Joint Danube Survey 2

icpdr International Commission for the Protection of the Danube River
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Full report on
Gas chromatography-mass spectrometry screening of unknown organic substances in surface water and sediment samples

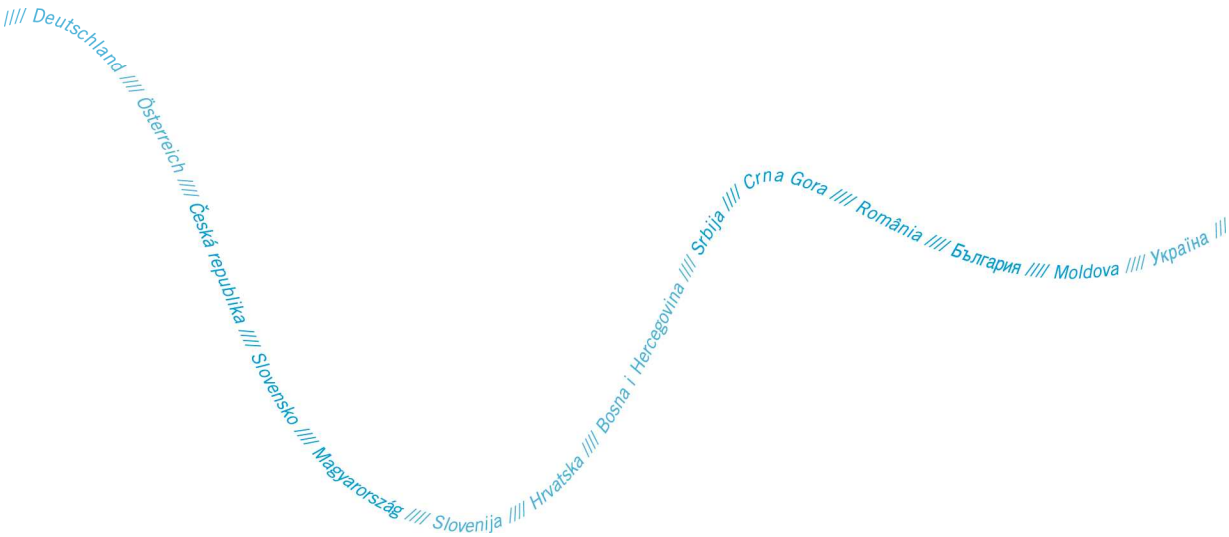

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Table of content

1 Introduction 4

2 Methods 5

3 Results 6

4 Conclusions 15

5 References **Error! Bookmark not defined.**

1 Introduction

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According to the Annex V of WFD countries are obliged to include *Other pollutants* discharged in significant quantities into a water body and specific for an individual river basin into the classification of the overall ecological status. Once identified, these pollutants must be included into the monitoring schemes and their environmental quality standards have to be derived. At present, only four metals - As, Cr, Cu and Zn are listed as *Other pollutants* specific for the Danube Basin. Similar to other basins in Europe, the inclusion of additional organic pollutants into the list is hampered by the lack and quality of available data. In a typical monitoring effort most of the Danube countries are focusing on the analysis of the 33 WFD Priority substances (Dangerous Substances Directive, 2006) determining the chemical status of the water bodies and overlooking the possible presence of tens of thousands substances which may potentially enter the environment.

The main objective of the gas chromatography-mass spectrometry (GC-MS) screening of the JDS2 samples was therefore to identify and trace pollution trends of 'unknown' substances, which are not included (i) in the routine monitoring schemes of the Danube countries and (ii) among the JDS2 target parameters. Such pollutants are often termed as 'emerging substances' and may be candidates for future regulation, depending on research on their (eco)toxicity, potential health effects, public perception and on monitoring data regarding their occurrence in the various environmental compartments. Emerging substances are of increasing concern to scientists, regulators and the public. They are not necessarily new chemicals and some of them have long been present in the environment, but their presence and significance are only now attracting closer attention. Personal care products, pharmaceuticals, fragrances, disinfection by-products, detergents, petrol additives, flame retardants and new types of pesticides are just some examples of the emerging substances frequently discussed today.

Mass spectra obtained from GC-MS screening in electron impact mode are widely accepted as unique fingerprints of individual organic compounds and can be compared against the existing databases (e.g. Wiley library containing more than 400,000 spectra). Despite the above, typically some 10 – 30% of the compounds detected in an environmental sample stay unidentified. Here, a decision can be made to judge whether additional targeted research is needed to identify the detected unknown substances, e.g., based on the overall ecotoxicity of the sample, frequency of occurrence, concentrations or evidence of biological impact in the vicinity of the sampling site(s).

The guidelines developed within the EU 6th Framework Programme research project NORMAN dealing with the emerging substances at the EU level (www.norman-network.net) were taken into account at the evaluation of results.

2 Methods

2.1 GC-MS screening of water samples

Water sample (100 ml) in a 250 ml glass bottle was spiked with 10 µl (10 ng/µl) methanolic perdeuterated anthracene internal standard solution to give a concentration of 1 µg/l and extracted using stir bar sorptive extraction (SBSE) for 60 min at 600 rpm. After extraction, stir bar was removed from the sample, dried and placed in the liner of a TDU thermal desorption system (Gerstel, Mühlheim a.d. Ruhr, Germany). The GC-MS screening analysis was performed using Agilent 6890 gas chromatograph coupled to Agilent 5973 mass spectrometric detector (MSD; Agilent Technologies, Little Falls, DE, USA). The system was equipped with a TDU and a CIS4 programmed temperature vaporisation (PTV) injector system (Gerstel). Solvent venting thermal desorption was performed by programming TDU from 40 to 250ºC (5 min) at a rate of 12 ºC/s. The analytes were cryo-focused in the PTV at -30ºC with liquid CO_2 prior to injection. For splitless injection (5 min) the PTV was ramped from -30ºC to 250ºC (5 min) at a rate of 12 ºC/s. Capillary GC analysis was performed on a 30 m × 250 µm I.D., 0.25 µm df HP-5MS column (Agilent Technologies). The oven was programmed from 70 ºC (2 min) at 25 ºC/min to 150 ºC, at 3 ºC/min to 200 ºC and finally at 8 ºC/min to 280 ºC (10 min). Helium was used as carrier gas. The head pressure was calculated using the retention locking (RTL) software so that chlorpyrifos methyl was eluting at a constant retention time of 16.596 min (Sandra et al. 2003, Sandra et al. 2003). The MSD was used in the scan mode (m/z 40–550) for all samples. Identification of compounds was performed using mass spectrum libraries Wiley 7n and NIST02, respectively.

