PAH and Petroleum Hydrocarbon Contamination in Water, Suspended Particulate Matter, Sediments, and Biota in the Danube

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Abstract Several analytical methods are used to measure petroleum hydrocarbons contamination in the environment. Each method provides different, specific information about the characteristics of the contamination. Only the results obtained with a particular analytical method can be used for a comparative study or a pollution trend analysis. The polluting aromatic hydrocarbons can be characterized in terms of fluorescence patterns; the contamination level/concentration can be calculated from the fluorescence intensity at specified excitation/emission wavelengths.

Interpretation of the fluorescence fingerprint of cyclohexane extracts of water, SPM, and bottom sediment samples, collected during the Joint Danube Surveys, as well as the results of the PAH analysis provided the following findings: (1) petroleum hydrocarbons in water were characterized by the fluorescence of gasoline; the concentrations varied in the range of 2–300 μg/L; (2) the level of oil contamination was similar in the SPM and the bottom sediment, characterized with the fluorescence of crude oil, and the concentrations varied between 5 and 500 mg/kg; (3) PAH determined in water, SPM, bottom sediment, and biota (mussels) showed similar trends in contamination as observed in the case of petroleum hydrocarbons. However, even the highest concentrations were usually below the EQS values according to the Directive 2013/39/EU, or the PELs in the Canadian Sediment Quality Guidelines.

Keywords Environmental quality standards, Fluorescence fingerprints, Fluorescence spectroscopy, Joint Danube Survey, Oil pollution

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1 Introduction

Among the organic pollutants, oil pollutants (petroleum compounds including PAHs) are one of the most common and frequently occurring organic pollutants, which are introduced into rivers, lakes, and marine waters from oil refineries, other industries, transportation, municipalities, and accidental spills. The oil pollutants, i.e., aliphatic, aromatic, cyclic, and naphthenic hydrocarbons or hetero-compounds, have mainly hydrophobic properties. They can float on the surface of the water and can be dispersed/dissolved in the water column or associated with the suspended particulate matter (SPM), and after settling of the suspended solids, they can accumulate in the bottom sediment. These compounds may undergo environmental weathering—biodegradation and/or chemical (photo-)oxidation, resulting in degradation products—and a number of the petroleum-related compounds may accumulate in aquatic organisms.

Petroleum hydrocarbons and PAHs have been studied in national research programs in several Danube countries; however, the first coordinated transnational survey, along the whole Danube, was conducted by a Cousteau team in 1991–1992 [1]. The sediment survey results indicated pollution hot spots and high variation of the oil pollution along the Danube between Vienna and Budapest. Therefore, one of the Danube Basin Applied Research Projects [2] aimed to make a collaborative study in this Danube reach. The Austrian, Slovak, and Hungarian institutions carried out this survey in 1995–1996. In 1997–1998, the MS Burgund survey [3] was carried out along the Danube reach between the confluence of the Rhein-Main channel and the Hungarian Danube section. Both of these surveys, limited to a specified Danube reach, reported about the similar level of oil pollutants as observed during the Cousteau survey.

Based on the results of these surveys, and the release of the EU Water Framework Directive (WFD) in 2000 [4], coordinated surveys, called Joint Danube Survey (JDS), were planned along the Danube to be implemented every 6 years, starting in 2001 [5].

Table 1 EQS for petroleum-related substances in surface waters and aquatic biota

	EQS as in Directive 2013/39/EC			
	Water		Biota	
Substance	AA (μg/L)	MAC (μg/L)	μg/kg wet wt.	Remarks
Anthracene	0.1	0.1		
Benzene	10	50		
Fluoranthene	0.0063	0.12	30	Crustaceans and Mollusks
Naphthalene	2	130		
Benzo[a]pyrene	0.00017	0.27	5	
Benzo[b] fluoranthene		0.017		
Benzo[k] fluoranthene		0.017		
Benzo[g,h,i]perylene		0.0082		

2 Guidelines/Standards for Assessing Petroleum Hydrocarbon and PAH Contamination in Surface Waters

Environmental quality guidelines for petroleum-related contamination are represented by aromatic hydrocarbons, particularly polyaromatic hydrocarbons, as shown in Table 1 for surface water and biota and in Table 2 for surface water sediment.

3 Methodologies

There is no single analytical method to characterize properly oil pollution due to the variable composition of complex mixture of compounds in the crude oil and its refined products. Different analytical methods have been and are being used for characterizing/estimating oil pollution in water, suspended solids (SPM), and bottom sediment. These methods are based on measuring groups of petroleum compounds or quantifying individual substances. Infrared and UV absorption and fluorescence measurements show group characteristics. Gas chromatograph with flame ionization detector (GC-FID), gas chromatograph with mass spectrometer (GC-MS), and high pressure liquid chromatograph (HPLC) methods can measure individual aliphatic hydrocarbons, volatile organic compounds (e.g., benzene), and/or polyaromatic hydrocarbons (PAHs).

