01$), suggesting that lower-brominated congeners preferentially bind to organic-rich sediment. The correlation matrix is visualized in Figure 4, where warmer colors denote

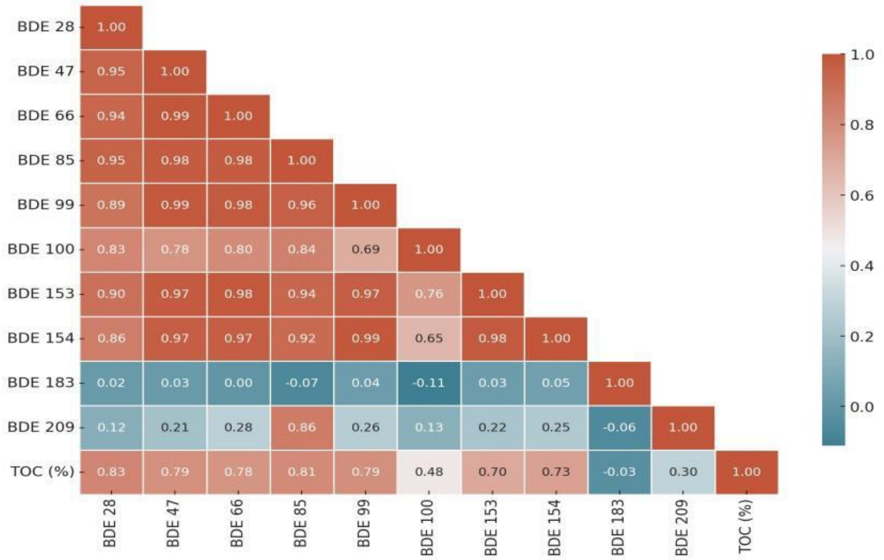

Figure 4. *Pearson correlations between the content of TOC and PBDE congeners in sediment samples*

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Table 1. *PBDEs concentrations (μg/kg d.w.) in sediments from different regions worldwide*

Location	Sampling year	N° of samples	N° of congeners	Tri- to hepta-BDE	BDE-209	Reference
Fuhe river, North, China	2008	19	18	0.13-6.39	11.8-292.7	Hu et al., 2010
Four major rivers, Korea	2005-2008	70	27	0.46-1760	0.34-1320	Lee et al., 2012
Niagara river, Canada	2003-2006	20	17	n.d.-18	n.d. -170	Richman et al., 2013,
Liaohe river, China	2013	29	8	0.04-4.65	0.12 to 13.50	Lv et al., 2015
Upper catchment of the Danube river	2007-2008	5		0.02-0.24	0.06-40.20	Kukucka et al., 2015
Vaal River, South Africa	2013	6	5	14-28		Chokwe et al., 2016
Three Gorges Reservoir, China	2012	5	37	0.08-0.30	0,08-10.00	Wang et al.,2017
Danshui river and Keelung river, Taiwan	2015	33	19	1.4-52.7 3.9-96.1	0.9-1388.1 18.7-332.9	Cheng and Ko, 2018
Sava river Serbia	2014-2015	11	8	nd to 14.0		Giulivo et al., 2017
Danube river, Serbia	2007	28	8	Penta BDE: 0.20-1.20 Hepta BDE: 0.03-0.42	1.5-51	JDS2. 2008
Danube river, Serbia	2022	10	10	0.03-24.05	0.49-23.37	Present study

n.d. – not defined

strong positive correlations with TOC and cooler colors indicate weak or negative associations. BDE-183 and BDE-209 exhibited weak or negative correlations with TOC, implying that their sorption is more influenced by sediment characteristics such as grain size or mineral composition. These larger, highly bromineted congeners may preferentially bind to coarse particles and exhibit lower affinity for organic fractions. In sum-mary, the spatial and compositional trends of PBDEs in Danube River sediments reflect a mixed influence of technical mixtures, with Deca-BDE predominance and localized contributions of lower-brominated species. The results also highlight the critical role of TOC in the distribution of less brominated congeners, while more brominated PBDEs appear to be governed by addi-tional sedimentological and physicochemical factors. A broader comparison with international data (Table 1) further contextualizes the observed concentrations. PBDE levels in the Danube sediments are generally comparable to or slightly lower than those reported in highly industrialized or urbanized regions worldwide, reflecting the mixed influence of local emissions, regulatory history, and hydromorphological dynamics.