2.2 GC-MS screening of sediment samples

Sediment sample (0.5 g) was weighed to an Eppendorf 1.5 ml micro test tube (Eppendorf AG, Hamburg, Germany) and spiked with 10 µl (10 µg/ml) methanolic perdeuterated anthracene internal standard solution to give a concentration of 0.2 µg/g. 1 ml of a solvent mixture methanol/dichloro-methane (9:1; v/v) was added and the mixture was sonicated for 2×15 min. After extraction, the mixture was centrifuged at 1000 rpm for 2 min and the supernatant was transferred to 100 ml Milli-Q water containing 10 ml methanol. Resulting solution was extracted using SBSE for 60 min at 600 rpm. The GC-MS screening analysis was performed in the same way as for water samples.

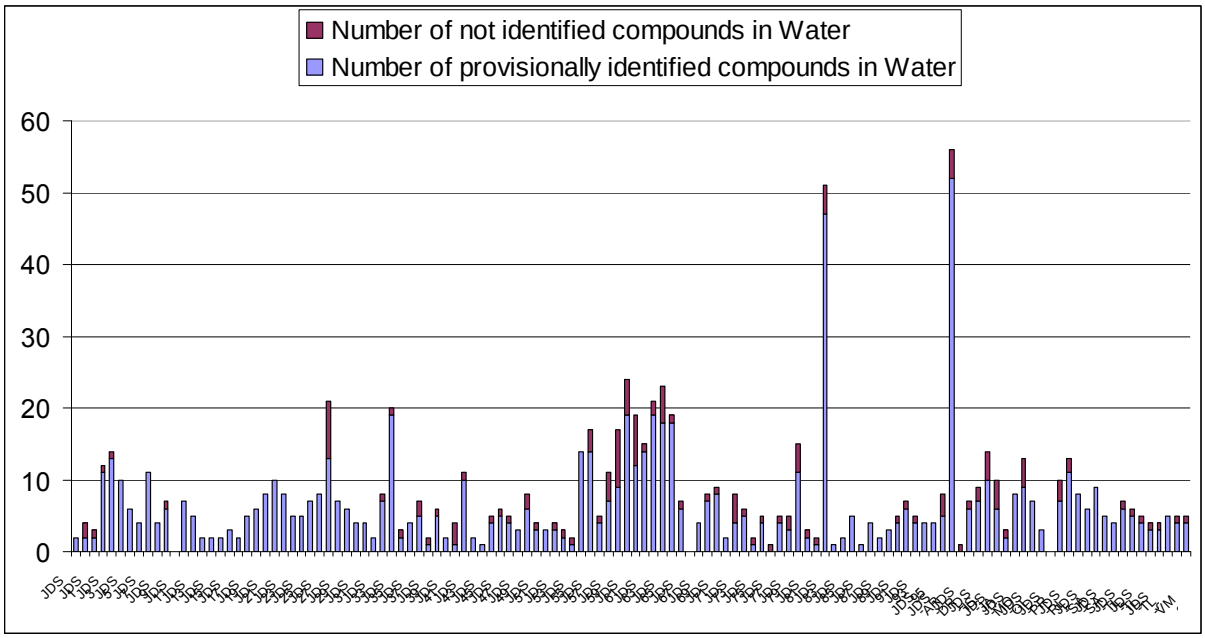
It should be stressed that the GC-MS analyses of water and sediment samples were performed in the SCAN mode, i.e., the mass ions created in the mass spectrometer were detected at the full spectral scale not to miss any information on created fragments. This operation mode, however, results in a limited sensitivity, which cannot be compared with the sensitivity of standard methods used for the determination of target organic compounds within the JDS2. Therefore, not all organic micropollutants determined by target analyses must have been necessarily detected by GC-MS.

3 Results

3.1 GC-MS screening of surface water samples

Altogether 124 water samples from the Danube River and its tributaries were analysed by GC-MS and in all but three samples (JDS12, 69 on the Danube and JDS-PR2 on the Prut tributary) several organic compounds were identified. Based on the obtained spectral information chemical structures of 158 analytes could be proposed (see Table 3.1-1 and Figure 3.1-1). Additional 43 compounds remained unidentified. For comparison, screening of 98 water samples in the JDS1 revealed the presence of 96 provisionally identified analytes. Here, one should take into account that in the JDS1 water samples were prepared using liquid-liquid extraction into dichloromethane whereas in the JDS2 a more advanced technique (SBSE, see Methods above) was applied. Both techniques concentrate hydrophobic compounds from water to an extraction solvent, and the extraction efficiency depends on the compound partition coefficient. Both methods have comparable performance characteristics, however, dichloromethane extraction enables detection of more volatile organic compounds (e.g., tetrachloroethene) and also more hydrophobic compounds that are usually adsorbed on the SPM (e.g., sterols). On the other hand the SBSE techniques is less laborious and showed that more compounds could be detected compared to the JDS1 despite the fact that the method allows only for extraction of organic substances dissolved in water.