Annex VIII of the WFD [4] shows the indicative list of the main pollutants, including the persistent hydrocarbons and persistent and bioaccumulable toxic organic substances. Among the petroleum hydrocarbons, the aliphatic hydrocarbons are easily biodegradable, whereas persistent hydrocarbons include usually aromatic or polyaromatic structures.

Table 2 Sediment quality guidelines for petroleum-related substances

Substances	Canadian Sediment Quality Guidelines [6]		EU Priority Substances data sheet [7] (μg/kg dry weight)
	Interim sediment quality guidelines (ISQGs) (μg/kg dry weight)	Probable effect levels (PELs) (μg/kg dry weight)	
Anthracene	46.9	245	24
Benzo[a] anthracene	74.8	693	
Benzo[a] pyrene	88.8	763	91.5
Chrysene	108	846	
Dibenz[a,h] anthracene	6.22	135	
Fluoranthene	113	1,494	2,000
Benzo[b] fluoranthene			70.7
Benzo[k] fluoranthene			67.5
Benzo[g,h,i] perylene			42
Phenanthrene	86.7	544	
Pyrene	153	1,398	

Regarding the analytical approach, infrared spectroscopy and the GC-FID methods provide information primarily on the presence of aliphatic hydrocarbons. The GC-FID chromatograms can be used to differentiate between biogenic and petrogenic hydrocarbons and between fresh and weathered oil pollution. UV and fluorescence spectrometry provides signals of the aromatic structures, indicating the persistent hydrocarbons. GC-MS and HPLC methods are used for measuring individual petroleum compounds, particularly those aromatic substances such as benzene or PAHs, which represent petroleum hydrocarbon contaminants among the priority substances and for which environmental quality standards (EQS) have been established [8].

Since the fluorescence measurements provided data/information for characterizing oil pollution of the water, suspended and bottom sediment samples during each of the three JDSs, the florescence fingerprints can be used for a comparative evaluation.

3.1 Determination and Interpretation of Fluorescence Fingerprints

Total fluorescence spectra (fingerprints) of cyclohexane extracts of water, SPM, and bottom sediment samples were recorded according to procedures described in

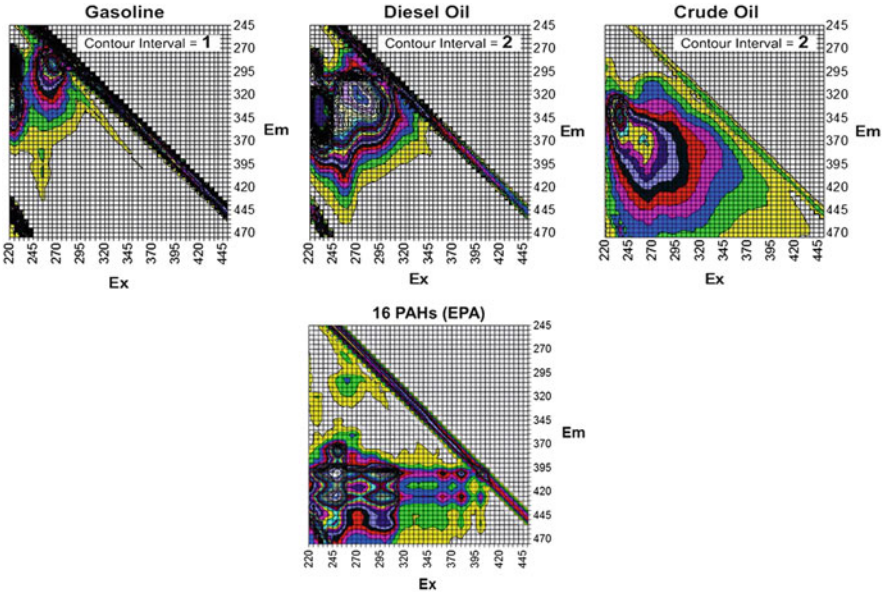


Fig. 1 Fluorescence fingerprints (contour diagrams) of arbitrary standards (gasoline, diesel, and crude oil, 1–1 μg/mL; 16 PAHs, each 3 ng/mL, in cyclohexane)

detail elsewhere [9, 10]. Fluorescence spectrophotometer (Hitachi Model 4500) was used to record the fluorescence spectra in the 220–450 nm excitation and 245–475 nm emission wavelength ranges. Figure 1 shows fluorescence fingerprints of the arbitrary standards (petroleum products) including 16-PAHs.

Determination of contamination type is based on the degree of correlation between the concatenated fluorescence spectra of the arbitrary standards and the environmental samples, which was achieved by decomposing each fingerprint into 22 emission spectra (Rayleigh scattering removed) as follows:

Spectrum number	Excitation wavelength	Emission range	Spectrum number	Excitation wavelength	Emission range
Spectrum 1	220 nm	250–365 nm	…	…	…
Spectrum 2	225 nm	255–370 nm	Spectrum 20	315 nm	345–460 nm
Spectrum 3	230 nm	260–375 nm	Spectrum 21	320 nm	350–465 nm
…	…	…	Spectrum 22	325 nm	355–470 nm

These fluorescence emission spectra were then concatenated. Examples of the concatenated spectra for the arbitrary standards are presented in Fig. 2.

