5.2.4.7 Pharmaceuticals

During the JDS, 19 surface water samples were taken from river Danube. The samples were analysed for the occurrence of several pharmaceuticals and corresponding metabolites, listed in Table C2.4.7.-1.

TABLE C2.4.7.-1: List of pharmaceuticals and their type analyzed during the JDS.

Substance	Type of drug
N-acetyl-4-aminoantipyrine	metabolite of metamizol
1-acetyl-1-methyl-2-phenylhydrazide	metabolite of phenazone
Ambroxol	expectorant
Bromhexine	expectorant
Clonidin	sympathomimetic
Clotrimazol	antimycotic
3,5-dibromoanthranilic acid methyl ester	metabolite of ambroxol and bromhexine
Dimetridazole	antimycotic
N-formyl-4-aminoantipyrine	metabolite of metamizol
Isopropylphenazone	analgesic
Methyl-2(methyl-sulphonamido)-benzoate	possible metabolite of diff. diuretics
Metronidazole	antiparasitic
Na873 = 6,8-dibromo-3-(trans-4-hydroxy-cyclo-hexyl)-1,2,3,4-tetrahydro-quinazoline	metabolite of ambroxol and bromhexine
Naphazoline	sympathomimetic
Oxyphenbutazone	metabolite of phenylbutazone
Paracetamol	analgesic
Phenylbutazone	antiphlogistic
Phenazone	analgesic
Ronidazole	antiparasitic
Timolol	betablocker
Xylometazoline	sympathomimetic

The results show that only few of the analytes extracted at neutral pH were detectable. The only substances present in nearly every sample were isopropylphenazone (an analgesic), which was found in low concentrations of 0.003-0.004 μg/l, and N-acetyl-4-aminoantipyrine (one main metabolite of metamizol, also an analgesic), whose concentrations showed larger variations. From Budapest downstream, the concentrations of N-acetyl-4-aminoantipyrine showed a relatively constant level of around 0.1 μg/l. Three samples showed significantly higher amounts of this substance, namely Iskar (Sofia discharge), Jantra and Arges (Bucharest discharge). These samples came from tributaries rather than the Danube stream itself. Another metabolite of metamizol, N-formyl-4-aminoantipyrine, shows the same behaviour, but cannot be detected in some cases due to a higher limit of detection. The three samples mentioned before also showed the analgesic phenazone in amounts from 0.029 – 0.46 μg/l. The results obtained are in good agreement with previous data obtained in our laboratory. The relatively constant "background level" in most of the samples is about one half of that found in river Elbe in two sampling campaigns in 1999 and 2000, with the same substances prevailing. The three samples with higher contamination show concentrations in a range that is typical for surface waters that are charged with effluents of wastewater treatment plants, e.g. Lake Tegel in Berlin [1].

The analytes extracted at acidic pH show a slightly different behavior due to better limits of detection. More substances can be found in most of the samples, mainly in the low ng/l range. Diclofenac and naproxen are present in every sample, clofibric acid and ibuprofen in most of them. A significant rise in the concentrations can be seen only in the Arges sample, a slight one (diclofenac and indometacin) in Iskar and none in Jantra.

Reference:

1. ***Schmidt, R., Brockmeyer, R., Vorkommen und Verhalten von Expektorantien, Analgetika, Xylometazolin und deren Metaboliten in Gewässern und bei der Uferfiltration, Vom Wasser, 98,*** 37-54 (2002, in press)

5.2.4.8 GC/MS Screening of Organic Pollutants

For assessment of organic micropollutants the use of available standardized target analytical techniques is not always realistic. Such an approach requires a thorough calibration using standard materials prior to the analysis. However, it is rather difficult task to make a complete calibration and optimizing of the analytical setup when the target analyte is unknown. Moreover, for most of the possible degradation products (metabolites) of the original organic compounds there are no standard materials available for calibration under full AQC conditions. Therefore, the best available solution for identification of unknown organic micropollutants is the use of screening techniques based on the multi-step separation of the analytes from the complex matrix of the aqueous sample and their consequent detection and structure recognition by studying their spectral properties. In a screening method, the analytes are extracted by a suitable sorbent or an organic solvent from the water sample. After the extraction the analytes are separated by high-resolution gas chromatography and detected by means of a mass spectrometry (hence, this method is usually referred to as GC-MS). Using the combined information on the separation behavior of a compound in gas chromatographic column (retention time) and on the mass spectrum of this compound, its structure can be proposed.