Figure 3.1-1 No. of compounds detected in the JDS2 surface water samples



In agreement with the results of the JDS1 **phthalates** and **fatty acids** belong to the most ubiquitous compounds detected. Phthalates are commonly used as plasticizers, industrial and lubricating oils, defoaming agents, cosmetics and insect repellents. **Dibutylphthalate**, which is already on the list of *Other substances* of Slovakia and Finland, with provisional AA-EQS of 10 µg/l, was detected in 27 samples with highest estimated concentrations found at JDS22 (Iza/Szony) and JDS23 (Sturovo/Esztergom; cf. Figure 3.1-2). Its significantly elevated concentrations detected in the river stretch between JDS20 (Komarno/Komarom) and JDS31 (Downstream Budapest) are a matter of concern and might be a topic for the follow up study. **Di(2-ethylhexyl) phthalate** which is on the list of WFD priority substances was detected in 40 samples, which confirms statements on its ubiquitous

presence in the Danube basin from target analyses (see Chapter on Priority substances). The most widespread representative of this group was **isobutylphthalate** present in 91 samples. Fatty acids are entering environment mainly from degradation of petroleum hydrocarbons and animal and vegetable fats, being often used as indicators of the efficiency of the treatment process in the waste water treatment plants.

In general, a significantly wider variety of **esters of fatty acids and other acids, derivatives of benzene and polycyclic aromatic hydrocarbons** were detected in the JDS2 compared to the JDS1. A large number of **derivatives of naphthalene and phenathrene** were characteristic for the JDS84 (Arges tributary), JDS-AR2 (Arges tributary Upstream Bucharest) and JDS-RL2 (Rusenski Lom tributary at Beli Lom, Pisanetz) sampling sites. **Phenanthrene**, present in nine JDS2 samples, is already included in the list of *Other substances* for Slovakia with a provisional AA-EQS of 0.38 µg/l.

Alkylsubstituted benzenes represent typical degradation products of petroleum hydrocarbons coming mainly from oil pollution due to navigation and combustion of fuels. They were found in larger numbers and quantities in the river stretch from river kilometer 1040 to 840 (Irongate reservoir (Golubac/Koronin) - Pristol/Novo Selo Harbour), at JDS36 (Paks, rkm 1533) and at JDS4, 5 and 6 sites (Deggendorf, Niederaltereich and Inn tributary).

A significant presence of **personal care products** as indicators of the waste water pollution or poor efficiency of the waste water treatment plants was identified in most of the samples. Among the detected compounds were, e.g.:

- Sun-screen agents: EHMC, drometrizole, acetophenone and benzophenone;
- Fragrances and musks: limonene, .alpha.-terpinene, junipene, longicyclene, isobornyl acetate, dihydro methyl jasmonate, dihydromyrcenol, menthol, galaxolide, 2,4,7,9-tetramethyl-5-decyne-4,7-diol, 1,4-dioxacycloheptadecane-5,17-dione (Musk T);
- Other cosmetic ingredients: ethylene-, diethylene-, triethylene- and pentaethylene glycol monododecyl ethers, 2-hydroxybenzoic acid pentyl ester, dipropyleneglycol dibenzoate, bis(2-ethylhexyl) maleate, tributyl acetylcitrate, 2-ethyl-1-hexanol, 3,7-dimethyl-3-octanol, 2-(dodecyloxy) ethanol, 2-(1,1-dimethylethyl) cyclohexanol, 1-methyl-2-pyrrolidinone, acetylcedrene, 2,4-toluenediamine.

Galaxolide, which was included also among the JDS2 target determinands in SPM (cf. chapter on Priority substances) was found at the highest level at JDS84 and JDS-AR2 (both on the Arges tributary) indicating pollution by urban waste water from Bucharest, and at ca. 10-fold lower concentration levels at JDS31 and 35 reflecting the pollution by waste water from Budapest. The compound was present also in tributaries Morava, Olt, Iskar and Russenski Lom. Galaxolide was detected at the JDS84 site (the Arges tributary) also during the JDS1 in 2001 at the same estimated concentration level of 0.4 µg/l as in 2007.

Other relevant groups of detected compounds included **chlorinated compounds** (e.g., 1,2-dichlorobenzene, bis(2-chloroisopropyl) ether), **organophosphate flame retardants (OPFR), benzothiazoles (**rubber accelerators in the tyre industry**), nitrogen containing compounds** (alkylnitrobenzenes, nitriles, amines) and a group of **pesticides** (hexachlorocyclohexane (alpha isomer), thiocarbamates).

Regarding the spatial distribution of identified compounds ubiquitous, section-specific, site-specific and random occurrence could be distinguished. The highest number of organic compounds, including a wide variety of **aromatic hydrocarbon derivatives** and **personal care products**, was identified in samples from the Arges tributary (47 and 52 compounds in JDS84 and JDS-AR2, respectively).

A site-specific contamination by **pesticides** was detected in the Olt tributary in Romania. The identified compounds include the insecticide **hexachlorocyclohexane** (alpha isomer) and two **thiocarbamate pesticide** related compounds (bis(2-methylpropyl)-, S-ethyl ester and

cyclohexylethyl-, S-ethyl ester of carbamothioic acid). Since these were the only samples from the JDS2 survey that contained detectable amounts of pesticides one could assume that pesticides in general might not be applied in larger amounts in the given period of year.