After calculating the correlation between the concatenated spectra of the samples and the arbitrary standards, the standard showing the highest correlation coefficient with the samples was used as calibration standard for estimating the

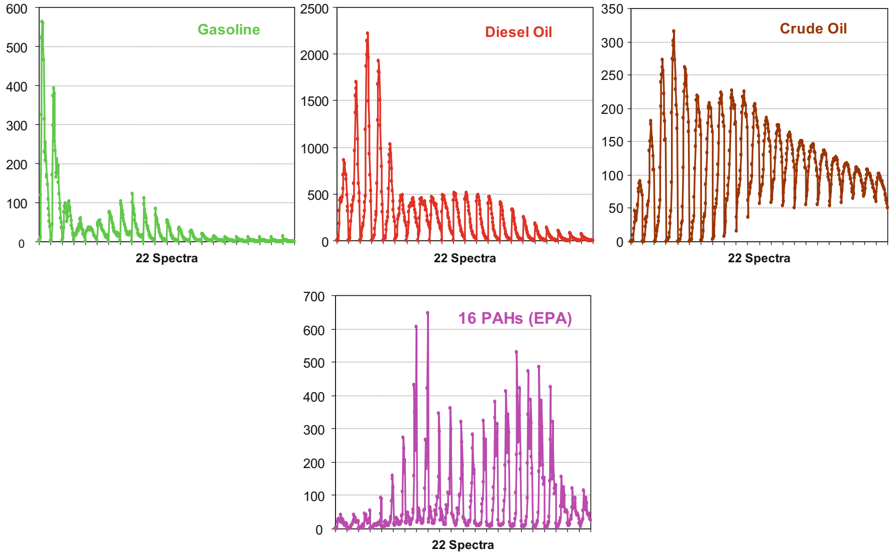


Fig. 2 Concatenated fluorescence spectra of the arbitrary standards, PAHs

concentration of the petroleum hydrocarbon contamination [9]. The fluorescence intensity at the excitation/emission (Ex/Em) wavelength, specified for each standard material, was used for this estimation.

The highest correlation was observed with the gasoline (fluorescence by monoaromatic compounds) in the case of the water and with the crude oil in both the SPM and bottom sediment samples. The specific Ex/Em wavelengths in the case of gasoline and the crude oil were 265/290 and 270/380 nm, respectively.

3.2 Determination of PAHs

PAHs were analyzed in water, SPM, and sediment samples after extraction with organic solvents and determined with HPLC-Fluo or GC-MS according to internationally accepted analytical protocols.

4 Results and Discussion

4.1 The Cousteau Survey in 1991–1992

The first coordinated survey along the Danube (excluding the then-Yugoslavian Danube reach due to the war activities) by the Cousteau team involved collection of

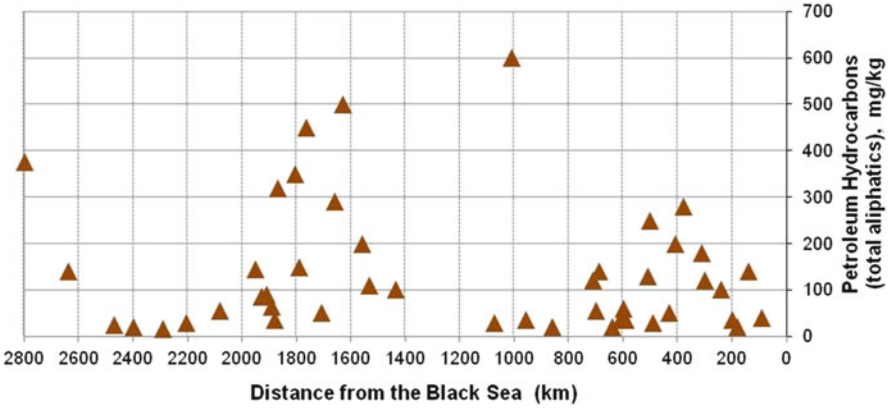


Fig. 3 Distribution of petroleum hydrocarbons in Danube sediments

sediment and bivalves samples. Petroleum-related contamination of the sediment samples was determined: (a) by analysis of *n*-alkanes as a measure of relatively fresh oil pollution using GC-FID method and (b) individual PAHs analyzed with HPLC-fluorescence detector.

Concentration of the petroleum hydrocarbons along the Danube is shown in Fig. 3, whereas Fig. 4 shows the benzo[a]pyrene concentrations.

Both figures show similar levels of petroleum hydrocarbon contamination, rather high in certain hot spot areas (e.g., the upper Danube reach in Germany, the middle reach between Austria and Hungary, and the lower Danube reach in the industrial areas of Romania and Bulgaria) but are generally inferior to similarly polluted rivers in other parts of the world. In the case of PAHs (e.g., phenanthrene, fluoranthene, benzo[a]anthracene, and benzo[a]pyrene), the concentrations were similar or slightly lower than those observed in the Lower Rhine and in the Mersey estuary in the UK.

