Alkylphenolic Compounds in the Danube River

Vesna Mićić and Thilo Hofmann

Abstract The occurrence of alkylphenolic compounds along the Danube River revealed a ubiquitous fingerprint of wastewater impact, recorded in various extents and being the most prominent in the main tributaries and side arms, as well as in vicinity of industrial areas and some Danubian capitals.

As revealed by the Joint Danube Survey 2 (JDS2) in 2007, there was a significant decrease in nonylphenol and octylphenol levels in both sediments and suspended particulate matter (SPM) compared to the findings of the Joint Danube Survey 1 (JDS1) in 2001, validating the effects of the EU regulations.

Nevertheless, the JDS2 results showed that the inputs of untreated or insufficiently treated wastewater mostly from metropolitan and industrial areas are still large enough to (occasionally) cause nonylphenol concentrations above environmental quality standards (EQS) for freshwater sediments.

Nonylphenol mono- and diethoxylates (NP1EO and NP2EO) often coexist with nonylphenol in sediments and SPM in comparable concentrations, which may induce additive mixture effects on Danube biota.

Given that there are no EQS for alkylphenolic compounds in SPM, it is difficult to estimate potential risks that SPM-linked contamination may exert on Danube biota. Slight nonylphenol accumulation in mussels was evident at the sites where nonylphenol levels in SPM were continuously high.

Based on the JDS2 findings, octylphenol and its lower ethoxylates rarely occur and in low concentrations, thus appear to be of no concern for the Danube environment.

Nonylphenol and nonylphenoxyacetic acid (NPE1C) were frequently found in water during the JDS2, exceeding the valid (or proposed) EQS for freshwater in

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some tributaries. Which possible additive or synergic effects these two compounds may have on aquatic organisms remains however unclear.

The results of the Danube surveys highlighted the necessity of reduction of untreated wastewater discharges, especially in areas where alkylphenolic compounds exceeded EQS, in order to protect quality and environmental conditions of the Danube River.

Keywords Alkylphenolic compounds, Danube River, Mussels, Sediments, Suspended particulate matter, Water

Contents

1 Introduction

Danube River flows through many different landscapes; the natural variations in topography, changes in land use, and human impacts causing pollution all affect the overall environmental quality of the river and restrict the use of water resources. Most river pollution is caused by wastewater that contains liquid waste from household, industrial, and agricultural practices. Wastewater largely contains surfactants and their metabolites, which inevitably enter rivers either through effluents from wastewater treatment plants (WWTPs) or through direct discharges and runoffs.

Some of the cost-effective surfactants widely used in industrial, institutional, and household applications, as well as in pesticide formulation, are alkylphenol polyethoxylates (APEOs) [1]. APEOs are manufactured from alkylphenols, which in addition have other industrial usages, such as in the preparation of phenolic resins, polymers, heat stabilizers, and antioxidants [2]. Approximately 80% of APEOs is built of nonylphenol polyethoxylates (NPEOs), while the remaining 20% are attributed to octylphenol polyethoxylates (OPEOs) [3]. In WWTPs as well as in rivers, both NPEOs and OPEOs biodegrade by a stepwise loss of ethoxy groups, resulting in the formation of shorter chain hydrophilic alkylphenoxy carboxylic acids and shorter chain hydrophobic alkylphenol ethoxylates. All these APEO metabolites ultimately degrade back to alkylphenols [2].

Either as constituents of WWTP effluents or of untreated wastewater, residual surfactants and their degradation products are discharged into surface water and then dispersed into different environmental compartments. Due to their physical/chemical properties, such as low water solubility and high hydrophobicity ($\log K_{\mathrm{OW}} = 4.0$–$4.5$, [4]), octylphenol, nonylphenol, and their mono- and diethoxylates accumulate in environmental compartments that are characterized by high organic content (sediments, suspended particulate matter (SPM), biota), where they persist. The acidic, more hydrophilic metabolites of APEOs remain in water and (depending on hydraulic conditions) may infiltrate into ground- and drinking water [5, 6]. All APEO metabolites with 0–2 ethoxy groups are hereafter called alkylphenolic compounds.

Alkylphenolic compounds are more toxic to aquatic life than their precursors and may have carcinogenic as well as estrogenic effects [1, 7–10]. Octyl- and nonylphenol are therefore defined as priority pollutants by the EU Water Framework Directive (EU WFD). Similar behavior may be expected for the earlier APEO metabolites, such as alkylphenoxy(ethoxy) acids and alkylphenol mono- and diethoxylates, regarding their physical, chemical, and structural characteristics [2, 11]. Therefore, they are frequently discussed as potential emerging pollutants by the network of reference laboratories for the monitoring of emerging environmental pollutants [12].

Taking into account the emissions and potential risks of alkylphenolic compounds, it is in the interest of river management to monitor the occurrence and spatial distribution of these compounds in the Danube River, to identify their sources, and to support pollution control and prevention, as well as the overall protection of the Danube River environment. This report summarizes the findings of the investigations of alkylphenolic compounds in the Danube River carried out during the two Joint Danube Surveys (JDS1 and JDS2) organized by the International Commission for the Protection of the Danube River (ICPDR).

2 Sampling Sites and Sample Collection

In the course of the Danube surveys, the core team collected environmental samples along a 2,600 km long river stretch at close to a hundred sites from the main river channel and the main tributaries and side arms.

Surface sediments were taken with a sampling net from the left and right sides of the main river channel. Either there was only one sediment sample taken per tributary (from the middle or from one side of the channel cross section) or two samples from the left and right sides of the channel were combined into a mixed sample prior to analysis. Sediments were wet-sieved shipboard through a 0.063 mm sieve, and the fine sediment fraction was preserved at 4°C for further analysis.

SPM samples were collected with a continuous flow centrifuge in the middle of the river, usually while underway between two neighboring sites (due to the long time required for collection of a sufficient amount, but also in order to minimize the collection of re-suspended sediments). The exceptions were only a few sites where a stationary sampling was carried out. SPM was deep frozen shipboard and freeze-dried and homogenized in the laboratories onshore along with the fine sediment fraction.