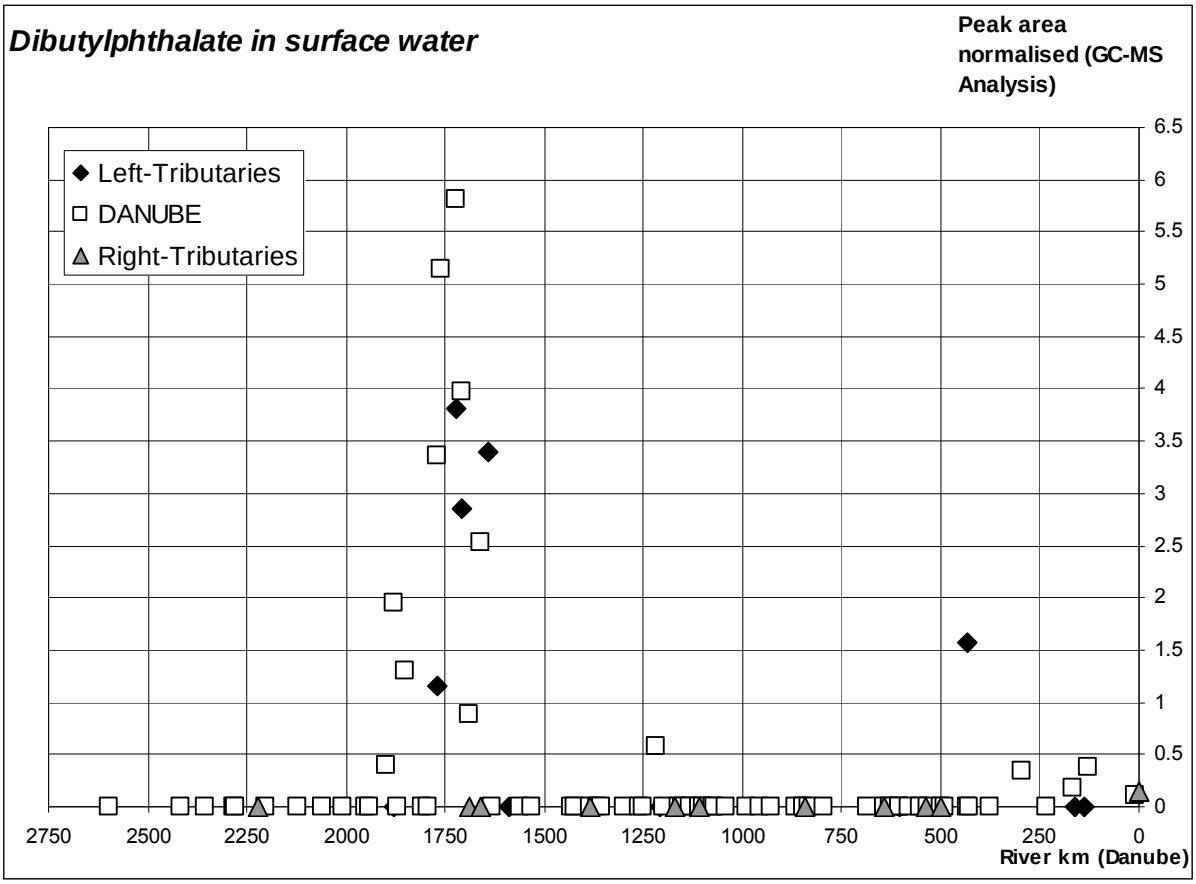
Compared to the JDS1 only one **sterolic compound** (androst-5,16-diene-3.beta.-ol) was identified and therefore no conclusions on the related faecal contamination could be made.

The WFD PS **nonylphenol** was detected in JDS-AR2 (Arges tributary Upstream Bucharest) which is in line with the findings of target analyses showing its highest concentration 3.28 µg/l at the same site (see chapter on Priority substances).

Benzothiazole, which is among the Slovak *Other pollutants* with provisional AA-EQS of 2 µg/l, was present in samples of 13 JDS1 sites but in the JDS2 only at JDS13 (Wildungsmauer). A related compound **2-methylthiobenzothiazole** was found in samples from 9 JDS2 sites in comparison to its previous occurrence at 31 JDS1 sites..

Tributyl phosphate belonging to the group of OPFR was present in most samples in the stretch from river kilometer 795 (JDS68 – Calafat) till the Black Sea, with the highest concentration at JDS84 (Arges tributary). In the upper part of the Danube the compound was present only in samples from JDS37 (Sio tributary) and JDS39 (Hercegszanto). The majority of the OPFR have been on the market since the 1950s being used mainly as flame retardants in furniture, electronic devices and building products, however, little data exists about degradation and end-of-life issues, like deposition, mobility, long-term effects or bioaccumulation. The OPFR have come under intense environmental scrutiny, due to their acute toxicity to algae, invertebrates and fish revealed in numerous environmental studies. The presence and toxic effects of the OPFR in the Danube river basin certainly deserves serious attention and further investigation.

Figure 3.1-2 Occurrence profile of dibutylphthalate in the JDS2 surface water samples



Semi-quantitative assessment

Even though there are no quantitative values of analytes identified by the GC-MS available, their concentrations were estimated using a semi-quantitative assessment. In the approach a relative abundance of an analyte is used, which is the ratio of its peak area and the peak area of the internal standard (anthracene-D10), both obtained from an extracted chromatogram at its base ion m/z. The obtained relative abundances allowed for a construction of the occurrence profiles of identified compounds (see e.g., Figures 3.1-1 and 3.2-1). Complete information on the occurrence profiles of all detected compounds is presented in the full report.

Archivation of data on unidentified compounds

Despite using the advanced identification methodologies, ca. 10 – 30% of the detected compounds in each sample remained unidentified due various interferences and/or missing spectra in the available libraries. In such cases the raw measurement data containing digital information of each mass spectrum were stored in a specifically developed GC-MS database of the ICPDR (next to the information on the provisionally identified compounds) in order to enable the retrospective identification of an unknown compound in the future.