Owing to the high acquisition and operation costs of the GC-MS this kind of analysis is not very frequent in common monitoring programs. In the Danube TNMN the GC-MS analysis is not included and it was also missing in the results of international expeditions on the Danube in the past. For the time being the GC-MS monitoring of the Danube River on a regular basis has been agreed only as a part of some bilateral intergovernmental agreements on transboundary water quality control (e.g., between Hungary and Slovakia, [1]). Therefore, to acquire the largest possible overview on the presence of the organic contaminants in the Danube River Basin the qualitative analysis of water and sediments was included in the list of determinands for the JDS. The intention was not only to see the present status but also to create the foundation of the future database of organic micropollutants. Considering the possible information output from the GC-MS analysis it was clear that even though recognized GC-MS laboratories would be charged with this task still many of the detected compounds would remain unidentified due to various interferences and/or missing spectra in the libraries applied. However, in the case that a spectral signal remains unresolved for the time being, the storage of raw, measurement data may enable to indicate the relation of these data with results obtained in the future. This may even lead to the retrospective identification of an unknown compound in the future.

It has to be stressed that the GC-MS analyses of water and sediment samples were performed in the SCAN mode, e.i., 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 target organic compounds within the JDS study. Therefore, not all organic micropollutants determined by target analyses must have been necessarily detected by GC-MS.

5.2.4.8.1 GC/MS Screening of Water Samples

Altogether 98 water samples were analysed by GC-MS and in each sample some organic compounds were identified. Based on the spectral information collected the chemical structure of 96 analytes could be proposed. The most ubiquitous compounds were phthalates, fatty acids, aliphatic chlorohydrocarbons and sterols. In addition to these compounds following groups were observed: aliphatic and aromatic hydrocarbons, phenols, hydroxy- and keto- aliphates and aromates, benzothiazoles and other sulfur and nitrogen containing compounds, organophosphates and limited number of herbicides. The list of all compounds found in the water of the Danube River and its tributaries is in Table C2.4.8.-1.

Regarding the spatial distribution of identified compounds a large variability exists depending on the type of the compound. There are several compounds, which were found in most of the sampling sites (squalene, 1,1,2,2-tetrachloroethane, isobutylphthalate, 2-methyl-2-propenamide, cholesterol). For some homological groups the observed abundancy profiles indicate the section-specific occurrence, mostly in the middle Reach of the Danube River (e.i., phenols, sterols and bentzothiazoles). However, many other identified compounds can be considered as site-specific or they occurred randomly at various locations. As for the situation in the tributaries, this is often more complex than that in the main stream, e.i., more organic compounds have been identified in some of the tributaries when compared to the samples taken from the Danube River upstream and/or downstream of the confluence of the respective tributary. This phenomenon can mostly be ascribed to the lower dilution of pollutants in streams with a low flow. The highest number of compounds was identified in Morava and, especially, in Rusinsky Lom and Arges. The latter two tributaries in the lower Reach of the Danube contained the largest amount of total identified organics expressed as the sum of the relative abundance of all identified compounds.

It has to be pointed out, that even though there are no concentration values of analytes identified by the GC-MS available, their semi-quantitative assessment has been done using their relative abundance. The relative abundance of an analyte is the ratio of its peak area and the peak area of the internal standard (4-bromofluorobenzene). Using this approach an estimation of the occurrence profiles of identified compounds can be done.

TABLE C2.4.8.-1: List of compounds identified in the Danube River and its tributaries