The sediment monitoring results are very useful for detecting pollution hot spots. The multiparameter approach uses the coincidence of two pollutants associated with a given human activity. Examples of this approach are shown in Fig. 5.

Using the multiparameter approach in the case of petroleum hydrocarbons and coprostanol, the coincidence highlights those sites where petroleum hydrocarbons are discharged in association with municipal sewage. The spectacular coincidences were observed in the Iron Gate reservoir, at Budapest, and downstream of the Arges (demonstrating the impact of Bucharest).

The coincidence of benzo[a]pyrene and lead shows a combination of compounds characteristic of fossil fuel combustion and using leaded fuels. The coincidence factor here shows peaks coinciding with industrial activities in Germany, along the Slovak-Hungarian Danube, and the accumulation in the Iron Gate reservoir and downstream of the Arges river introducing waste discharges from Bucharest.

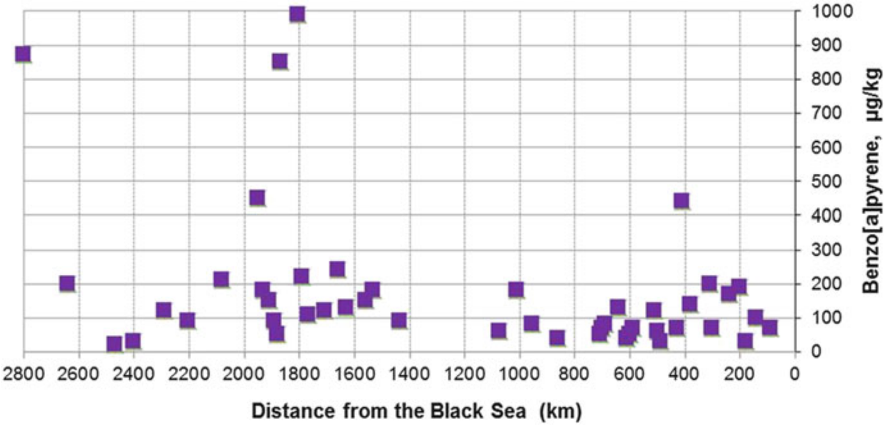


Fig. 4 Distribution of benzo[a]pyrene in Danube sediments

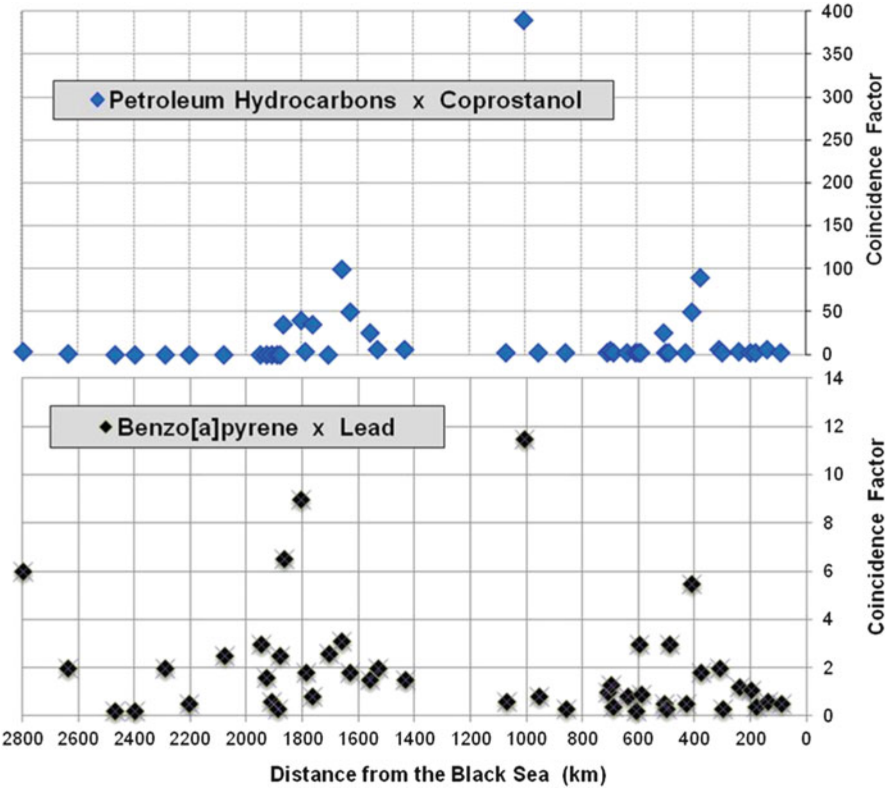


Fig. 5 Hot spot identification with coincidence factors

4.2 The Joint Danube Surveys

As continuation of the "along the Danube survey" by the Cousteau team, the ICPDR initiated Danube surveys with joint participation of the riparian Danube country institutions. The first Joint Danube Survey (JDS) was conducted in 2001, planned on the basis of the lessons learned from the previous surveys and also considering the requirements of the EU Water Framework Directive [4].