Table 3.1-1 List of compounds provisionally identified in surface water of the Danube River and its tributaries. Compounds highlighted in colour are present on the EU list of NORMAN emerging substances.

Compound	CAS no.	Compound	CAS No.
2,6-Di(t-butyl)-4-hydroxybenzaldehyde	n/a	Diethylene glycol monododecyl ether	3055-93-4
.alpha.-iso-Methyl ionone	127-51-5	Dihydro methyl jasmonate	24851-98-7

Compound	CAS no.	Compound	CAS No.
.alpha.-Lindane (HCH)	319-84-6	Dihydromyrcenol	53219-21-9
.alpha.-Terpinene	99-86-5	Diisopropylnaphthalene	38640-62-9
1,1'-Biphenyl, 4-methyl-	644-08-6	Dipropyleneglycol dibenzoate	n/a
1,4-Dioxacycloheptadecane-5,17-dione	105-95-3	Dodecanal	112-54-9
1-Decene	872-05-9	Dodecane	112-40-3
1-Dodecanol	112-53-8	Dodecanoic acid	143-07-7
1-Hexanol, 2-ethyl-	104-76-7	Dodecanoic acid, 1-methylethyl ester	10233-13-3
1H-Indene, 2,3-dihydro-	496-11-7	Drometrizole	2440-22-4
1H-Indene, 2,3-dihydro-4,7-dimethyl-	6682-71-9	Ethanol, 2-(dodecyloxy)-	4536-30-5
1-Tetradecene	1120-36-1	Ethylene glycol monododecyl ether	4536-30-5
1-Tridecene	2437-56-1	Fluorene	86-73-7
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	126-86-3	Galaxolide 1 and 2	n/a
2,4-Toluenediamine	95-80-7	Heptadecane	629-78-7
2,6-Di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	n/a	Heptanoic acid	111-14-8
2,6-Diisocyanatotoluene	91-08-7	Hexadecane	544-76-3
2,6-Diisopropylnaphthalene	24157-81-1	Hexadecanenitrile	629-79-8
2,6-Di-tert-butyl-4-(dimethylaminomethyl)phenol	88-27-7	Hexadecanoic acid	57-10-3
2-Benzimidazolinone, 5-methyl-	5400-75-9	Hexadecanoic acid, butyl ester	111-06-8
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	80-71-7	Hexanedioic acid, diisooctyl ester	1330-86-5
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	5466-77-3	Isobornyl acetate	125-12-2
2-Pyrrolidinone, 1-methyl-	872-50-4	Isobutyl phthalate	84-69-5
2-Pyrrolidinone, 1-methyl-	872-50-4	Junipene	475-20-7
3-Ethylbenzophenone	66067-43-4	Limonene	138-86-3
3-Octanol, 3,7-dimethyl-	78-69-3	Longicyclene	1137-12-8
4,8,12-Tetradecatrienal, 5,9,13-trimethyl-	66408-55-7	Menthol	1490-04-6
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	n/a	Naphthalene	91-20-3
7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	n/a	Naphthalene, 1,2,3,4-tetrahydro-1,4-dimethyl-	4175-54-6
9-Hexadecenoic acid	2091-29-4	Naphthalene, 1,2,3,4-tetrahydro-1-methyl-	1559-81-5
9H-Fluorene, 2-methyl-	1430-97-3	Naphthalene, 1,2,3,4-tetrahydro-2-methyl-	3877-19-8
9-Octadecenamide	301-02-0	Naphthalene, 1,2,3,4-tetrahydro-5-methyl-	2809-64-5
9-Octadecenoic acid	112-80-1	Naphthalene, 1,2,3,4-tetrahydro-6-methyl-	1680-51-9
Acenaphthene	83-32-9	Naphthalene, 1,4,6-trimethyl-	2131-42-2
Acetophenone	98-86-2	Naphthalene, 1,4-dimethyl-	571-58-4
Acetylcedrene	n/a	Naphthalene, 1,6,7-trimethyl-	2245-38-7
Androst-5,16-diene-3.beta.-ol	1224-94-8	Naphthalene, 1-methyl-	90-12-0
Benzene, (1-butylheptyl)-	4537-15-9	Naphthalene, 2,3,6-trimethyl-	829-26-5
Benzene, (1-butylhexyl)-	4537-11-5	Naphthalene, 2,3-dimethyl-	581-40-8
Benzene, (1-butylnonyl)-	4534-50-3	Naphthalene, 2,6-dimethyl-	581-42-0
Benzene, (1-butyloctyl)-	2719-63-3	Naphthalene, 2-methyl-	91-57-6
Benzene, (1-ethyldecyl)-	2400-00-2	Nonadecanenitrile	28623-46-3
Benzene, (1-methyldecyl)-	4536-88-3	Nonanoic acid	112-05-0
Benzene, (1-methyldodecyl)-	4534-53-6	Nonylphenol	25154-52-3
Benzene, (1-methylundecyl)-	2719-61-1	Octadecane	593-45-3