1-heptadecene
Heptadecane
Octadecane
10-heneicosene
Ethylbenzene
1,2-dimethylbenzene
1,3-dimethylbenzene
1-ethyl-2-methylbenzene
1-ethyl-3-methylbenzene
1-ethyl-4-methylbenzene
1,2,3-trimethylbenzene
1,2,4-trimethylbenzene
Propylbenzene
Isopropylbenzene
1-methyl-3-propylbenzene
(1-methylpropyl)benzene
1-methyl-4-(1-methylethyl)benzene
1-ethyl-2,3-dimethylbenzene
1-ethyl-3,5-dimethylbenzene
4-ethyl-1,2-dimethylbenzene
1,2,3,4-tetramethylbenzene
1,2-dimethoxy-4-(2-propenyl)benzene
1,4-dimethylnaphthalene
2,6-dimethylnaphthalene
1,3,6-trimethylnaphthalene
1,6,7-trimethylnaphthalene
1,2,3,4-tetrahydronaphthalene
Galaxolide 1 and 2
Neophytadiene
Squalene
1,1,2,2-tetrachloroethane
Tetrachloroethene
Dodecanoic acid
Tetradecanoic acid
Pentadecanoic acid
Hexadecanoic acid
9-hexadecenoic acid
Heptadecanoic acid
Octadecanoic acid
9-octadecenoic acid
1-methylethyl ester hexadecanoic acid
Ethyl linoleolate
1-methylbutyl methacrylate
Methyl phthalate
Ethyl phthalate
Butyl phthalate
Isobutyl phthalate
Bis(2-ethylhexyl)phthalate
Tributyl acetylcitrate
5-methyl-2-hexanol
Benzeneethanol
9-octadecen-1-ol (Z)
p-tert-amyl phenoxy ethanol
2-butanone
3,3-dimethoxy-2-butanone
6,6-dimethyl-2,4-cyclohexadien-1-one
1,3-dihydro-2H-indol-2-one
Propylene glycol trimer 1
Tripropylene glycol 2
3,4-dimethoxyphenylacetone
4-methylphenol
3,5-dimethylphenol
2,6-dimethylphenol
4-(1,1-dimethylethyl)phenol
2,4,7,9-tetramethyl-5-dicyne-4,7-diol
2,4-bis(1,1-dimethylethyl)phenol
4-(1-methyl-1-phenylethyl)phenol
Nonylphenol isomer
Benzothiazole
2-(methylthio)benzothiazole
1H-indole
N,N-diethyl-3-methylbenzamide
2-methyl-2-propenamide (methacrylamide)
Triphenyl phosphate
Tributyl ester phosphoric acid
1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane
3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane
N,N' -diphenyloxamide
Hexadecanamide
Octadecanamide
9-octadecenamide
Isothiocyanato ethane
Dimethyltrisulfide
1,4-dimethyltetrasulfide
Sulfur, mol.
Cholesterol
Dihydrocholesterol
22-dehydrocholesterol
Crinosterol
.delta.5-ergostenol
Stigmasterol
Clionasterol
7-dehydrostigmasterol
26,27-dinorergosta-5,22-dien-3-ol (3.beta., 22Z)
Atrazine
Carbamazepine

Petroleum hydrocarbons

Aliphatic, alicyclic and aromatic hydrocarbons belong to the most frequent pollutants in the Danube River. Small part of occurring hydrocarbons is of natural origin coming from biological sources such as land plants, animals and algae. Certain hydrocarbons are produced from bacterial and chemical degradation of naturally occurring lipids. However, the major hydrocarbon load to the Danube River is coming from direct petroleum inputs (mostly from the navigation) and from combustion of fuels. The Danube River is also considered as the major source of oil input to the Black Sea [2]. Petroleum hydrocarbons are regularly measured in the Danube River water and they are detected/quantified at many profiles within the TNMN monitoring [3]. Therefore, positive responses for petroleum hydrocarbons might have been expected in the results from the JDS. However, here it must be stressed that the result of the total analysis of petroleum hydrocarbons represent the sum of all hydrocarbons absorbing light at a certain wavelength. This sum is created by tens to hundreds of different compounds. Using a GC-MS technique, each compound provides a separate signal, which is usually one or more magnitudes lower that the summary signal. This means that the GC-MS analysis can detect only elevated concentrations of hydrocarbons otherwise its sensitivity is not sufficient. This was also the case in the JDS results, in which, except of the ubiquitous squalene, the aliphatic and aromatic hydrocarbons were found scarcely in the Danube mainstream (they were found only at 8 sampling points). Larger diversity of hydrocarbons was observed in the tributaries due to their lower flow. Five or more hydrocarbons were identified in the Morava, Mosony, Ipel, Sio and Arges. A specific situation was found in Rusinsky Lom, which contained a wide variety of hydrocarbons at elevated concentrations. Remarkable is the extreme value of relative abundancy of the 1,3-dimethylbenzene in this tributary.

Volatile chlorinated aliphatic hydrocarbons

The major sources of volatile chlorohydrocarbons are industrial effluents. Only two chlorinated volatiles were identified in the JDS samples. The analytical results, relative abundance of the compounds, are shown in Figure C2.4.8.-1.

1,1,2,2-tetrachloroethane was found in more than 80% of the samples having slightly elevated profile in the middle Reach of the Danube River and in some of its tributaries. Traces of tetrachloroethene were identified mostly in between the rkm 1659 and 1161 and in the Rusinsky Lom and Arges.