Source identification

Although multivariate statistical approaches such as PCA are typically applied to large datasets, in this study the method was employed on a limited number of sediment samples due to the restricted spatial coverage. Nevertheless, the PCA yielded clear and consistent patterns that allow meaningful interpre-tation of PBDE sources and transformations, sup-porting its use as an exploratory tool in this context. PCA with varimax rotation was applied to the correlation matrix of PBDE concentrations to identify potential sources and patterns in the data. Although based on a relatively limited dataset, the analysis yielded three well-defined principal components (PCs), together explaining 94.37% of the total variance (Figure 5), with PC1 accounting for 74.30%, PC2 for 10.55%, and PC3 for 9.51%. The three components represent distinct yet complementary pollution sources. PC1 grouped congeners typically found in Penta- and Octa-BDE technical formu-lations (BDE 28, 47, 66, 85, 99, 100, 153, and 154), which were extensively used until their restriction in 2004. Despite the ban, residual materials remain in circulation, and improper disposal continues to contribute to environmental contamination (Zhang et al., 2025). Once deposited, these compounds can undergo debromination processes—such as photoly-sis, microbial activity, or anaerobic degradation—producing more toxic and persistent lower-bromine-ted congeners (Sodré et al., 2024). PC1 thus reflects both transport and debromination of higher-bromina-

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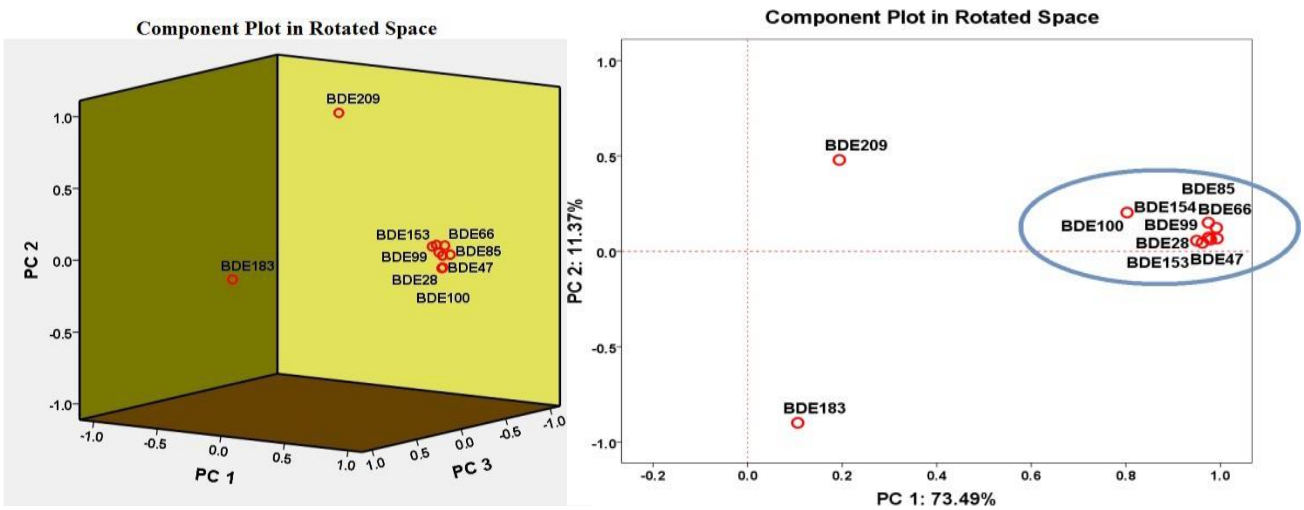

Figure 5. *Component plots for PBDE congeners (3d and 2d rendering*

ted congeners. PC2 was primarily defined by BDE 209, the dominant congener in Deca-BDE mixtures, which is characterized by extremely low mobility (log Kow ≈ 10) and rapid sedimentation near emission points. Its presence highlights recent or direct inputs from industrial or urban activities.Finally, PC3 was associated with BDE 183 and congeners related to e-waste and polymer materials, such as those used in acrylonitrile butadiene styrene (ABS), polyamides, and polystyrene. Higher levels of BDE 183 were observed downstream from Novi Sad, a highly industrialized city with approximately 0.5 million inhabitants, suggesting localized impacts from inadequate electronic waste disposal and leachates. Altogether, the PCA effectively delineated pollution originating from historical product use, ongoing point-source releases, and diffuse contributions from consumer plastics and e-waste. This multi-source profile underscores the complexity of PBDE contamination and the importance of integrated source-control measures.These findings demonstrate the value of PCA in disentangling overlapping contamination signals and reinforce the need for comprehensive source control strategies.