Alkylphenolic compounds in water and mussels were analyzed during the JDS2 only. Water samples were taken with a grab sampler in the middle channel of the river below the water surface. Different mussel species were collected from the selected locations. The whole soft tissues were used, deep frozen shipboard and then freeze-dried and homogenized in the laboratory onshore.

3 Laboratories and Methodologies

Nonylphenol and octylphenol in the fine sediment fraction collected during the JDS1 and JDS2, as well as in the SPM samples collected during the JDS1, were analyzed in the laboratories of the Water Technology Center (Karlsruhe, Germany). Samples were ultrasonic extracted in a cyclohexane–acetone (9:1) mixture, followed by centrifugal separation of the liquid extract. After derivatization by a mixture of (trimethylsilyl)trifluoroacetamide (MSTFA) and trimethyliodosilane (TMIS) (1,000:2), the extracts were analyzed by means of gas chromatography/mass spectrometry (GC/MS). The compounds were quantified using 4-*n*-nonylphenol as internal standard, with quantification limits of 0.01 and 0.005 mg/kg for nonylphenol and octylphenol, respectively. More details on the analytics are given in the JDS1 Technical Report [13].

Nonylphenol and octylphenol in the SPM samples collected during the JDS2 were analyzed in the laboratories of the Bavarian Environment Agency (Munich, Germany). Samples were Soxhlet extracted in a hexane–dichloromethane (1:1) mixture, followed by extract purification via column chromatography with silica gel as stationary phase. After derivatization by MSTFA, the extracts were analyzed with a GC/MS and compounds were quantified using $^{13}C_6$-ring-labeled 363-nonylphenol and 4-octylphenol, with the quantification limits of 0.01 and 0.005 mg/kg, for nonylphenol and octylphenol, respectively. More details on analytics are given in the JDS2 Technical Report [14].

Nonylphenol and octylphenol in water were analyzed in the laboratories of the TG Masaryk Water Research Institute (Prague, Czech Republic). Non-filtered water samples were liquid–liquid extracted and purified via column chromatography using silica gel as stationary phase. Without derivatization, samples were analyzed with a GC/MS, and compounds were quantified using $^{13}C_6$-ring-labeled 363-nonylphenol, following the ISO 18857-1 protocol [15], with quantification limits of 0.02 μg/L for nonylphenol and 0.005 μg/L for octylphenol.

Table 1 Locations of the 23 sites selected during the JDS2 by the MA EG for detailed studies, with the distance from the Danube Delta (in river km), ISO 3166-1 alpha-2 country code, and mussel species

River km	Site name	Country code	Mussel species
2,412	Kelheim	DE	n.a
2,205	Jochenstein	DE/AT	n.a
1,942	Klosterneuburg	AT	n.a
1,869	Bratislava	SK	n.a
1,761	Iža/Szőny	SK/HU	*Unio tumidus* (25)
1,707	Szob	HU	*Unio pictorum* (18)
1,580	Dunaföldvár	HU	*Unio tumidus* (17) *Unio pictorum* (11)
1,434	Hercegszántó	HU	*Unio tumidus* (20) *Anodonta anatina* (8)
1,379	Drava*	HR/RS	*Sinanodonta woodiana* (6)
1,300	Ilok/Bačka Palanka	HR/RS	*Unio tumidus* (22)
1,252	Ds. Novi Sad	RS	*Unio tumidus* (?) *Anodonta anatina* (20)
1,170	Sava*	RS	*Unio tumidus* (30)
1,151	Ds. Pančevo	RS	*Unio tumidus* (21)
1,103	Velika Morava*	RS	*Unio tumidus* (27)
1,077	Stara Palanka/Ram	RS	*Unio tumidus* (35)
579	Ds. Turnu Măgurele/Nikopol	RO/BG	n.a
500	Us. Ruse	RO/BG	n.a
434	Us. Argeş*	RO/BG	n.a
429	Ds. Argeş*	RO/BG	n.a
378	Chiciu/Silistra	RO/BG	n.a
167	Brăila	RO	n.a
130	Reni	RO/UA	n.a
0	Sulina arm	RO	n.a

The numbers in brackets show the number of mussels collected per site. Tributary names are marked with an *asterisk*

Ds downstream, *Us* upstream, (?) unknown, *n.a.* not available

Nonylphenoxy acetic acid in water was analyzed in the laboratories of Joint Research Centre (Ispra, Italy). Non-filtered water samples were extracted by solid-phase extraction, followed by elution with methanol. The analyses were carried out on a liquid chromatography coupled to tandem mass spectrometry (LC–MS^2). The quantification was performed using deuterated 4-*n*-nonylphenol (4-*n*-NP-D8), with the quantification limit of 0.002 μg/L. More details on the analytics are given in Loos et al. [5].

During the JDS2, the Monitoring and Assessment Expert Group of the ICPDR (MA EG) has selected 23 sampling sites for a more detailed investigation (Table 1). Using the samples from these sites, a cross-matrices study (including fine sediments, SPM, mussels, and water) was carried out at the laboratories of the Department of Environmental Geosciences, University of Vienna (Vienna, Austria). A suite of six alkylphenolic compounds including nonylphenol, nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), octylphenol, octylphenol monoethoxylate (OP1EO), and octylphenol diethoxylate (OP2EO) was simultaneously investigated in all matrices. Sediments and SPM samples were extracted by an accelerated solvent extractor (ASE) using methanol as extraction solvent and partitioned in *n*-hexane. Mussels were extracted by an ASE with an acetone–*n*-hexane (1:1) mixture. After the partitioning in *n*-hexane, the mussel extracts were purified using an open column chromatography with Florisil as stationary phase. All sediment, SPM, and mussel extracts were derivatized by a mixture of MSTFA and TMIS (1,000:2) and analyzed on a GC/MS with 4-*n*-nonylphenol and 4-*n*-NP2EO as quantification standards. Water samples provided by the JRC laboratory were spiked with the same internal standard mixture, derivatized, and further analyzed in the same way as the solid matrices. The quantification limits in solid matrices were as follows: 0.02 mg/kg for nonylphenol, NP1EO, and NP2EO; 0.0015 mg/kg for octylphenol; 0.0025 mg/kg for OP1EO; and 0.003 mg/kg for OP2EO. Quantification limits in water were 0.1 μg/L for nonylphenol, NP1EO, and NP2EO and 0.005 μg/L for octylphenol, OP1EO, and OP2EO. More details on the analytics are given in Micić and Hofmann [16] and Micić et al. [17].