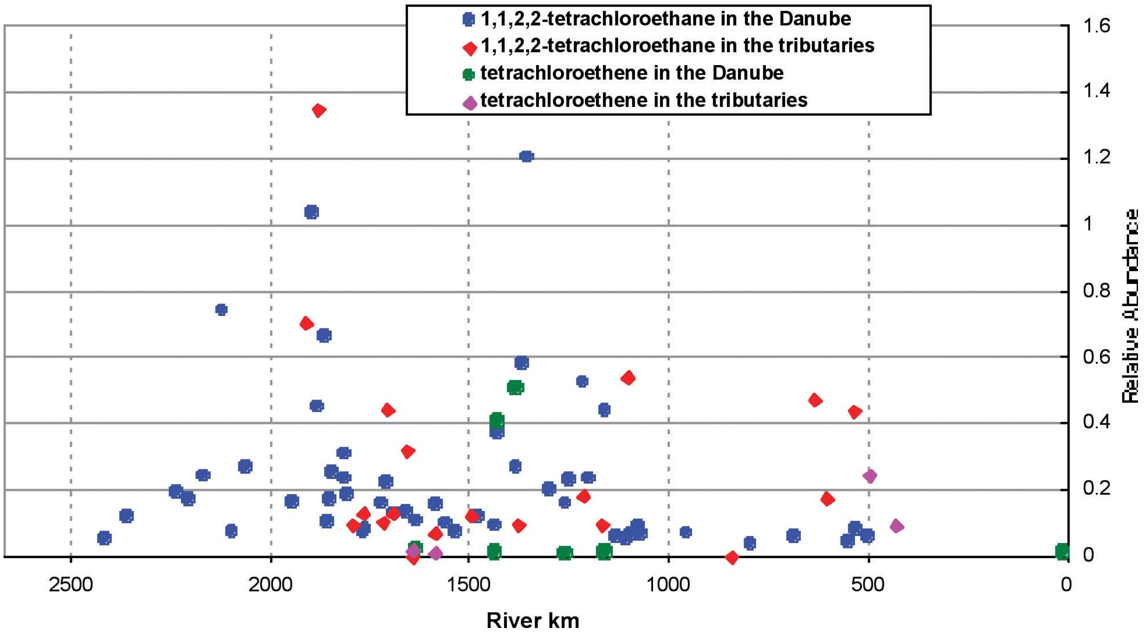


FIGURE 2.4.8.-1: Relative abundance of 1,1,2,2,-tertachloroethane and tertrachloroethene in the water samples along the Danube and in the tributaries during the JDS

Fatty acids

Fatty acids are products of oxidation of linear aliphatic hydrocarbons. Their presence in riverine environment is to a lower extent result of natural processes, but the major part of fatty acids results from degradation of petroleum hydrocarbons and animal and vegetable fats. Important sources of these compounds are municipal discharges. In wastewater treatment technologies the fatty acids are often used for the characterization of the efficiency of the treatment process. Their presence in stream indicates intensive anthropogenic activities in the region.

Saturated and unsaturated C12 – C18 fatty acids were found throughout the whole Danube mainstream. Elevated abundance profiles were observed between rkm 1650 and 500, gradually increasing downstream of Budapest (one of the major municipal waste discharger), as shown in Figure C2.4.8.-2. No fatty acids were found in the Danube delta. Among the tributaries the Morava, Drava, Velika Morava and Rusinsky Lom were found to contain most of the n-alkanoic and alkenoic acids.