Among the chemical characteristics, petroleum hydrocarbons and PAHs were analyzed in water, SPM, bottom sediment and biota (mussels) samples. The first joint survey (JDS1) was followed by JDS2 (2007) and JDS3 (2013). The petroleum hydrocarbons were determined with different analytical methods during the JDS1. Based on the first results, the method based on measurement of fluorescence (fluorescence fingerprinting as detailed in Sect. 3.1) was agreed to be used during the following surveys.

Figure 6 demonstrates visual comparison between the different samples collected from representative sampling sites along the Danube.

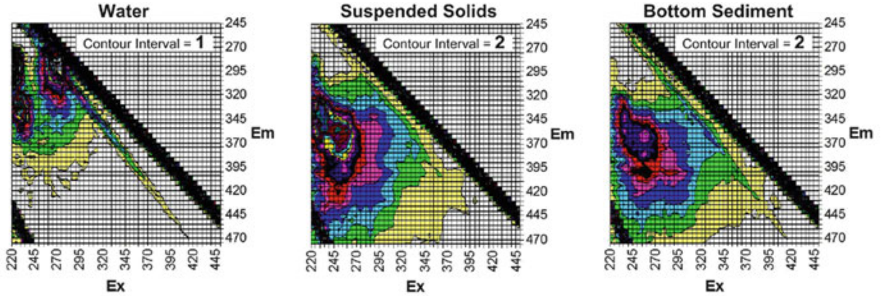
The fingerprints in Fig. 6 show the results of the analysis of the cyclohexane extracts of the water samples. They demonstrate that the most water-soluble monoaromatic (BTEX) compounds are dominating in samples from rkm 2,204 and rkm 532, likely originating from pollution with gasoline. In the case of the Morava river, the fingerprint indicated that the water was polluted with gasoline, diesel, and even with some crude oil residues.

The fingerprints of the Danube suspended solids and bottom sediment extracts demonstrate the presence of higher ring-number aromatic compounds, a mixture of diesel and crude oils, as well as weathered petroleum residues. It is interesting to note that these fingerprints look similar at different sampling sites; however, considering the contour intervals, the contamination of SPM and bottom sediment in the Morava river was about 10 times higher compared to the upstream Danube site (rkm 2,204). The oil pollution inputs discharged into the Danube between Vienna and Bratislava significantly increased the petroleum contamination in both the SPM and bottom sediment between Bratislava and the end of the Slovak-Hungarian Danube reach (1,707 rkm).

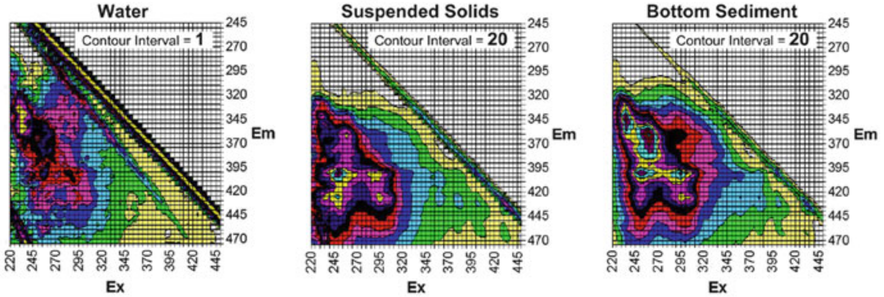
4.2.1 Petroleum Hydrocarbons in Water, SPM, and Bottom Sediment

Petroleum Hydrocarbons in Water

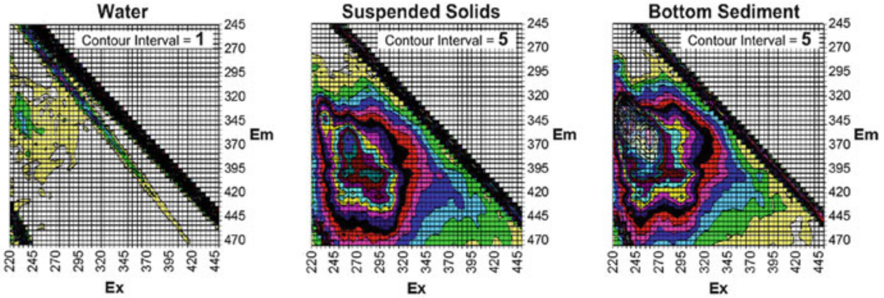
The calculation of the correlation between the concatenated spectra of the cyclohexane extracts of the water samples and the arbitrary standards resulted in highest correlation with gasoline in 16, with diesel oil in 44, and with crude oil in eight water samples. The petroleum hydrocarbon concentration in each water sample was calculated from the calibration with the relevant standard. The results are shown in Fig. 7.