4 Results and Discussion

4.1 *Alkylphenols in Surface Sediments*

During the JDS1 nonylphenol was identified in almost all sediment samples, both from the main Danube channel (gray-filled triangles) and from its tributaries and side arms (gray hollow triangles, Fig. 1).

The concentrations were evidently higher in the tributaries and in the side arms than in the main channel (Fig. 1). The peak concentrations of 160 mg/kg in the Bulgarian tributary Rusenski Lom and 46 mg/kg in the Romanian tributary Argeş were a clear sign of an extended use of NPEO-based surfactants in these areas and a lack of wastewater treatment.

The elevated nonylphenol concentrations in Schwechat (AT), Váh (SK), Drava (HR), Timok (BG), Tisa, and Velika Morava (RS) and in side arms such as Kelheim (DE) and Chilia arm (UA/RO) evidenced that also these tributaries and arms were among the main receivers of untreated NPEO surfactant-containing wastewaters.

In the main Danube channel, the nonylphenol concentrations were significantly lower, often below 0.1 mg/kg. Levels above this threshold were commonly recorded in the middle river stretch, 1,700–1,000 km from the Danube Delta and at a few downstream locations. The highest concentrations were mostly found downstream of the confluences with the biggest tributaries, such as downstream

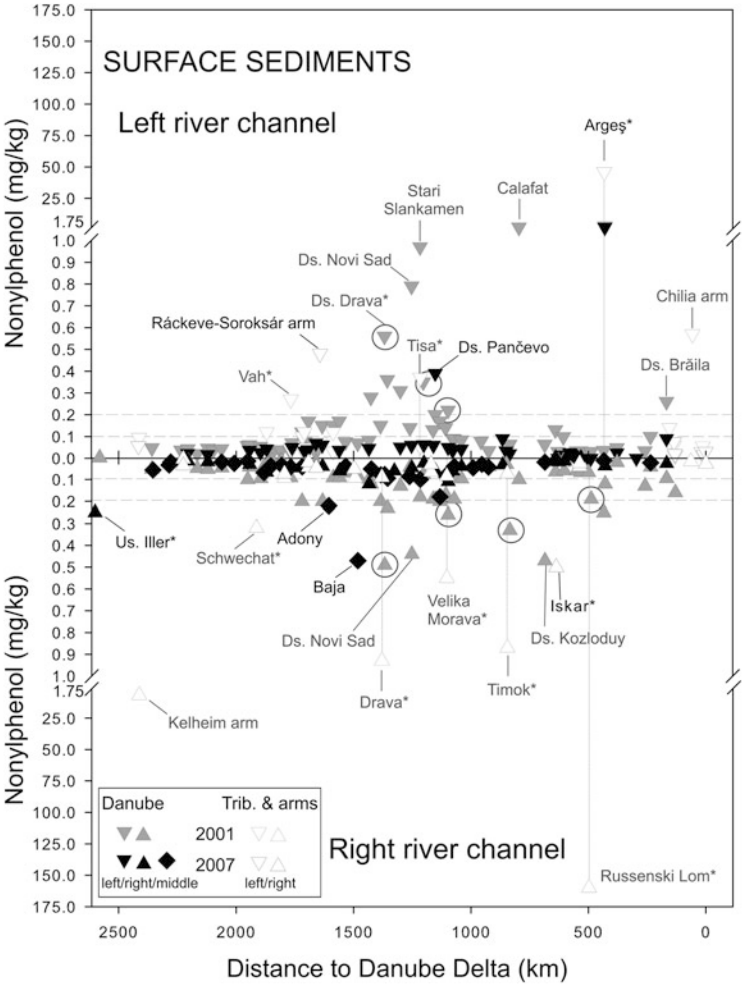

Fig. 1 Spatial distribution of nonylphenol along the main Danube channel and in its main tributaries and side arms, as revealed during the JDS1 (2001) and JDS2 (2007). Note that during the JDS2, sediments from the left and the right sides of the river channel were combined at some sites and the results are reported as from the middle river channel. *Ds* downstream, *Us* upstream. Tributary (trib.) names are marked with an *asterisk*

of Argeş (RO, 2.8 mg/kg) and Drava (~0.5 mg/kg) and also downstream of Tisa, Velika Morava, and Timok (gray-filled triangles marked with cycles, Fig. 1). Other locations with elevated nonylphenol concentrations were downstream of bigger and industrial cities, such as Novi Sad (RS), Brăila (RO), and Kozloduy (BG), but also upstream of the Tisa River (at Stari Slankamen, RS) and at Calafat (RO).

Octylphenol was present only in half of the sediments at levels above the quantification limit. As for nonylphenol, octylphenol was generally higher in the middle Danube stretch. Maximal concentration along the main Danube channel was recorded in the Serbian stretch, reaching 0.84 mg/kg downstream of Pančevo and 0.76 mg/kg downstream of the Velika Morava–Danube confluence. Outside of the Serbian stretch, a remarkably high octylphenol concentration (0.6 mg/kg) was recorded at Giurgeni (RO).