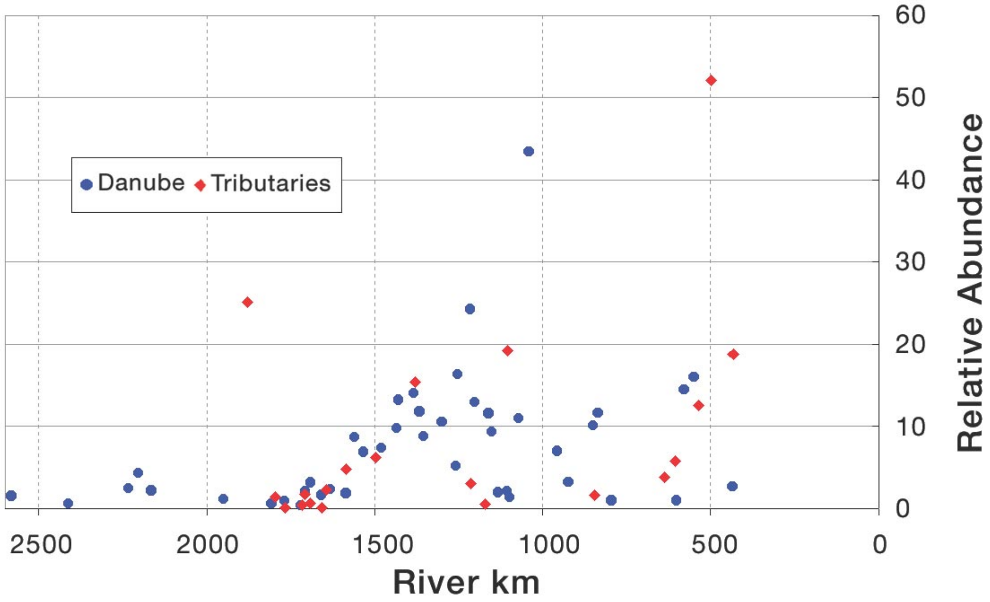


FIGURE 2.4.8.-2: Relative abundance of the fatty acids in the water samples along the Danube and in the tributaries during the JDS

Phthalates

The esters of 1,2-benzenedicarboxylic acid are frequently used in different branches of industry (plasticizers of polymeric materials, insect repellents, industrial and lubricating oils, defoaming agents, cosmetics, etc.) and hence they are contained in many common products of a daily use. As a result of their wide-spread application phthalates are usually found in most of the water samples during screening campaigns. Similar situation was observed in the Danube River and its tributaries, as it is demonstrated in Figure C2.4.8.-3.

Phthalates were identified in all, but three, JDS water samples. The highest total abundancies of phthalates were observed in the main stream and tributaries in the middle Danube Reach, particularly between rkm 2120 and 1481 and in the tributaries Arges and Rusinsky Lom. Similar occurrence pattern can be seen also for the diethylhexylphthalate, one of the EU WFD priority substances.

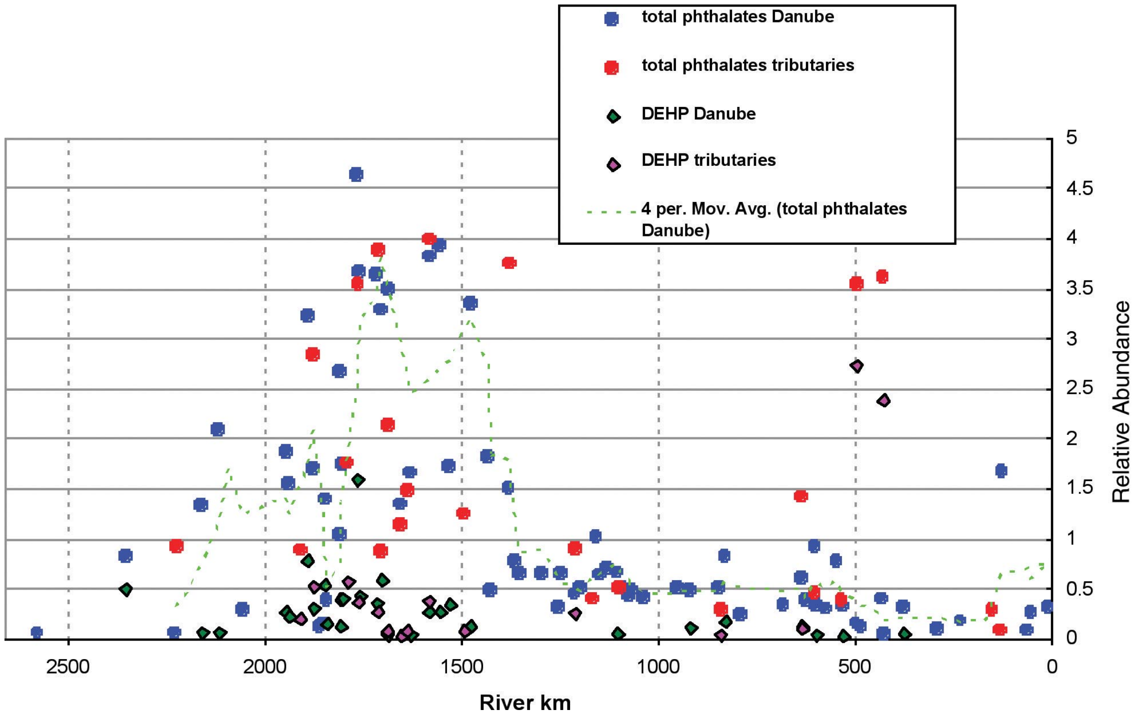


FIGURE 2.4.8.-3: Relative abundance of phthalates in the water samples along the Danube and in the tributaries during the JDS