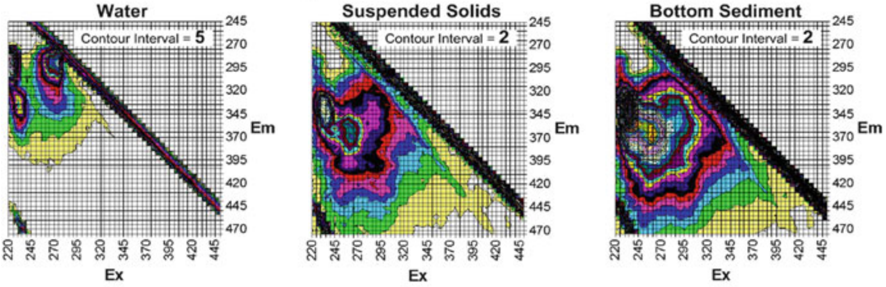
Danube at 2204 rkm (downstream of the Inn confluence)



Morava River confluence at the 1880 rkm of the Danube



Danube at 1707 rkm (downstream of the Slovak-Hungarian border



Danube at 532 rkm (downstream of the Jantra confluence)

Fig. 6 Fluorescence fingerprints of water, SPM, and bottom sediment samples collected at selected sampling sites during JDS1

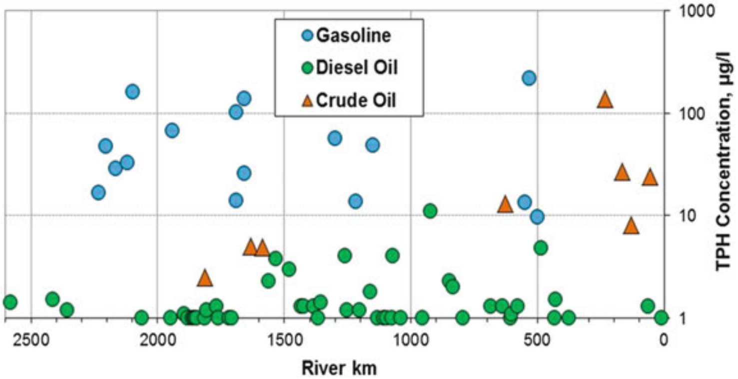


Fig. 7 Petroleum hydrocarbon contamination in the Danube water during JDS1

The concentration of the petroleum hydrocarbons was high in the samples with gasoline-type contamination likely due to the higher solubility of the mono-aromatic hydrocarbons. The relatively high crude oil type contamination in the lower Danube reach was likely from the oil industrial discharges.

The usefulness of the one-time analysis of oil contamination in the water has been questioned after JDS1; therefore, this type of petroleum hydrocarbon analysis was discontinued. Instead, determination of PAHs in water was carried out as required by the EU WFD.

Petroleum Hydrocarbons in SPM

In both SPM and bottom sediment samples and during all three surveys, the highest correlation was observed with the crude oil standard, and the petroleum hydrocarbon contamination was calculated and expressed in crude oil equivalent.

Figure 8 shows the variation in the petroleum hydrocarbon contamination in the SPM along the Danube during the JDS1, JDS2, and JDS3 surveys.

The survey results distinguished three characteristic sections along the Danube: (1) upstream of the Gabčikovo reservoir, (2) section between the Gabčikovo and the Iron Gate dams, and (3) downstream of the Iron Gate reservoir, similar to the observation during the bottom sediment survey by the Cousteau team. The most significant variation in contamination levels was observed along the middle section.

At most of the sampling sites, the highest concentrations of petroleum hydrocarbons were observed during JDS2, the lowest during JDS1, while during JDS3, the contamination level was between the results of JDS1 and JDS2, with few exemptions when the highest contamination level was found during JDS3. This was particularly significant downstream of the Arges confluence (at rkm 432).

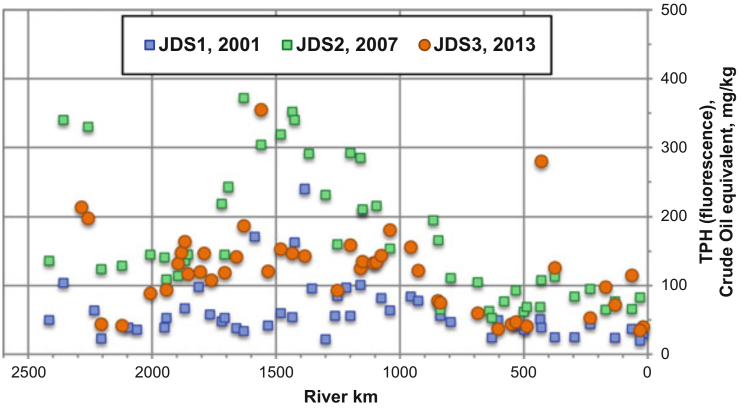


Fig. 8 Variation in petroleum hydrocarbon contamination in the SPM along the Danube River during Joint Danube Surveys

Petroleum Hydrocarbons in the Bottom Sediment

Figure 9 shows the variation in the petroleum hydrocarbon contamination in the bottom sediment along the Danube during the JDS1, JDS2, and JDS3 surveys.

The three characteristic Danube sections can be distinguished also by the results obtained for the bottom sediment samples. The highest variation was observed along the middle section of the Danube. It is likely that the highly contaminated SPM (observed in the period of JDS2) mainly settled to the bottom which resulted in an increase in the oil contamination of the bottom sediment from JDS1 through JDS2 to JDS3. The high concentration of oil pollution in the upper Danube (in Germany) as well as upstream of the Iron Gate reservoir can also be due to sedimentation of the contaminated SPM.