Compound	CAS no.	Compound	CAS No.
Benzene, (1-pentylheptyl)-	2719-62-2	Octadecanoic acid	57-11-4
Benzene, (1-pentylhexyl)-	4537-14-8	Octadecanoic acid, butyl ester	123-95-5
Benzene, (1-propyldecyl)-	4534-51-4	Octanal, 2-(phenylmethylene)-	101-86-0
Benzene, (1-propylnonyl)-	2719-64-4	Octanoic acid	124-07-2
Benzene, (1-propyloctyl)-	4536-86-1	O-Diethoxybenzene	n/a
Benzene, 1,2,3-trimethyl-	526-73-8	Pentadecane	629-62-9
Benzene, 1,2,4,5-tetramethyl-	95-93-2	Pentadecanenitrile	18300-91-9
Benzene, 1,2-dichloro-	95-50-1	Pentadecanoic acid	1002-84-2
Benzene, 1,2-diethyl-	135-01-3	Pentaethylene glycol monododecyl ether	3055-95-6
Benzene, 1,3,5-trimethyl-	108-67-8	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	1000140-77-5
Benzene, 1,3-bis(1-methylethyl)-	99-62-7	Phenanthrene	85-01-8
Benzene, 1,4-bis(1-methylethyl)-	100-18-5	Phenanthrene, 1-methyl-	832-69-9
Benzene, 1-ethyl-2,3-dimethyl-	933-98-2	Phenanthrene, 2-methyl-	2531-84-2
Benzene, 1-ethyl-4-nitro-	100-12-9	Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4
Benzene, 1-methyl-2-(1-methylethyl)-	527-84-4	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	128-37-0
Benzene, 1-methyl-4-nitro-	99-99-0	Phthalic anhydride	85-44-9
Benzoic acid, 2-hydroxy-, pentyl ester	2050-08-0	Piperonyl butoxide	51-03-6
Benzophenone	119-61-9	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	74367-34-3
Benzothiazole	95-16-9	Squalene	7683-64-9
Benzothiazole, 2-(methylthio)-	615-22-5	Tetradecane	629-59-4
Bibenzyl	103-29-7	Tetradecanenitrile	629-63-0
Bis(2-chloroisopropyl) ether	39638-32-9	Tetradecanoic acid	544-63-8
Bis(2-ethylhexyl) ether	n/a	Tetradecanoic acid, 12-methyl-	5746-58-7
Bis(2-ethylhexyl) maleate	142-16-5	Tetradecanoic acid, 1-methylethyl ester	110-27-0
Bis(2-ethylhexyl) phthalate	117-81-7	Tri(butoxyethyl) phosphate	78-51-3
Carbamothioic acid, bis(2-methylpropyl)-, S-ethyl ester	2008-41-5	Tributyl acetylcitrate	77-90-7
Carbamothioic acid, cyclohexylethyl-, S-ethyl ester	1134-23-2	Tributyl phosphate	126-73-8
Cyclododecane	294-62-2	Tridecane	629-50-5
Cyclohexanol, 2-(1,1-dimethylethyl)-	13491-79-7	Tridecanoic acid	638-53-9
Cyclohexanone, 4-(1,1-dimethylethyl)-	98-53-3	Triethylene glycol monododecyl ether	3055-94-5
Decane	124-18-5	Tri-n-butylamine	102-82-9
Decanoic acid	334-48-5	Triphenyl phosphate	115-86-6
Dibenzofuran	132-64-9	Undecane	1120-21-4
Dibutyl phthalate	84-74-2		
Diethyl phthalate	84-66-2		

3.2 GC-MS screening of sediment samples

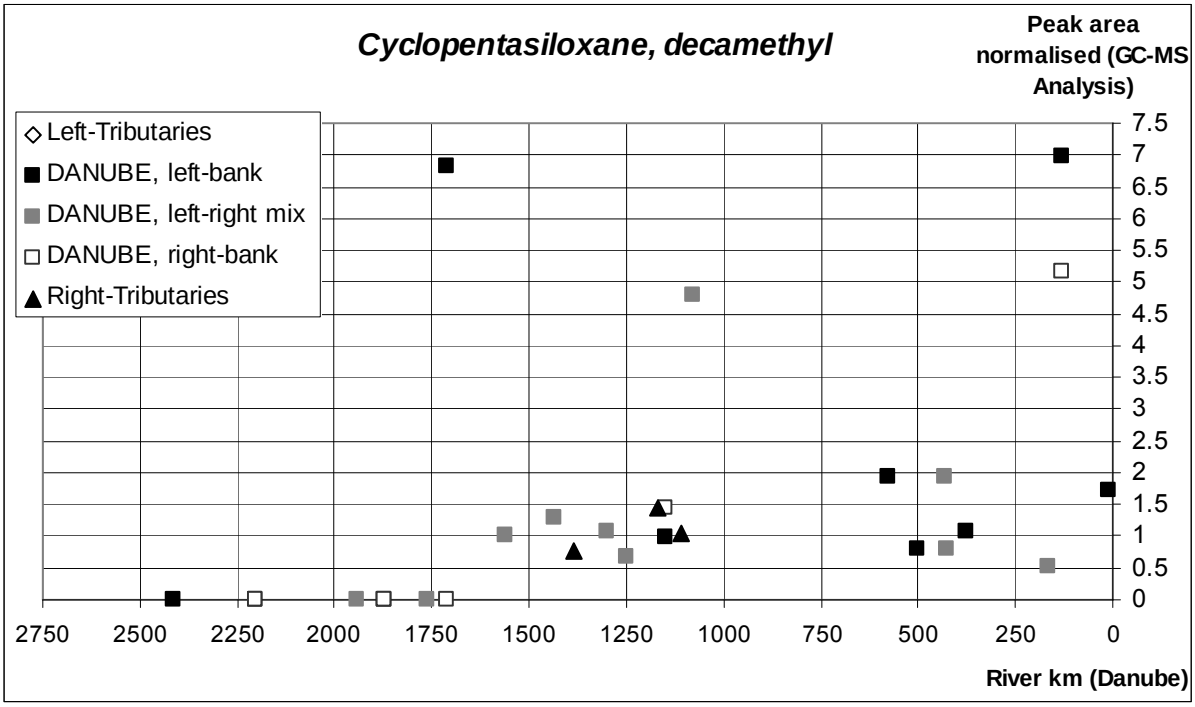
In total 28 sediment samples were analysed and 87 organic compounds with proposed chemical structures were identified in sediment extracts (for a list see Table 3.2-1), whereas 18 compounds remained unidentified. The sediment samples were taken from the 'top' 23 JDS2 sites (see Chapter Survey preparation) and analysed as a mix of left and right side for 11 sampling sites (JDS 7, 12, 22,

35, 39, 45, 47, 58, 83, 85, 89) and separately as left and right from the sites JDS 16, 26, 53 and 92. Only one left or right sediment sample was available for analysis from the sites JDS 2, 42, 51, 56, 76, 80, 86 and 95.