Hydroxylated and ketonic compounds

The major sources of hydroxy- and keto- aliphates and aromates in surface waters are industrial discharges and the in-stream oxidation of aliphatic and aromatic hydrocarbons. Most of the ketones and alcohols are not very persistent in the aquatic environment, they biodegrade slowly and evaporate to the ambient air (2-butanone evaporation half-life in rivers and lakes is estimated to 3-12 days). Lower ketones and alcohols are not expected to bioconcentrate in aquatic organisms and their adsorption to solid particles is not a significant loss process. Based on these properties their occurrence in the JDS samples was only occasional. The most frequent occurrence was observed for 3,3-dimethoxy-2-butanone in the whole Reach of the Danube River at a random pattern. Noticeable phenomenon is the presence of 2-butanone (methyl ethyl ketone) in the Soroksar arm of the Danube River. 2-butanone is a frequently used solvent in the surface coating industry and in production of cosmetics, pharmaceuticals and paint removers, etc. In the Budapest area also 3,4 dimethoxyphenylacetone was identified.

Phenols

Similarly as for the previous group of oxo-compounds, the major source of phenols (monohydroxybenzenes) are industrial discharges, however, phenolic compounds can be formed also by oxidation processes. Phenols were identified mostly in the middle Reach of the Danube River including its tributaries and then in the lower Reach tributaries - the Arges and Rusinsky Lom, as shown in Figure C2.4.8.-4.

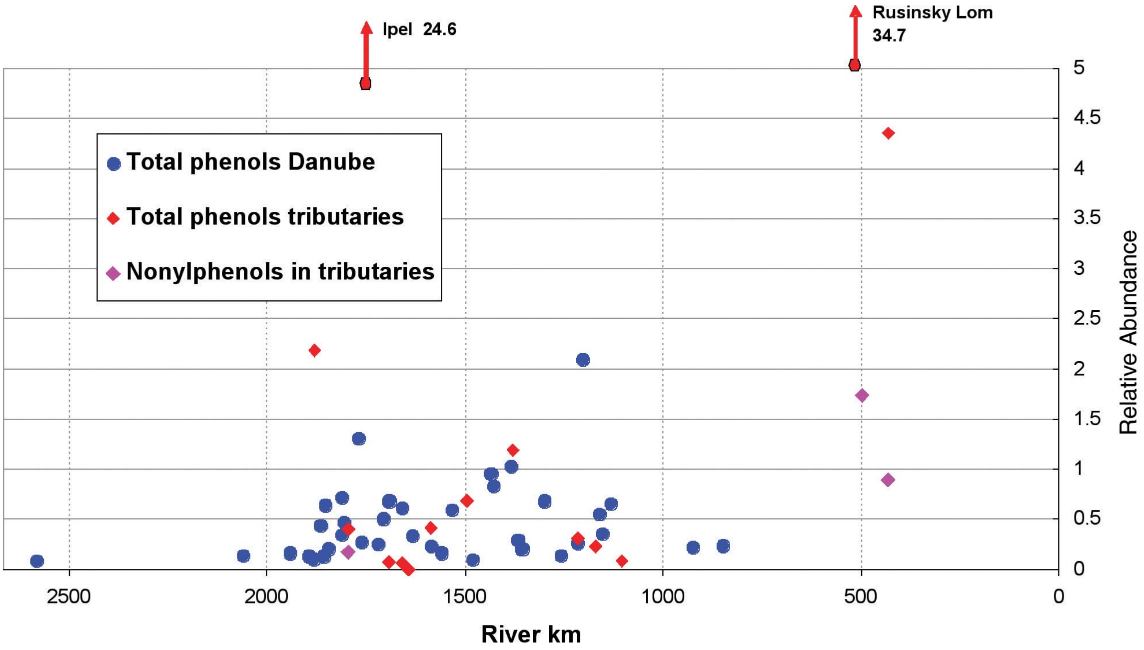
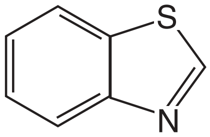


FIGURE 2.4.8.-4: Relative abundance of phenols in the water samples along the Danube and in the tributaries during the JDS

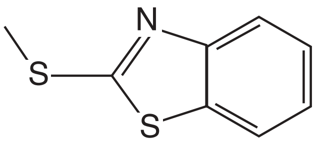
Most frequently observed compound was 4-(1,1-dimethylethyl)phenol. This phenol derivative is used in manufacturing of bactericidal detergents. Noticeable are also elevated abundancies of 3,5-dimethylphenol in the Rusinsky Lom and 2,4-bis(1,1-dimethylethyl)phenol in the Ipel. Elevated abundancies of nonylphenols were found in the Rusinsky Lom and Arges. This phenomenon closely corresponds with the results of the target analysis of the JDS sediments.