The main Danube tributaries exhibited peak concentrations, the highest being in Ipel (SK/HU) and Iskar (BG), with 1.7 and 1.4 mg/kg, respectively. Other elevated octylphenol levels were found in Tisa, Sava, and Velika Morava (RS), Rusenski Lom (BG), and Argeş (RO).

The occurrence of elevated alkylphenol concentrations in 2001 along the Danube revealed that tributaries and (to a smaller extent) discharges from the industries and municipalities along the main channel are the major pathways through which these compounds reached the Danube River. In approximately 20% of sediments investigated during the JDS1, the provisional environmental quality standards (EQS) for freshwater sediments for both nonylphenol and octylphenol (0.18 and 0.034 mg/kg, respectively [18, 19]) were exceeded, raising a concern about the river degradation.

Compared to the previous survey, the JDS2 revealed a significant decrease in nonylphenol levels in sediments from both the main river channel (black-filled triangles) and the tributaries and side arms (black hollow triangles, Fig. 1). Most of the recorded concentrations were below 0.05 mg/kg. Such a decrease after 6 years reflected a reduction of the NPEOs use in commonly applied detergent formulations. In the year 2003, in fact, the time between the two Danube surveys, the European Commission (EC) passed an EU-wide restriction of marketing and use of all products and product formulations that contain more than 0.1% of NPEOs or nonylphenol [20]. These restrictions, together with the natural attenuation processes, resulted in an improved status of the Danube sediment quality regarding the nonylphenol levels. Similar to the year 2001, also during the JDS2, the highest levels of nonylphenol recorded in sediments were found in the middle stretch of the river, with concentrations remaining mostly below or close to 0.1 mg/kg. Exceptions were only a few locations such as the Ráckeve-Soroksár arm downstream of Budapest (~0.5 mg/kg), in the vicinity of the Hungarian cities Baja (~0.5 mg/kg) and Adony (~0.2 mg/kg), downstream of the Serbian cities Pančevo (~0.4 mg/kg) and Grocka (~0.2 mg/kg), and upstream of the Iller–Danube confluence in Germany (~0.25 mg/kg). These increased concentrations (compared to the previous survey) were probably a consequence of an extended use of products containing nonylphenol and/or NPEOs, increased industrial activity, and more untreated wastewater discharges in these areas.

The peak nonylphenol concentration remained close to 2 mg/kg in the sediments downstream of the Argeş–Danube confluence, reflecting that the wastewater composition and the amount discharged into the Argeş River remained almost unchanged between the surveys.

Results of the surveys highlighted the necessity of nonylphenol reduction in sediments at all locations where the provisional EQS for freshwater sediments of 0.18 mg/kg dry wt. (proposed by the Common Implementation Strategy for the Water Framework Directive [18]) was exceeded, in order to protect the benthic organisms in these areas.

Moreover, no information on nonylphenol levels in the JDS1 hotspot locations (tributaries Rusenski Lom and Argeş and the Kelheim arm) was available in the year 2007. Even though there is only few data on the toxicity of sedimentary alkylphenolic compounds to benthic organisms, the nonylphenol concentrations recorded in 2001 in the Rusenski Lom and Argeş tributaries were ~2–6-fold higher than the lowest reported effect concentration for subacute toxicity of nonylphenol to shrimps, 26 mg/kg [21].

During the JDS2 (after the 6-year period), the decrease in octylphenol levels in sediments was even more prominent. In fact, octylphenol was recorded only in approximately one fifth of the samples, lying mostly in the range from 0.005 to 0.01 mg/kg. Values above this range were recorded at sites with the elevated nonylphenol levels, the highest being downstream of Pančevo (RS, 0.026 mg/kg), in the tributary Iskar (BG, 0.022 mg/kg), at Baja (HU, 0.019 mg/kg), at Klosterneuburg (AT, 0.015 mg/kg), downstream of the Argeş–Danube confluence (RO, 0.014 mg/kg), and close to Budapest (HU, 0.011 mg/kg).

All octylphenol concentrations recorded in the JDS2 were nevertheless clearly lower than the provisional EQS of 0.034 mg/kg dry wt., proposed by the CIRCA [19], and therefore did not pose any threat to the benthic organisms.

4.2 Alkylphenols in Suspended Particulate Matter

Both Danube surveys revealed the presence of nonylphenol in SPM at the majority of the sampling sites. The observed "background concentration" in the year 2001 was close to 0.05 mg/kg (grey-filled circles, Fig. 2). The values above this threshold were distributed along the Danube in the form of two bell-shaped curves. The first increase starting at ~60 km downstream from Bratislava (SK) reached its maximum of 0.1 mg/kg at ~70 km downstream from Budapest (Dunaföldvár, HU). Then the concentrations continuously decreased to the quantification limit but were rising again in the Serbian sector downstream of Novi Sad. They reached the second maximum of ~0.2 mg/kg in the main river channel downstream of the confluences with the tributaries Tisa and Sava and of 1.4 mg/kg in the tributary Velika Morava. Nonylphenol levels decreased again toward the lower river stretch and exhibited a constant but elevated value of ~0.08 mg/kg in the area downstream of the confluence with the Argeş River until the Danube Delta, with a peak downstream of the Olt–Danube confluence in Romania (0.12 mg/kg).

Octylphenol in concentrations above the quantification limit of 0.005 mg/kg was not found in any of the SPM samples collected during the JDS1.

