5 CHEMICAL STATUS CHARACTERIZATION

According to the EU Water Framework Directive (WFD), characterization of the chemical status of surface waters (river basins) requires the monitoring of general characteristics, nutrients, and specific pollutants. These characteristics were selected as JDS target determinands.

Concerning the specific pollutants, the target determinands were selected:

(a) from the list of 33 single compounds in Annex X of the EU WFD from different chemical groups, such as
- 4 heavy metals (in Section on Heavy Metals),
- 5 VOCs (in Section on Organic Pollutants: VOCs)
- 9 polar pesticides (in Section on Organic Pollutants: Polar Pesticides),
- 4 OCP´s (in Section on Organic Pollutants: Organochlorine Compounds),
- 4 polyaromatic hydrocarbons (including 1 group of 5 individual substances; in Section on Organic Pollutants: Petroleum Hydrocarbons),
- 7 substances or groups of substances from different origin (in Section on Organic Pollutants: Other WFD Priority Pollutants);

(b) from the TNMN determinand list, such as additional heavy metals, etc.;

(c) from pharmaceuticals as pollutants with recent concerns in water quality assessment.

5.1 METHODS

5.1.1 Sample Collection

Collection of water samples

Water samples were collected with the built-in pump of the Argus laboratory ship, with the exception of some tributaries that were not accessible by ship. In those cases water samples were collected with a bucket from the small boat used for the collection of biological samples.

Water samples were stored in appropriate glass or plastic containers. For the analysis of the dissolved form of compounds the samples were filtered through 0.45 µm pore-size membranes.

On-board analysis of nutrients, nitrogen-forms and orthophosphate-P was carried out from the membrane filtered water. Original and filtered samples were collected and preserved for the analysis of dissolved and total metals.

Collection of suspended solid and bottom sediment samples

Sampling of suspended solids

-Suspended solids were sampled with a continuous flow centrifuge (Carl Padberg, Type 61, 17 000 rpm) mounted on board the Argus ship equipped with an electronic pump (Gardena 6000/4). The pump flow of 35 l/min was regularly checked.

To avoid collection of resuspended sediments, centrifugation was either carried out mostly between stations or it was stopped during the sampling of zoobenthos and sediments on the

river banks. Depending on the expected amount of suspended solids (as estimated by transparency of the water column and chlorophyll determination), centrifugation time varied between 20 min and 6:55 hours. The collected suspended matter was transferred into a 500 ml PP beaker. Suspended solids were stored frozen (-18°C) until they were transported to VITUKI, Budapest.

Sampling of bottom sediment

Sediments were sampled at every station on the left and right river banks. They were taken either by the grab sampler of the Argus ship or manually dredged at the same location where mussel samples were taken. In tributaries, only one location was sampled or sediments of both sites of the tributary were combined before further treatment.

Sediments were filled into PVC buckets and thoroughly homogenised. A part of the sediment was taken as the total sample. The rest was mixed with distilled water (at an approximate ratio of 3:2 v/v). The sediment/water mixture was put on top of a set of sieves with different mesh sizes (5 mm, 1 mm, 224 µm, 63 µm) for separation of the below-63-µm fraction. Wet sieving was carried out on a sieving machine (Retsch AS 200 control "g") set between 0.5 and 1.5 mm amplitude and 10 s interval sieving. Approximately 1,5 l of the wet fraction < 63 µm was collected in a 2 L PE-bottle and filled into appropriate sample storage containers (Table C1.1.1). The remaining residue on the 63 µm and 224 µm sieves was washed with about 2 litres of distilled water using the same adjustments of the Retsch machine. The fractions between 63 - 224 µm and 224 - 1000 µm were collected. Every container was labeled with JDS position number, a capital letter (L - left bank, R - right bank or MIX - tributaries), the sampling date, the location and the sample type (Table C1.1.1). Additional total sediment was sampled at selected locations in 500 ml glass bottles for the analysis of polybrominated diphenylethers.