The significant difference between the correlation with the crude oil and the other two standards showed that: (a) gasoline-type discharges evaporate relatively fast; BTEX compounds are more soluble in the water (this was demonstrated during JDS1, showing the highest correlation with the gasoline in the water samples) and show limited adsorption to the particulate matter and (b) decreasing correlation with crude oil and increasing correlation with the diesel oil from the Iron gate reservoir to the Danube Delta indicate higher inputs from refined petroleum products (mainly diesel oil) and limited weathering of the hydrocarbon pollutants.

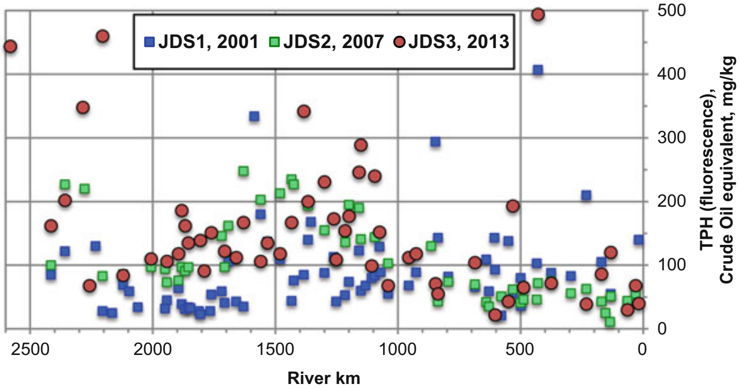


Fig. 9 Variation in petroleum hydrocarbon contamination in the bottom sediment along the Danube River during Joint Danube Surveys

4.2.2 Polyaromatic Hydrocarbons (PAHs) in Water, SPM, Bottom Sediment, and Biota

PAHs in the Water Samples

Table 3 shows the maximum concentration of individual PAH substances listed among the priority or priority hazardous substances in Directive 2013/39/EU in water samples collected during JDS3.

With the exception of benzo[g,h,i]perylene, the maximum concentration of the other PAH substances on the list was significantly below the relevant maximum allowable concentration, the MAC-EQS. It is also important to note that the detection limit of benzo[a]pyrene, benzo[b]fluoranthene, and benzo[k]fluoranthene was exceeded in three, five, and one water samples, respectively. Furthermore, in case of a one-time sampling and analysis of water, only the comparison to the MAC-EQS is appropriate.

PAHs in the SPM Samples

Table 4 shows the maximum concentrations of individual PAH substances in the SPM samples during JDS3.

The maximum concentration of most of the PAH substances was found at the most upstream site (at Böfinger Halde). Only the maximum concentration of benzo [a]pyrene and benzo[k]fluoranthene exceeded limit concentration indicated in the EU Priority Substances data sheet. However, even the maximum concentration of benzo[a]pyrene was far below the PELs = 763 μg/kg (see Table 2).

Table 3 Concentration of PAHs in water samples during JDS3

Substance	MAC (μg/L)	LOQ (μg/L)	Number of samples > LOQ	Maximum concentration (μg/L)
Anthracene	0.1	0.002	67	0.0401
Fluoranthene	0.12	0.002	17	0.0098
Naphthalene	130	0.002	59	0.0204
Benzo[a]pyrene	0.27	0.002	3	0.0024
Benzo[b] fluoranthene	0.017	0.002	5	0.0027
Benzo[k] fluoranthene	0.017	0.002	1	0.0022
Benzo[g,h,i] perylene	0.0082	0.0005	66	0.029

Table 4 Concentration of PAHs in SPM samples during JDS3

Substance	EU Priority data sheet (μg/kg)	LOQ (μg/kg)	Number of samples > LOQ	Maximum concentration (μg/kg)
Anthracene	24	20	2	21
Fluoranthene	2,000	20	48	191
Benzo[a] pyrene	91.5	20	35	110
Benzo[b] fluoranthene	70.7	20	39	122
Benzo[k] fluoranthene	67.5	20	25	55
Benzo[g,h,i] perylene	42	20	33	75

PAHs in the Bottom Sediment Samples

Table 5 shows the maximum concentration of individual PAH substances in the bottom sediment.

With the exception of the fluoranthene, the maximum concentrations of the other PAH substances on the list exceeded the limit concentration indicated in the EU priority substances data sheet. However, in the case of anthracene and benzo[a] pyrene, even the maximum concentration was far below the PELs = 245 and 763 μg/kg, respectively, in the Canadian Sediment Quality Guidelines (see Table 2).

Polyaromatic hydrocarbons are major contributors to the fluorescence in the cyclohexane extracts of environmental samples. The cyclohexane extract of some selected bottom sediment samples used for fluorescence fingerprinting was analyzed for PAHs. The particular reason was to compare the concentration of selected PAHs to the results of the fluorescence fingerprints. Table 6 shows the results for comparison.