Environmental hotspots: HCA and KSOM applications

Hierarchical Cluster Analysis revealed four distinct clusters, reflecting variations in PBDE contamination across the study area (Figure 6). The Neštin site (Cluster 1) stood out as the most contaminated, marked by elevated concentrations of penta- and octa-BDEs. In the absence of a clear point source, these levels likely result from atmospheric deposition, hydrodynamic redistribution, and potentially debromination of higher-brominated congeners. Industrial emissions from nearby Bačka Palanka may also contribute. A second hotspot emerged at site D10, with high levels of deca-BDE (BDE 209), plausibly linked to textile-related industrial activity in Smederevo. This localized input distinguishes Dubravica from the more diffuse contamination observed in Neštin. Further downstream, site D6 formed a separate cluster, notable for the highest concentration of BDE 183. Its proximity to a major controlled solid waste landfill suggests that waste leachates and decomposition processes are likely sources. This highlights landfills as significant secondary reservoirs of PBDEs. In contrast, the final cluster grouped sites with generally lower contamination, indicative of minimal anthropogenic influence or effective pollutant dilution. The heatmap in Figure 6 comple-

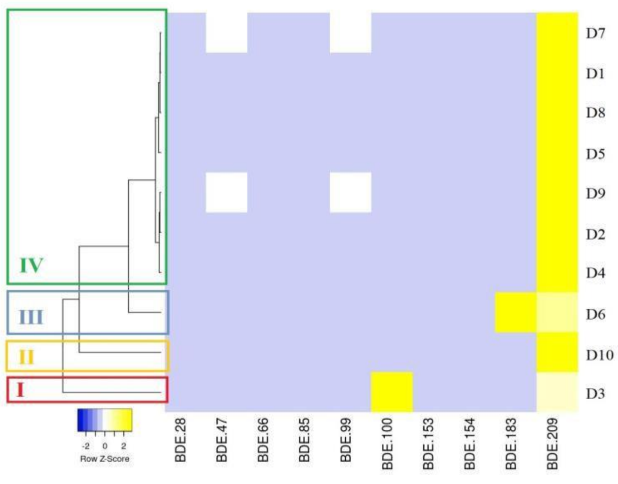

Figure 6. *Cluster dendograms of PBDEs identified in ten tested samples*

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ments the clustering results, mapping the relative abundance of PBDE congeners through a gradient from blue (low) to yellow (high). BDE 209 was enriched at sites D3 and D10, while BDE 183 peaked at D6. Localized hotspots at D4 and D6 also point to combined effects of diffuse and point-source inputs. Overall, the HCA patterns support findings from PCA, confirming complex interactions between industrial activity, waste management, and contaminant transport pathways. To enhance the interpretability of complex, high-dimensional data, KSOM, a subclass of artificial neural networks (ANN) employing unsupervised learning, were applied. KSOM enables intuitive and detailed visualization of inter-variable relationships and spatial patterns that are often obscured in conventional statistical methods (Yang et al., 2015). The component panels produced by KSOM, trained on the concentrations of all examined PBDE congeners, are shown in Figure 7. These panels use color gradients - yellow indicating high and blue indicating low values - to visualize relative abundance and correlations across sampling locations. By comparing these panels, inter dependencies between congeners become evident; similar color regions suggest positive correlations, while opposing patterns point to negative associations.