At Gabcikovo Reservoir (station JDS20), Rackeve-Soroksar Danube Arm (station JDS36) and Iron Gate Reservoir (stations JDS63-L and JDS63-R) sediment core samples were taken using a PE tube. 10 cm segments of the sampled cores were immediately frozen in plastic containers. The overall summary of the samples and sample containers is given in Table C1.1.-1. Sediment samples were stored frozen (-18°C, plastic containers) or refrigerated (5°C, glass bottles) until they were transported to VITUKI, Budapest, which usually happened every week.

At stations JDS18-L and JDS86-R two samples were independently taken to test the sampling and sieving procedure for contamination and to get an idea of the spatial heterogeneity of heavy metal concentrations in the investigated sediments.

TABLE C1.1.-1: Samples and storage containers of JDS sediment samples

Sediment Sample	Storage Container	Intended Use
Total Sediment	1 L HDPE wide mouth box	grain-size analysis
Sediment fraction < 63 µm	1 L HDPE wide mouth box	heavy metal analysis, sample bank
Sediment fraction < 63 µm	500 ml glass bottle	analysis of organic contaminants
Sediment fraction 63 – 200 µm	250 mL PVC beaker	sample bank
Sediment fraction 200 – 1000 µm	250 mL PVC beaker	sample bank
Sediment core (10 cm segments)	500 mL PVC beaker	heavy metal analysis, sample bank

For most of the investigated metals, the concentrations are comparable in the parallel samples (Table C1.1.-2). Even for Zinc as the most sensitive element to contamination, there are no significant differences in its concentration in samples JDS18-L and JDS18-LB or JDS86-R A and JDS86-R B, respectively. However, the different Cu- and Cd-concentrations at position JDS86-R indicate an uneven distribution of these metals in the Danube sediments.

TABLE C1.1.-2: Heavy metal concentrations in parallel samples taken at the same location

Sample River km	JDS18-L 1856	JDS18-LB 1856	JDS86-R A 434	JDS86-R B 434
Zinc [µg/g]	124	118	250	222
Aluminium [µg/g]	23400	22600	42800	38500
Iron [µg/g]	23500	25300	36800	33900
Manganese [µg/g]	608	703	1032	825
Arsenic [µg/g]	11.5	12.4	26.9	23.7
Cadmium [µg/g]	0.9	0.6	2.5	8.4
Chromium [µg/g]	47.7	45.6	89.7	96.6
Copper [µg/g]	39.0	39.2	188	131
Lead [µg/g]	25.3	23.4	76.9	77.2
Mercury [µg/g]	0.124	< 0.1	0.369	0.432
Nickel [µg/g]	34.3	35.3	70.6	64.4

Collection of mussel samples

The different species of mussels were collected as part of macrozoobenthos sampling either with the polyp grab or manually. The samples were frozen and transported to the laboratory (VITUKI Plc.) where the flesh of the mussels was separated and freeze-dried. The freeze-dried, grinded and homogenized samples were stored in refrigerators until they were analysed. Aliquots of the samples were used for the analysis of heavy metals and organic micropollutants, i.e. PAHs and chlorinated hydrocarbons.

5.1.2 Analysis of General Characteristics

Determinands and methods

The general characteristics of water and sediment, e.g., temperature, pH, conductivity, alkalinity and grain-size distribution were determined according to international standards as were the relevant methods selected for use during the Danube basin TNMN.

Analytical quality control

Calibration was performed according to the procedure described in the relevant standard.

5.1.3 Analysis of Nutrients

Determinands and methods

Biological growth of aquatic plants requires the presence of chemicals. The necessary elements include carbon, oxygen, hydrogen, nitrogen, phosphorus, sulphur, silica and other essential elements, which must be present at least in trace amounts.

JDS included those macroelements (nitrogen, phosphorus, silica) that can be a "limiting nutrient" in aquatic systems.