Similarly to the sediments, a clear decrease in nonylphenol concentration was noticeable in the SPM samples taken during the JDS2, possibly also as a consequence of the EC regulations and natural attenuation. The "background values" were four- to fivefold lower compared to those from 2001, between 0.01 and 0.02 mg/kg. A double bell-shaped increase above this threshold was apparent along the same river stretches as in the year 2001, with again one of the highest

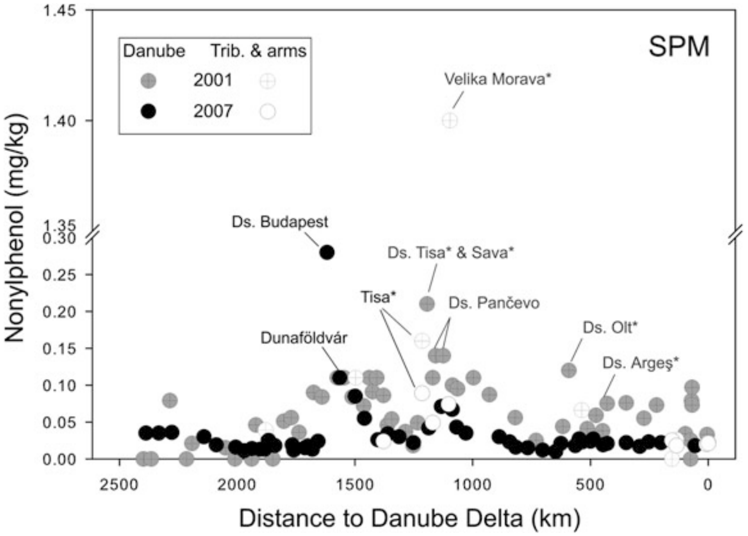

Fig. 2 Nonylphenol concentrations in SPM along the Danube and in its main tributaries and side arms in samples collected during the JDS1 and JDS2. Note that since SPM was mostly sampled while underway between two neighboring sites, nonylphenol concentrations are plotted against the middle distance to the Danube Delta between these neighboring locations. *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

(and unchanged) values at ~70 km downstream of Budapest (0.1 mg/kg at Dunaföldvár, HU). The only increase in nonylphenol concentration (compared to the the JDS1) was identified in the sample taken even closer to Budapest (~35 km downstream), therewith being the peak concentration of 0.28 mg/kg observed in 2007. This was most likely caused by an intrusion of untreated and/or insufficiently treated effluents from the city of Budapest, since at the time of the JDS2 sampling, the new Budapest central wastewater treatment plant was still under construction [14].

Octylphenol was recorded at only few locations at levels higher than the quantification limit. The highest levels were recorded along ~200 km river stretch downstream of Budapest (HU), reflecting the intrusion of wastewater from the Budapest metropolitan area. The peak concentrations recorded at the Hungarian sites Baja (0.043 mg/kg) and Dunaföldvár (0.038 mg/kg) were only slightly higher than the provisional EQS for freshwater sediments [19] but still highlighted the necessity of a reduction of the alkylphenol release from these areas.

4.3 Alkylphenols and Nonylphenoxyacetic Acid in Water

During the JDS2, nonylphenol was present in water along the whole river stretch in concentrations above the quantification limit (0.02 μg/L, Fig. 3) but rarely exhibited levels above 0.1 μg/L in the main channel. The concentrations reached this

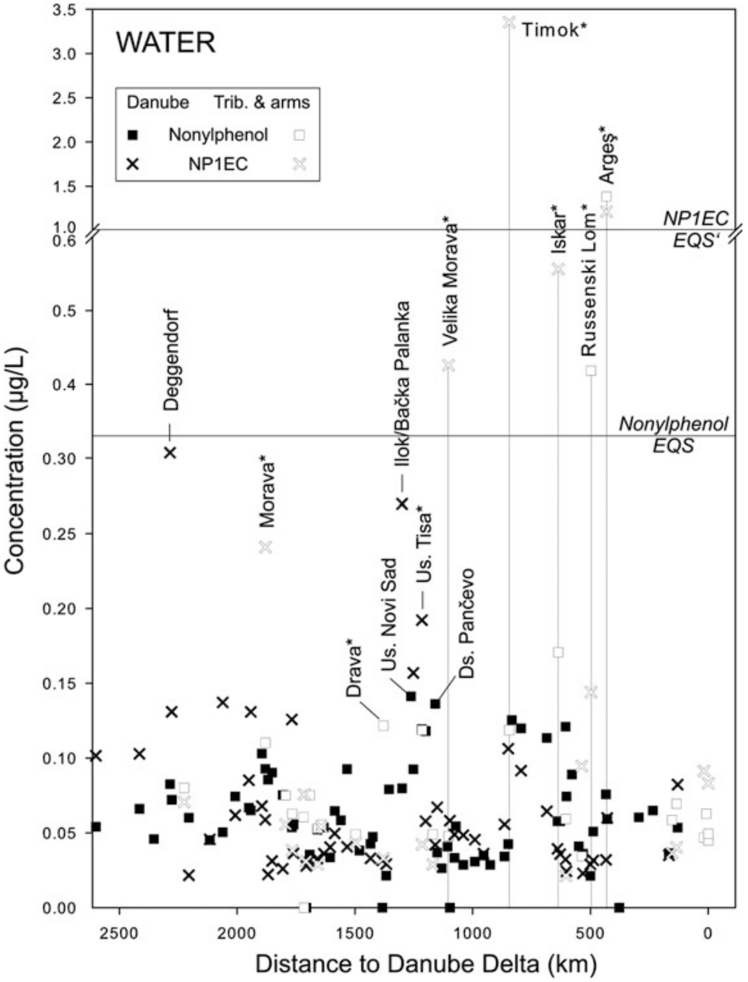

Fig. 3 Concentration and spatial distribution of nonylphenol and NPE1C in water samples collected during the JDS2. *Ds* downstream, *Us* upstream. Tributary (trib.) names are marked with an *asterisk*. EQS′ proposed EQS

threshold in the Morava tributary close to Bratislava (SK) and then again in the Serbian stretch of the river, from the tributary Drava (HR/RS) until a site downstream of the city of Pančevo, with the maximal concentration in the main channel found upstream of Novi Sad (0.41 µg/L). Downstream, nonylphenol levels remained low (mostly ~0.03 µg/L) up to the Bulgarian stretch where the concentrations once more increased in the tributary Timok (0.12 µg/L), downstream of the Timok–Danube confluence, and in the tributaries Iskar (0.17 µg/L) and Rusenski Lom (0.42 µg/L).