Benzothiazoles

The occurrence of benzothiazole derivatives in aqueous environment is due to their use as rubber accelerators in the tyre industry. An intensive car/truck transport results in the transfer of benzothiazoles via the rubber dust to the hydrosphere. The increased abundance profiles of benzothiazole and 2-(methylthio)benzothiazole



benzothiazole



2-(methylthio)benzothiazole

were observed in the middle Reach of the Danube including its tributaries, as demonstrated in Figure C2.4.8.-5.

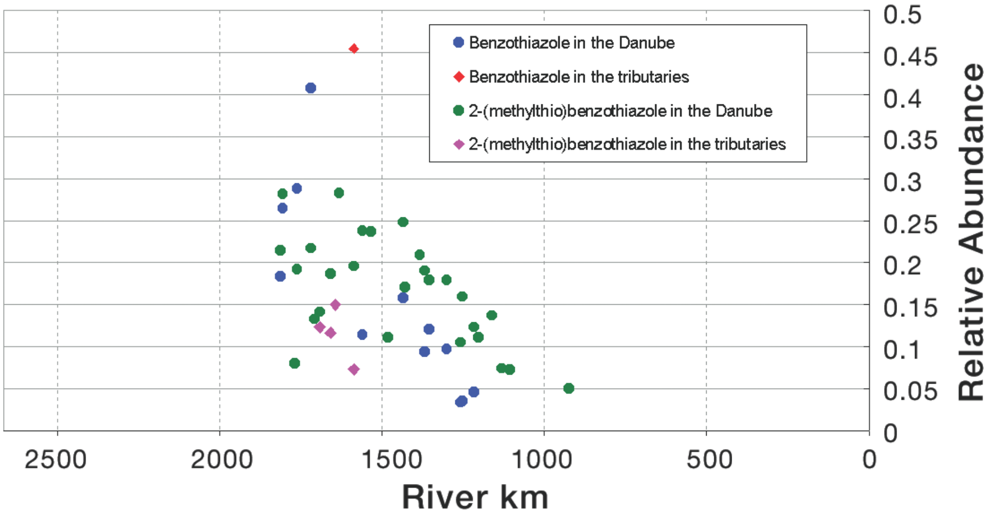
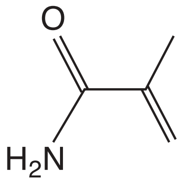


FIGURE 2.4.8.-5: Relative abundance of benzothiazoles in the water samples along the Danube and in the tributaries during the JDS

Sulphur and nitrogen-containing compounds

Amides, diaza-compounds, organophosphates and organosulfides were randomly identified in the analysed samples. The most frequently found compound was methacrylamide. Methacrylamide



is used in the process of the manufacturing of methyl methacrylate, however, its occurrence in aqueous environment might be also derived from acrylamide. Polyacrylamides are used in the water treatment industry (flocculants in sewage and waste treatment), pulp and paper industry and mineral processing.

Two alkylamides were found in Arges. Noticeable is also the occurrence of the diaza-compounds in the Danube mainstream, rkm 924.

Sterols

The presence of sterols in the water is often used as an indicator of sewage pollution. In the JDS samples several sterol derivatives were identified having the highest abundance in the middle Reach of the Danube (Figure C2.4.8.-6). The abundancy profile of total sterols roughly corresponds to the concentration profile of coprostanol in sediments as found by the Cousteau Survey [4].