JDS studies covered the nitrogen species ammonium, nitrite, nitrate, organic nitrogen, phosphorus form orto-phosphate and total phosphorus, and silica in the form of dissolved silicate. Some of the above species are typical in dissolved form and others in solid forms. Ammonium, nitrite, nitrate, ortho-phosphate, total phosphorus and dissolved silicate were measured in the water samples. Suspended solids and sediment samples were analysed for organic nitrogen and total phosphorus.

Ammonium, nitrite, nitrate, and orto-phosphate components were analysed on board and other nutrient forms in the laboratory by using the following analytical methods:

N–NH_4: ISO 7150/1: Determination of ammonium – spectrometric method;
N–NO_2: Standard Methods for the Examination of Water and Wastewater – sixteenth edition, Method no. 419;
N–NO_3: ISO 7890-3 Part 3: Spectrometric method using sulphosalycilic acid;
P–PO_4: Standard Methods for the Examination of Water and Wastewater – Sixteenth edition, Method no. 424 F, ascorbic acid method;
Total (Organic) nitrogen: application of Kjeldahl method ammonium analysis by spectrophotometric method;
Total phosphorus: persulphate digestion, molibdate color reaction, photometric analysis;
Dissolved silicate: spectrophotometric method, color measured at 670 nm.

Analysis carried out at ARGE:

Determinand	Unit	Method Used	Method	Limit of Quantification [LOQ]
Organic nitrogen: Total Kjeldahl nitrogen of the sediment	mg/kg	Titrimetric	Equivalent to EN 25663	10,0
Ammonia nitrogen, as eluate	mg/kg	Photometric	Equivalent to DIN 38406-E5-1, EN ISO 11732	1,0
Nitrate nitrogen, as eluate	mg/kg	Photometric	Equivalent to EN 26777, EN 12014-7	1,0
Total phosphorus	mg/kg	ICAP	Digestion, Equivalent to DIN 38414, Teil 7, analogue EPA, 200.15	10,0

Analytical Quality Control

During on-board analyses, a control sample was analysed for each parameter at the beginning of each working day.

5.1.4 Analysis of Heavy Metals

Determinands and methods

Determination of heavy metals in water

Surface water samples, filtered and original, were analysed at ARGE according to the following procedures:

Determinand	Unit	Method Used	Method	Limit of Quantification [LOQ]
Aluminium (Al)	µg/l	ICAP	Equivalent to EPA 200.15	10
Arsenic (As)	µg/l	ICAP	DIN EN ISO 11969	1,0
Cadmium (Cd)	µg/l	ICAP	Equivalent to EPA 200.15	0,2
Chromium (Cr) - total	µg/l	ICAP	Equivalent to EPA 200.15	1,0
Copper (Cu)	µg/l	ICAP	Equivalent to EPA 200.15	1,0
Lead (Pb)	µg/l	ICAP	Equivalent to EPA 200.15	1,0
Mercury (Hg)	µg/l	AAS	Equivalent to DIN 38406-E12	0,2
Nickel (Ni)	µg/l	ICAP	Equivalent to EPA 200.15	1,0
Zinc (Zn)	µg/l	ICAP	Equivalent to EPA 200.15	1,0

Determination of heavy metals in suspended solids and bottom sediments

Homogenised and freeze-dried samples of sediment (fraction < 63 µm), sediment cores and suspended solids were provided in amber glass bottles. Samples were stored at room temperature until they were analysed.

Extraction of heavy metals followed the ASTM D5258 protocol [1]. Essentialy, 0.500 or 1.000 g of dry sediment were weighed into PTFE-insets of microwave digestion vessels. Microwave-assisted heavy metal extraction was carried out with a mixture of conc. nitric acid/water (3:2 v/v, 12.5 or 25 ml) in a CEM MSP 1000 unit at 160°C for 25 min. The solution was brought to 50 ml/100 ml in a glass flask and filtered through paper filters (Schleicher&Schüll Folded Filters, S&S 595 $^{1}/_{2}$) into PE bottles.

Determination of aluminium, cadmium, chromium, copper, iron, lead, nickel, manganese and zinc in the extracts was carried out by ICP-AES (Perkin Elmer OPTIMA 2000 DV) according to ISO 11885 [2].