The highest concentrations of nonylphenol in water (1.38 μg/L) were found in the Romanian tributary Argeş. An even higher concentration of 3.28 μg/L (not shown in Fig. 3) was recorded in the Argeş sample taken closer to the Romanian capital Bucharest by a local sampling team [14]. These concentrations recorded in the tributaries Rusenski Lom and Argeş exceeded the EQS for freshwater of 0.33 μg/L [18]. The two peaks in the Argeş River are most likely caused by a significant amount of untreated and/or inadequately treated wastewater deriving from the municipality of Bucharest and its surroundings. The same three sites were the only ones where octylphenol was found in levels equal to or above the quantification limit: 0.005 μg/L (in Rusenski Lom), 0.011 μg/L (Argeş River, close to the confluence with the Danube), and 0.022 μg/L (Argeş River, closer to Bucharest, [14]), but did not exceed the water EQS for octylphenol (0.12 μg/L) [19].

Nonylphenoxyacetic acid (NPE1C) was also present in all water samples. Owing to its slightly better solubility in water compared to that of nonylphenol [22], NPE1C levels were generally higher but remained below 0.1 μg/L at the majority of sites. In the main river channel, levels above this threshold were found mostly at sites where nonylphenol was elevated: in the area around Bratislava and in the Croatian and Serbian stretch between Ilok (HR) and Bačka Palanka (RS) (0.27 μg/L), as well as close to Novi Sad (RS) and the Tisa–Danube confluence (HR/RS). The highest NPE1C concentration in the main channel (0.31 μg/L) was however recorded in the upper course of the Danube, close to Deggendorf (DE).

Similarly as for other alkylphenolic compounds, the tributaries exhibited generally higher NPE1C concentrations compared to the main channel. The highest concentrations were recorded in Timok (BG, 3.35 μg/L), Argeş (RO, 1.21 μg/L), Iskar (BG, 0.56 μg/L), Velika Morava (RS, 0.43 μg/L), Morava (SV, 0.24 μg/L), and Rusenski Lom (BG, 0.14 μg/L). In the Timok and in the Argeş, the proposed EQS of 1 μg/L [23] had been exceeded.

4.4 Alkylphenols and Their Lower Ethoxylates at the Selected Sites Along the Danube

During the JDS2, it was revealed for the first time that NP1EO generally and NP2EO sporadically co-occur with nonylphenol in the Danube sediments. The abundance of these NPEO metabolites in sediments was found to decrease in the following order: nonylphenol > NP1EO > NP2EO at the majority of the 23 selected sites (Fig. 4).

The highest concentrations of all target compounds in sediments detected downstream from the confluence with the Argeş River (RO, 2.83, 2.10, and 0.28 mg/kg for nonylphenol, NP1EO, and NP2EO, respectively) were among the highest reported in European sediments [16]. In the upper and the middle Danube stretch, nonylphenol mostly dominated over its lower ethoxylates. This indicated that (1) nonylphenol discharge may be higher compared to other compounds due to its

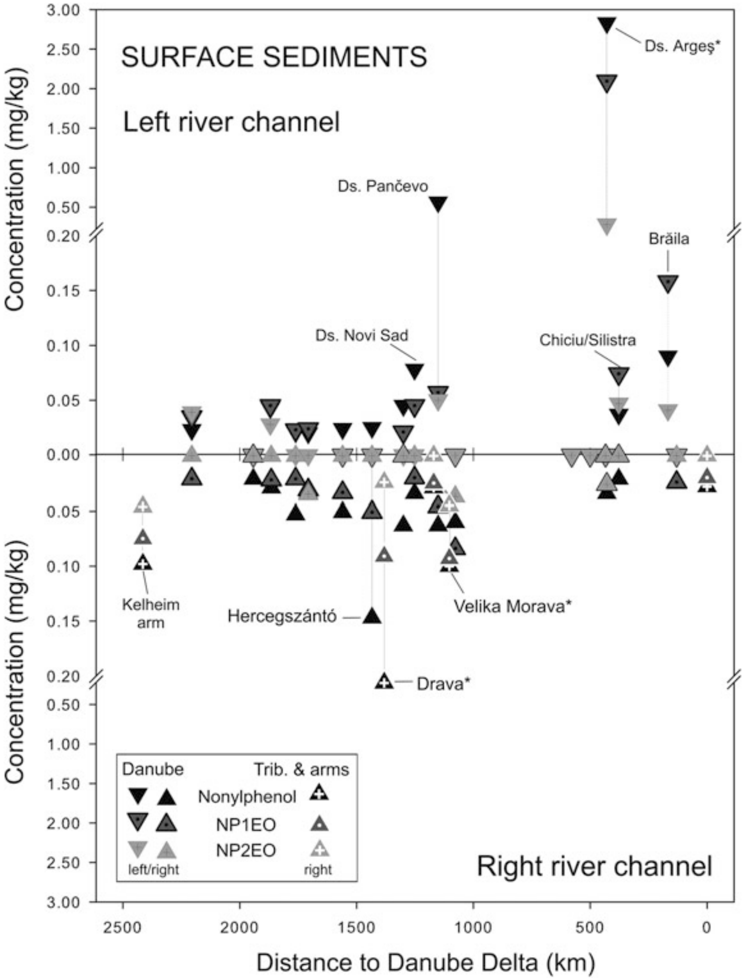

Fig. 4 Hydrophobic nonylphenolic compounds (nonylphenol, NP1EO, NP2EO) in sediments from 23 selected locations during the JDS2 (Table 1). *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

additional applications [2], (2) nonylphenol may be the most abundant NPEO metabolite in WWTP effluents [24], or (3) nonylphenol prevalence is additionally caused by in situ production from its precursors (NPE1C, NP1EO, and NP2EO) [25, 26]. All three nonylphenolic compounds were found elevated in sediments downstream of the Serbian cities Novi Sad and Pančevo, in the tributary Drava (HR/RS), and close to Kelheim in Germany (Fig. 4).