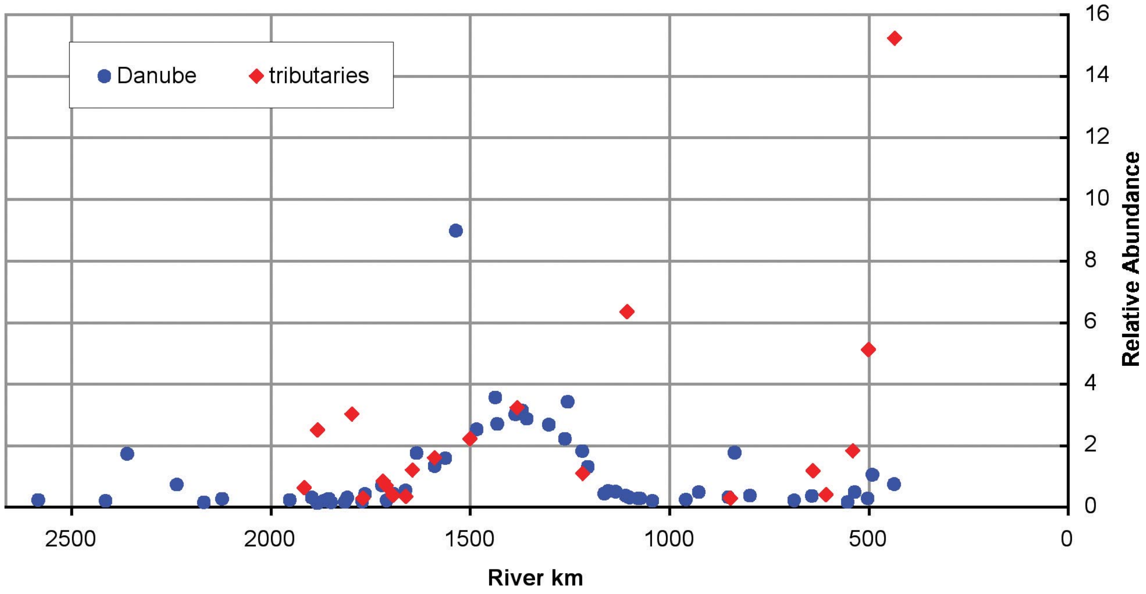


FIGURE 2.4.8.-6: Relative abundance of sterols in the water samples along the Danube and in the tributaries during the JDS

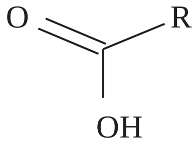
Pesticides

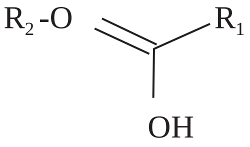
Owing to the limited sensitivity of the GC-MS and to the fact that application period for most of the pesticides finished before the JDS, only atrazine and carbamazepine were identified in the survey samples. Atrazine was found in the Sio and Sava and in the Danube Reaches downstream of the confluences of these two tributaries. This pattern corresponds perfectly to the results of the target analysis of atrazine in JDS water samples. Traces of carbamazepine were identified in the Sio.

5.2.4.8.2 GC/MS Screening of Sediment and Suspended Solids Samples

Over 150 organic compounds were detected in the sediment and suspended solids samples. The following groups of compounds were identified:

- n-Alkanes from C15 – C31 and branched alkanes;
- Alkyl benzenes with alkyl chain containing 12 carbons (benzene, pentylheptyl-);
- Alkyl cyclohexanes with alkyl chain containing 7-10 carbon atoms;
- Saturated and unsaturated carboxylic acids mostly with even number of carbon atoms (from dodecanoic (C12) to octadecanoic (C18)), and their esters with alkyl chain containing 3 to 16 carbon atoms;

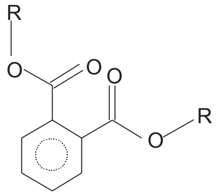




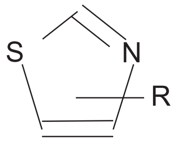
alkyl R = C11 to C17;

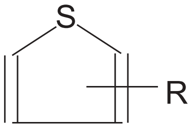
R1 = C11 to C17 and R2 = C3 to C16

- PAHs and alkyl PAHs containing 4 – 6 benzene rings;
- 1,2-Dihydronaphthalene isomers with one, two and three methyl substituents;
- Phthalate esters with alkyl chain R = C1 (methyl) to C8 (ethylhexyl);



- Sulphur (S6, S7, S8) and sulphur-containing compounds (alkyl thiazole, alkyl thiophene);





alkyl R = C1 – methyl; C4 – methylpropyl, C16...

- Steroid compounds containing 1,2-cyclopentaphenanthrene base with different substituents (-OH e.g., cholest-5-en-3-ol; =O e.g., cholestan-3-one; alkyl chain) and various degree of ring and alkyl substituent unsaturation (e.g. cholest-3-ene, ergosta-5,22-dien-3-ol, 19-norcholesta-1,3,5(10)-trien-6-one). These compounds are not easily identified as they elute at the end of chromatographic run, under isocratic conditions (constant oven temperature 300 C), which results in a great degree of overlapping.

References

1. ***J. Makovinská, F. László, Tendency and Dynamics of Water Quality Changes of the Danube River and its Tributaries (1989 – 1995),*** Práce a štúdie No. 134, Water Research Institute, Bratislava, 1997, p. 20

2. ***Black Sea Pollution Assessment (L.D. Mee, G. Topping, Eds),*** United Nations Publications, New York, 1998

3. ***Water Quality in the Danube River Basin 1998 (TNMN Yearbook),*** ICPDR Vienna, 2002

4. ***The Danube... For whom and for what?*** Final Report, Equipe Cousteau, Paris 1993.