Table 5 Concentration of PAHs in the bottom sediment samples during JDS3

Substance	EU Priority data sheet (μg/kg)	LOQ (μg/kg)	Number of samples > LOQ	Maximum concentration (μg/kg)
Anthracene	24	20	3	57
Fluoranthene	2,000	20	55	690
Benzo[a] pyrene	91.5	20	41	370
Benzo[b] fluoranthene	70.7	20	49	489
Benzo[k] fluoranthene	67.5	20	16	259
Benzo[g,h,i] perylene	42	20	33	328

Table 6 Concentration of selected PAHs in selected bottom sediments during JDS3

Substance	Unit	High TPH samples	Low TPH samples	Min–max during JDS2
Fluoranthene	μg/kg	215–265	21–45	15 and 853
Benzo[a]pyrene	μg/kg	104–114	41–52	10 and 115
Benzo[a]anthracene	μg/kg	66–71	26–32	
Benzo[b] fluoranthene	μg/kg	183–214	35–56	
TPH (fluorescence)	mg/kg	444–550	56–90	11 and 248

The results in Table 6 demonstrate that the higher TPH concentrations correspond to higher concentration of the PAHs. Unfortunately, the recent Directive 2013/39/EU shows EQS for water and biota only. However, considering the Canadian Sediment Quality Guidelines (CCME, 2001), even the maximum concentration of the selected PAHs is far below the PELs (probable effect limits), being 2,355, 782, and 385 μg/kg for fluoranthene, benzo[a]pyrene, and benzo[a]anthracene, respectively.

PAHs in Biota (Mussel) Samples

Mussel samples were analyzed for PAHs during JDS1. Figure 10 shows the sum of the individual PAH substances in biota.

The mussel samples contained PAHs at similar levels as during earlier surveys [1, 2]. A slight increasing trend can be observed downwards along the Danube to the Delta. The highest accumulation was measured in mussels collected from tributaries in the middle Danube reach where petroleum hydrocarbon contamination was the highest in other matrices.

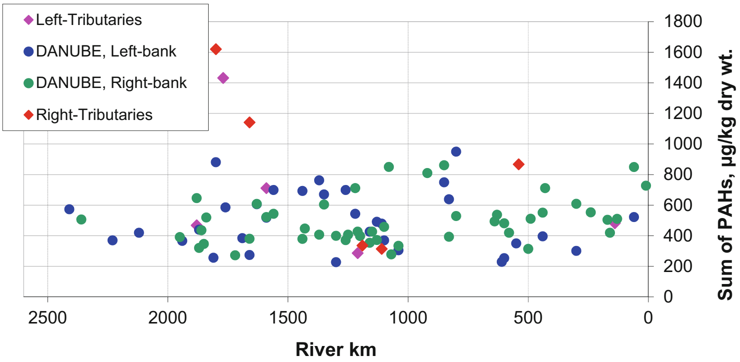


Fig. 10 Variation in the concentration of PAHs in the mussel samples collected from the Danube and its tributaries during JDS1

5 Conclusions

There are several analytical methods to measure petroleum hydrocarbons in the environment. Each method can provide information about the characteristics of the contamination. Comparison and interpretation of the data (usually called as "TPH") obtained with different analytical methods require specific treatment and considerations.

The fluorescence spectroscopy for characterizing fluorescing compounds being mostly persistent hydrocarbons (i.e., pollutants with aromatic rings, usually causing adverse effects to the environment) provided a sensitive, moderately selective, and cost-effective analytical tool for monitoring and assessment of oil pollution. The polluting aromatic hydrocarbons can be characterized in terms of fluorescence patterns of the fluorescence fingerprints; the concentration of the petroleum hydrocarbons can be calculated from the fluorescence intensity at specified excitation/emission wavelengths.

Interpretation of the fluorescence fingerprint of cyclohexane extracts of water, SPM, and bottom sediment samples, collected during the Joint Danube Surveys (in 2001, 2007, and 2013), provided information on the characteristics and level of the petroleum hydrocarbon contamination, concluding as follows:

- Petroleum hydrocarbons contamination in water was mainly characterized with the fluorescence of gasoline. The concentrations varied in the range of 2–300 µg/L, in gasoline equivalent.
- The level of oil contamination was similar in the SPM and the bottom sediment, characterized with the fluorescence of crude oil. The concentrations varied between 5 and 500 mg/kg, in crude oil equivalent.
- The petroleum hydrocarbon contamination in the bottom sediment showed slowly increasing trends during the three surveys, characterized with the highest contamination in 2013, likely caused by settling of the contaminated SPM, which showed the highest TPH concentration in 2007.

- Characteristics of the petroleum hydrocarbon contamination divided the Danube into three sections: (1) upstream of the Gabčikovo reservoir, (2) section between the Gabčikovo and the Iron Gate dams, and (3) downstream of the Iron Gate reservoir. High contamination was detected in the upper Danube reach, and significant variation in the contamination levels was observed along the middle section.
- The PAH compounds determined in water, SPM, bottom sediment, and biota (mussels) showed similar trends in contamination as observed in the case of petroleum hydrocarbons. However, even the highest concentrations in the different matrices were usually below the EQS according to EU Directive 2013/39/EU or the PELs in the Canadian Sediment Quality Guidelines.

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