Among the most ubiquitous compounds found were **polycyclic aromatic hydrocarbons** (mainly phenanthrene, fluoranthene and pyrene) **and their alkylderivatives** (e.g., diisopropylnaphthalane), **alkanes, cycloalkanes and aldehydes.** Most of these substances are originating from the chemical and bacterial degradation of petroleum hydrocarbons, the source being probably the oil pollution and fuel combustion.

Alarmingly, most of the sediments contained **siloxanes**, which are of increasing concern worldwide and present on the list of the emerging substances of NORMAN. Siloxanes belong to a group of substances used in a number of industrial applications and in consumer products such as additives in fuel, car polish, cleaners, anti-foamers and car waxes. Besides this, they are widely used in, e.g., personal care and biomedical products. The wide-spread use of siloxanes, their broad application as well as their high volatility has raised the concern for these compounds within various disciplines of environmental science. The results of monitoring programmes indicate that there is a general pollution of siloxanes in all matrices except soils. They seem to be emitted through diffuse pathways and they enter the aquatic food chain. The use of siloxanes is extensive and it is possible that continued use will lead to increased environmental levels, eventually reaching effect concentrations.
An important large volume chemical in this group, **decamethylcyclopentasiloxane (D5)**, is currently undergoing an in-depth risk assessment in the EU and may well soon be classified as a very persistent and bioaccumulating substance. This has already prompted some regulatory jurisdictions and industry to push development in this area.
The low molecular weight siloxanes: octamethylcyclotetrasiloxane, decamethyltetrasiloxane, D5, dodecamethylpentasiloxane, dodecamethylcyclohexasiloxane, tetradecamethylhexasiloxane and tetradecamethylcycloheptasiloxane were identified in majority of the samples. A wide range of representatives of this group of compounds was present also in 15 out of 30 investigated sediment sampling sites during the Aquaterra Danube Survey in 2004 (ADS), however, marked as “not identified” or provisionally identified as “silicone oil” due to the less developed identification software and databases available at that time.
A pollution profile of the analysed sediments by the most abundant and ubiquitous D5 is shown in Figure 3.2-1. Similar to the findings of the JDS2 and ADS a separate study of siloxanes in various environmental (including sludge and biota) and food matrices in Sweden in 2004 showed that D5 was the dominating siloxane in most samples (Swedish National Screening Programme 2004). The results trigger the need to investigate the occurrence of siloxanes in the Danube river basin on a more systematic basis.

Figure 3.2-1 Occurrence profile of decamethylcyclopentasiloxane (D5) in the JDS2 sediment samples



In general, most of the sediment contaminants showed a diffuse pollution patterns. The site-specific contamination could only be observed at several sampling locations, such as e.g. JDS85 - Downstream Arges/Oltenita. Here, the highest number of organic compounds in a single sample was detected (35 compounds) from which 31 analytes showed the highest estimated concentration values of the individually detected compounds from among all samples. The contamination pattern was matching that of water samples taken from the Danube tributary Arges, in which *i.a.* **galaxolide** and numerous **derivatives of naphthalene and phenanthrene** were identified. This pattern indicates the pollution by waste water from Bucharest. **Triphenyl phosphate** – flame retardant present also at the NORMAN list of emerging substances was found only at JDS16 in Bratislava (right bank).

Significantly smaller number of compounds was detected in the JDS2 compared to the ADS, in which the sediments from 30 sampling sites (from Vienna, Austria to Calafat, Romania) contained typically around 120 compounds. Similarly to the JDS2, the ADS report states that a general trend of increase of the number of detected compounds in the lower reach of the Danube (typically over 300 detected compounds after the Sava confluence) was observed. In average, about 35% of detected compounds could not be provisionally identified in sediment samples. The GC-MS screening provided a lot of useful information on the design of a general pollution pattern of the Danube river, e.g., indicating significant pollution impacts from either left or right bank sources. The detailed information on all detected compounds is presented in the full report.

Table 3.2-1 List of compounds provisionally identified in the Danube river sediments samples. Compounds highlighted in colour are present on the EU list of NORMAN emerging substances.

Compound	CAS No.	Compound	CAS No.
1,15-Hexadecadiene	21964-51-2	Hexanedioic acid, bis(2-ethylhexyl) ester	103-23-1
1,1'-Biphenyl, bis(1-methylethyl)-	69009-90-1	Hexasiloxane, tetradecamethyl-	107-52-8
1-Hexadecene	629-73-2	Naphthalene, 1,2(or 2,3)-diethyl-	74710-00-2
1H-Indene, 2,3-dihydro-1,1,4,5,6-pentamethyl-	16204-67-4	Naphthalene, 1,2,3,4-tetramethyl-	3031-15-0
1-Pentadecene	13360-61-7	Naphthalene, 1,4,5-trimethyl-	2131-41-1