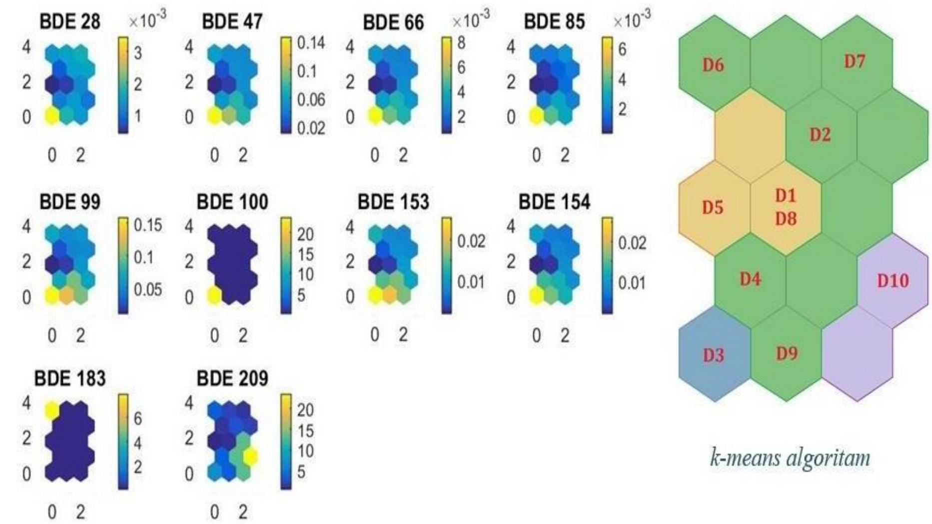

Figure 7
Component panels and k-means algorithm for PBDEs obtained using Kohonen self- organizing maps

KSOM results showed a distinct separation of deca-BDE (BDE 209) from the rest of the PBDE profile, in alignment with trends observed in both conventional multivariate statistical techniques. Spatial organization of the sampling sites within the self-organizing map further revealed four major clusters, derived via the k-means algorithm. Sites D3 and D10 clearly stood out as contamination hotspots, with D3 showing high concentrations of BDE-100 and D10 dominated by BDE-209. Other locations displayed moderate pollution profiles and grouped into green or yellow-toned clusters. What sets KSOM apart is its capacity to translate complex nonlinear relationships into accessible visual outputs, enhancing pattern recognition and allowing for faster data interpretation compared to PCA or HCA. Unlike linear reduction techniques, KSOM operates without presupposed data distributions or correlation structures, offering a more flexible, adaptive approach to clustering. Still, interpretation should be guided by a solid understanding of the dataset, as overreliance on color alone may oversimplify subtle patterns. Nevertheless, in this study, KSOM not only corroborated the findings from PCA and HCA but also offered nuanced insights into the compositional separation and spatial behavior of PBDEs across the Danube sediment matrix. The results of HCA and KSOM demonstrated strong complementarity, with both methods identifying similar site groupings and highlighting congruent pollution patterns. Their mutual agreement with PCA findings reinforces the robustness of the clustering structure obtained. Given the high degree of overlap among hierarchical, non-hierarchical, and neural network-based approaches, future studies could selectively apply fewer techniques, thereby saving time and analytical resources without compromising interpretive quality.

Conclusions

This study provides a comprehensive assessment of PBDEs in subaqueous sediments of the Serbian section of the Danube River, highlighting the spatial

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distribution of contamination and its potential sources. The high detection frequencies and measurable concentrations of all ten targeted PBDE congeners confirm the persistence and widespread legacy of these flame-retardant pollutants in aquatic environments. Among the examined congeners, BDE-209 was identified as the most dominant, followed by high levels of BDE-183 and BDE-100. All three are considered emerging contaminants due to their potential for bioaccumulation, endocrine-disrupting effects, and persistent presence in various environ-.mental matrices even at low concentrations over prolonged exposure. These compounds were most prominently detected at sites D10, Novi Sad, and Neštin, respectively, which emerged as the most impacted locations and thus represent critical areas for continued monitoring and management. Advanced multivariate and machine learning approaches, including PCA, HCA, and KSOM, proved highly effective in identifying pollution sources and characterizing spatial variability. Despite being based on a relatively small dataset, the analysis revealed clear and interpretable contamination patterns and robust statistical groupings, underscoring the value of these tools even under data-limited conditions. Three primary contamination pathways were identified: long-range transport and transformation of higher brominated compounds, direct industrial and urban e-missions, and leakage from PBDE-laden waste materials. The complementary application of unsupervised machine learning through KSOM further enhanced data interpretability and enabled fine-scale visualization of contamination hotspots. This study demonstrates the value of combining classical statistical tools with artificial intelligence–driven methods to better understand contaminant behavior in freshwater ecosystems. It highlights the importance of continued monitoring of both legacy and emerging pollutants, particularly in regions such as the Balkans that may be increasingly vulnerable to global e-waste redistribution. The results offer a replicable framework for sediment quality surveillance and evidence-based environmental decision-making. Future research should prioritize expanded spatial coverage, targeted bioavailability assessments, and integration of ecological and human health models to support long-term pollution control and mitigation strategies.

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Disclosure statement

The authors declare no competing interests.

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