Moreover, for the first time it was revealed that NP1EO was occasionally present in comparable concentrations with nonylphenol and that in the middle and lower

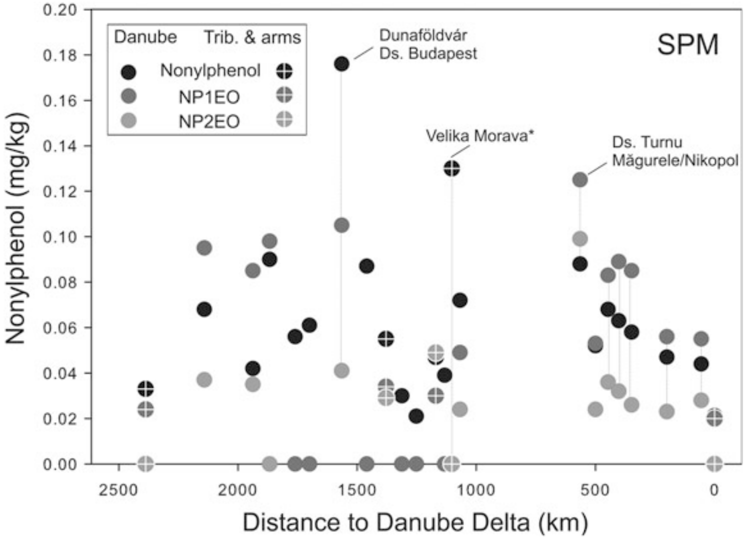

Fig. 5 Hydrophobic nonylphenolic compounds (nonylphenol, NP1EO, NP2EO) in SPM from 23 selected sites during the JDS2 (Table 1). *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

river stretch, it sporadically dominated (i.e., in Velika Morava tributary (RS), close to Chiciu/Silistra (RO/BG), and at Brăila (RO)). At these sites NP1EO was found in concentrations of 0.09, 0.16, and 0.07 mg/kg, respectively (Fig. 4), therewith being approximately twofold more abundant than nonylphenol. The NP1EO dominance suggested a fresh input of NPEO-containing untreated wastewater.

Octylphenol was recorded only at a few locations mostly at levels slightly higher than the quantification limit. The highest octylphenol concentrations were identified at the locations where nonylphenol was elevated; downstream from Pančevo (RS, 0.035 mg/kg, slightly above the EQS of 0.034 mg/kg) and from the Argeş–Danube confluence (RO, 0.017 mg/kg), indicating the use of the mixed surfactants in these areas. OP1EO and OP2EO were recorded at only one location, downstream of the Argeş River (RO) in concentrations of 0.005 and 0.007 mg/kg, respectively.

In SPM, nonylphenol was detected in the range from 0.02 to 0.18 mg/kg, NP1EO from 0.02 to 0.12 mg/kg, and NP2EO from below the quantification limit to 0.10 mg/kg. Even though peak concentrations in sediments were higher than peak concentrations in SPM, the most frequently found nonylphenol and NP1EO levels in SPM were higher than those found in sediments and often above 0.04 mg/kg (Fig. 5). Since SPM generally represents current and sediment historical pollution, this indicated higher recent inputs of nonylphenolic compounds. It is also possible that the SPM-associated contamination was subject to alteration before settling on the river bottom and that the sediment-associated contamination is additionally diluted by clastic, non-contaminated constituents.

The highest concentrations of nonylphenolic compounds were detected at Dunaföldvár, 72 km downstream from Budapest (HU): nonylphenol (0.18 mg/kg), NP1EO (0.10 mg/kg), and NP2EO (0.04 mg/kg), most likely resulting from untreated/insufficiently treated wastewater discharges from Budapest, as explained above. Among the highest concentrations identified were the ones in Velika Morava tributary (RS, nonylphenol: 0.13 mg/kg) and downstream from the cities of Turnu Măgurele and Nikopol (RO/BG, nonylphenol: 0.09 mg/kg, NP1EO: 0.12 mg/kg, NP2EO: 0.10 mg/kg).

In SPM octylphenol was found at only five sites in concentrations slightly higher than the quantification limit (0.002–0.003 mg/kg), while OP1EO and OP2EO were below the quantification limits. None of the alkylphenol lower ethoxylates were recorded in investigated water samples.

4.5 Cross-Matrices Study of Nonylphenol at the Selected Sites

A cross-matrices study of nonylphenol was carried out along the ~700 km long middle river stretch, where this compound was recorded in all environmental compartments studied: sediments, SPM, water, and mussels (Fig. 6).

Nonylphenol concentrations in sediments were lower than the ones in SPM at the majority of selected sites, except for sites downstream of Pančevo (RS) and in the Drava tributary (HR/RS). The sites with SPM peak concentrations did not

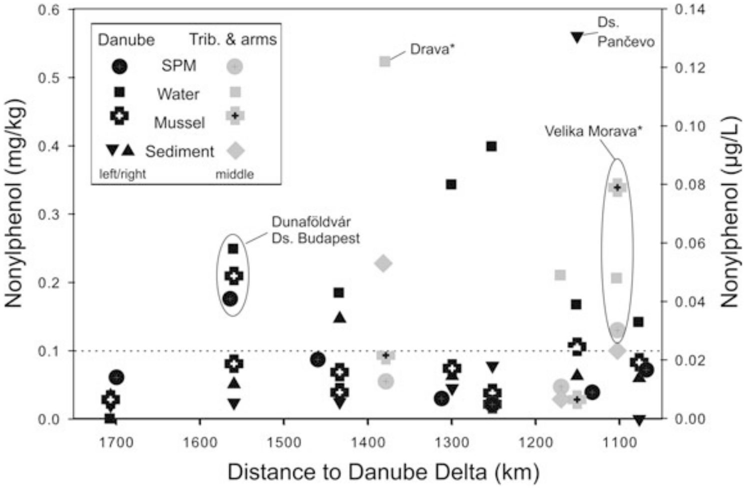

Fig. 6 Cross-matrices comparison of nonylphenol at sites where this compound was recorded in all matrices studied during the JDS2. Aqueous concentrations plotted were delivered by the TGM laboratories. *Ds* downstream. Tributary (trib.) names are marked with an *asterisk*

correspond with those with sedimentary peaks. This can be explained by the fact that SPM does not fully represent re-suspended bottom sediments but is instead a mixture of re-suspended sediments and recent inputs of particulate phase in water.