Compound	CAS No.	Compound	CAS No.
1S,cis-Calamenene	483-77-2	Naphthalene, 1,4,6-trimethyl-	2131-42-2
2,6-Diisopropylnaphthalene	24157-81-1	Naphthalene, 1,6,7-trimethyl-	2245-38-7
2-Decenal, (Z)-	2497-25-8	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	483-78-3
2-Undecenal	2463-77-6	Naphthalene, 1-methyl-	90-12-0
3-Methyl-2-(3,7,11-trimethyldodecyl) furan	n/a	Naphthalene, 1-propyl-	2765-18-6
4b,8-Dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,8a,9,10-octahydro-	n/a	Naphthalene, 2,3,6-trimethyl-	829-26-5
7-Isopropyl-4-methylazulene	n/a	Naphthalene, 2,3-dimethyl-	581-40-8
9-Octadecenoic acid	112-80-1	Naphthalene, 2,6-dimethyl-	581-42-0
Azulene, 1,4-dimethyl-7-(1-methylethyl)-	489-84-9	Naphthalene, 2,7-dimethyl-	582-16-1
Azulene, 7-ethyl-1,4-dimethyl-	529-05-5	Naphthalene, 2-methyl-	91-57-6
Benzene, 1,1'-(chloroethenylidene)bis-	4541-89-3	Octadecanoic acid	57-11-4
Benzene, 1-methyl-4-(1,2,2-trimethylcyclopentyl)-	16982-00-6	Pentadecanoic acid	1002-84-2
Bis(2-ethylhexyl) phthalate	117-81-7	Pentadecanoic acid, methyl ester	7132-64-1
Cholesta-3,5-diene	747-90-0	Pentasiloxane, dodecamethyl-	141-63-9
Cholestan-3-one, (5.beta.)-	601-53-6	Phenanthrene	85-01-8
Chrysene	218-01-9	Phenanthrene, 1-methyl-	832-69-9
Cyclododecane	294-62-2	Phenanthrene, 1-methyl-7-(1-methylethyl)-	483-65-8
Cycloheptasiloxane, tetradecamethyl-	107-50-6	Phenanthrene, 1-methyl-7-(1-methylethyl)-	483-65-8
Cyclohexasiloxane, dodecamethyl-	540-97-6	Phenanthrene, 2,3,5-trimethyl-	3674-73-5
Cyclopentadecane	295-48-7	Phenanthrene, 2,3-dimethyl-	3674-65-5
Cyclopentasiloxane, decamethyl-	541-02-6	Phenanthrene, 2,5-dimethyl-	3674-66-6
Cyclotetradecane	295-17-0	Phenanthrene, 2,7-dimethyl-	1576-69-8
Cyclotetrasiloxane, octamethyl-	556-67-2	Phenanthrene, 2-methyl-	2531-84-2
Decanal	112-31-2	Phenanthrene, 3,6-dimethyl-	1576-67-6
Decane	124-18-5	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-	128-37-0
Decanedioic acid, bis(2-ethylhexyl) ester	122-62-3	Phthalic anhydride	85-44-9
Dihydrocholesterol	80-97-7	Pyrene	129-00-0
Diisopropylnaphthalene	38640-62-9	Simonellite	n/a
Dodecanal	112-54-9	Squalene	7683-64-9
Dodecane	112-40-3	Tetradecanal	124-25-4
Dodecanoic acid	143-07-7	Tetradecanoic acid	544-63-8
Dodecanoic acid, methyl ester	111-82-0	Tetradecanoic acid, methyl ester	124-10-7
Fluoranthene	206-44-0	Tetrasiloxane, decamethyl-	141-62-8
Galaxolide 1 and 2	n/a	Tridecanal	10486-19-8
Heptadecanoic acid	506-12-7	Tridecane	629-50-5
Hexadecanamide	629-54-9	Triphenyl phosphate	115-86-6
Hexadecanoic acid	57-10-3	Undecanal	112-44-7
Hexadecanoic acid, butyl ester	111-06-8	Undecane	1120-21-4
Hexadecanoic acid, methyl ester	112-39-0		

4 Conclusions

159 and 87 organic compounds were detected and provisionally identified by GC-MS screening analysis in the 124 JDS2 surface water and 28 sediment samples, respectively. Among the main groups of substances, which are ubiquitous in the Danube river basin, were found plasticisers, degradation products of petroleum hydrocarbons, fuel additives, personal care products and organophosphate flame retardants. Some of the detected compounds, such as plasticiser dibutylphthalate, polyaromatic hydrocarbon phenanthrene and rubber accelerators benzothiazole are already included in the national monitoring of some Danube countries as *Other pollutants* with provisional EQS (e.g. Slovak Republic, 10, 0.38 and 2 µg/l, respectively).

Despite its lower sensitivity, GC-MS screening of unknown substances provided relevant complementary information to the analyses of target compounds. It also confirmed the findings of the other JDS2 laboratories in terms of identification of the most relevant diffuse and point pollution patterns in the Danube basin (e.g. for nonylphenol, DEHP, galaxolide etc.). The most polluted part of the basin in terms of number of the detected compounds and their concentrations was the Arges tributary draining waste water from the Bucharest area.

Only three pesticides were detected in the JDS2 samples in the Olt tributary, which indicates that sampling in August – September might not be representative for this group of compounds. Here, the deployment of passive samplers providing integrated information over longer time period (months) would certainly be of more use in future investigations.

Many of the detected compounds from the groups of personal care products (e.g., siloxanes, sun-screen agents, fragrances and musks), plasticizers (phthalates) and flame retardants (organophosphates) are on the list of emerging substances of European relevance. Carefully selected representatives of the above groups of compounds may be considered as candidates for future monitoring on a basin scale. Their toxic effects and widespread occurrence in Europe are under the scrutiny of the NORMAN project, which starts to operate as a permanent network from 2009 supporting the EC Chemical Monitoring Activity (CMA). A close cooperation with the NORMAN network in the coming years is recommended in order to harmonise strategies for deriving the compounds' EQSs, development of methodologies for their analysis and setting up schemes for investigative monitoring and developing eventual measures for their removal. The remaining challenge is to identify compounds which stayed unidentified due to their missing mass spectra in the currently available libraries.

5 References

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