Also higher aqueous concentrations recorded were not reflected in any other matrices at these locations, indicating that water-related contaminants are subject to, e.g., dissolution and photodegradation before they finally settle at the river bottom.

It has been revealed for the first time during the JDS2 that nonylphenol was present in all mussels' tissues investigated (Fig. 6). The concentration range in the Danube River from 0.03 to 0.34 mg/kg was in accordance with the range observed in the mussel species worldwide [27, 28]. There was a similar trend of nonylphenol concentrations in mussels and in SPM, with the levels in mussels being slightly higher than those in SPM. The highest concentrations were detected in *Unio tumidus* from the tributary Velika Morava (RS, 0.34 mg/kg) and in *Unio pictorum* at Dunaföldvár (HU, 0.21 mg/kg), exactly at locations where the SPM level exceeded a threshold of 0.1 mg/kg. Since it is known that mussels can filter several liters of water per hour (and with it associated fine particles), this most likely resulted in a slight nonylphenol accumulation at sites with a higher and long-term exposure to nonylphenol in SPM.

Octylphenol was only detected in *Unio pictorum* at Dunaföldvár (HU, 0.03 mg/kg), where the nonylphenol level was also elevated, while NP1EO, NP2EO, OP1EO, and OP2EO were not detected in any of mussel samples.

5 Conclusions

The occurrence of alkylphenolic compounds along the Danube River revealed a ubiquitous fingerprint of wastewater impact.

The first Joint Danube Survey (2001) raised concerns about alkylphenol levels in Danube sediments, with up to 160 mg/kg of nonylphenol recorded in sediment from the Rusenski Lom tributary (BG). The results of the second Joint Danube Survey (2007) revealed a significant decrease in alkylphenol concentrations, thus validating the effects of the EC legislation regarding marketing and use of nonylphenol- and NPEO-containing formulations since 2003, as well as the effects of natural attenuation.

Nevertheless, results from the survey in 2007 revealed a continuous input of APEO-containing wastewater from metropolitan areas, such as Budapest (HU) and Bucharest (RO), as well as from the industrial cities close to Belgrade (RS), such as Pančevo and Grocka. In these areas, as well as at the site upstream of the Iller–Danube confluence in Germany, and in the vicinity of the Hungarian city Baja, the provisional EQS of 0.18 mg/kg for freshwater sediments was still exceeded, highlighting the necessity for improvement of wastewater treatment in these areas.

Also the presence of alkylphenolic compounds in SPM in 2007, reflecting more recent inputs into the river, revealed that despite the EC regulations, there was still an occasionally higher input of this contamination. Since there are no available EQS for SPM for none of the alkylphenolic compounds studied, it is difficult to estimate the potential risk the recorded concentration may pose to aquatic organisms. However, it was shown that in areas with peak nonylphenol concentrations in SPM (in both JDS1 and JDS2), nonylphenol tends to slightly accumulate in mussel tissues, as found in *Unio tumidus* from the Velika Morava tributary (RS, 0.34 mg/kg) and in *Unio pictorum* from the location downstream of Budapest (HU, 0.21 mg/kg). These observations revealed a need for further reduction of nonylphenol-containing discharges and monitoring of nonylphenol in mussels in the areas where high concentrations in SPM were recorded. In addition, it pointed out necessity for regulating concentrations of alkylphenols in SPM. The question of which effects these accumulations may have on mussels remains open for the future ecotoxicological studies.

The simultaneous study of alkylphenolic compounds in different riverine compartments revealed the co-occurrence of nonylphenol and its mono- and diethoxylates in sediments and SPM in occasionally comparable concentrations. This observation raises concern about potential additive mixture effects on riverine organisms, as shown for aqueous concentrations [29], which remain yet another challenge for future ecotoxicological studies.

Nonylphenol and NPE1C were frequently recorded at low concentrations along the main river course but at substantially higher levels in the tributaries. Nonylphenol concentration in tributaries Argeş (RO) and Rusenski Lom (BG) exceeded the EQS for freshwater of 0.33 μg/L. Also NPE1C levels were high in Argeş (1.21 μg/L) and in Timok (BG, 3.35 μg/L), exceeding the proposed EQS of 1 μg/L. The aqueous concentrations once again demonstrated insufficient or missing wastewater treatment in these areas and the necessity to study what possible additive or synergic effects these two compounds may have on aquatic organisms.

Since octylphenol was rarely found during the survey in 2007 (and if so, then in levels mostly lower than the provisional EQS) and its mono- and diethoxylates were recorded only at one site (at low concentrations), it is apparent that these compounds are generally of no major concern in the Danube environment any longer.

Overall, judging on the occurrence and spatial distribution on nonylphenolic compounds, it is evident that as a result of insufficient or nonexistent treatment of wastewaters, the Danube continues to show signs of degradation downstream of metropolitan and industrial areas, as well as in a number of main tributaries, and that improvement of wastewater treatment is needed.

Because of considerable lack of ecotoxicological data and estrogenic effect studies for benthic organisms, as well as scientific uncertainties regarding exposure, there are currently only provisional EQS available for freshwater sediments. Therefore, one of the priorities for the protection of benthic organisms is to carry out further ecotoxicological and estrogenic potential studies with the individual and mixed alkylphenolic compounds, in order to amend the provisional European EQS.

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