Arsenic and mercury were determined by flow injection-graphite furnace-AAS using the hydride/cold vapor principle (Perkin Elmer 4100 ZL with FIAS 200) [3,4].

Analytical quality control

To control accuracy and to determine the uncertainty of the heavy metal determination, every extraction batch of 12 samples included a blank extraction and reference material (RM-3, Qualco Danube Sediment Sample or NIST SRM 2704, Buffalo River Sediment). Table C1.4.-1 summarises the analytical results of the quality control samples.

The data in Table C1.4.-1 indicate satisfactory performance of the heavy metal determinations in the sediments and suspended solids of the Danube. A blank correction was carried out for cad-

mium only in all sediment and suspended solids samples. No significant differences were detected between determined and certified concentrations of the reference materials for most of the investigated heavy metals. The lower-than-certified concentrations of aluminium, chromium, iron and manganese in the NIST SRM 2704 Buffalo River Sediment can be attributed to the partial extraction procedure, because this material is certified for a total digestion of sediments. Uncertainties between 5% and 15% for the determined element concentrations are sufficient to detect the expected variation of heavy metals along the Danube River. The method quantification limits are in the range of or above the published element background concentrations and allow the control of heavy metal quality targets for river sediments and suspended solids.

Metals	MQL** [µg/g]	Blank Values [µg/g]***	RM-3, Qualco Danube Sediment [µg/g]		NIST SRM 2704 Buffalo River Sediment [µg/g]	
			Analysed	Assigned	Analysed	Certified
Aluminium	4	2 ± 2	19100 ± 2300	?	30700 ± 4800	61100 ± 1600
Arsenic	1.0	0.59 ± 0.41	10.4 ± 1.3	?	24.8 ± 3.8	23.4 ± 0.8
Cadmium	1.1	0.5 ± 0.6	(0.4 ± 0.6)	0.281	3.5 ± 0.4	3.45 ± 0.22
Chromium	0.3	0.1 ± 0.2	34.9 ± 4.0	32.60	98.7 ± 4.8	135 ± 5
Copper	1.5	0.9 ± 0.6	21.9 ± 1.6	18.97	95.2 ± 3.6	98.6 ± 5.0
Iron	3	1 ± 2	19800 ± 2000	?	34000 ± 3900	41100 ± 1000
Lead	1.1	0.5 ± 0.6	11.9 ± 1.6	9.83	154 ± 22	161 ± 17
Manganese	0.7	0.3 ± 0.4	439 ± 44	?	502 ± 30	555 ± 19
Mercury	0.1	0.016 ± 0.06	(0.034 ± 0.080)	0.064	1.27 ± 0.18	1.47 ± 0.07
Nickel	0.5	0.3 ± 0.2	35.4 ± 3.0	31.68	39.7 ± 2.6	44.1 ± 3.0
Zinc	4.7	2.1 ± 2.6	59.9 ± 6.0	55.0	410 ± 26	438 ± 12

TABLE C1.4.-1: Heavy metal concentrations (overall means ± uncertainty*) in extraction blanks and reference materials analysed together with the Danube sediment and suspended solids samples

* The uncertainty is calculated using control chart data of heavy metal determinations in reference materials. The value is twice the standard deviation of the overall mean.

** MQL (method quantification limit) - The MQL is the heavy metal concentration in sediments and suspended solids that can be determined quantitatively with the applied analytical procedure (MQL = blank value + uncertainty of blank)

*** Blank Values are procedural blanks, transformed from measurements of the blank extract into sediment concentrations

To confirm extreme values of metal concentrations, selected sediment samples were subjected to an additional analysis. Fig. C1.4.-1 provides a comparison of the obtained data. The regression line of extraction B = f(extraction A) indicates that there are no significant differences between the two extractions and ensures the accuracy of even very high element concentrations in the Danube sediments. The outlying cadmium value of one sample can only be explained by inhomogeneities and is supported by similar findings in parallel sampling (Table C1.1.-2).

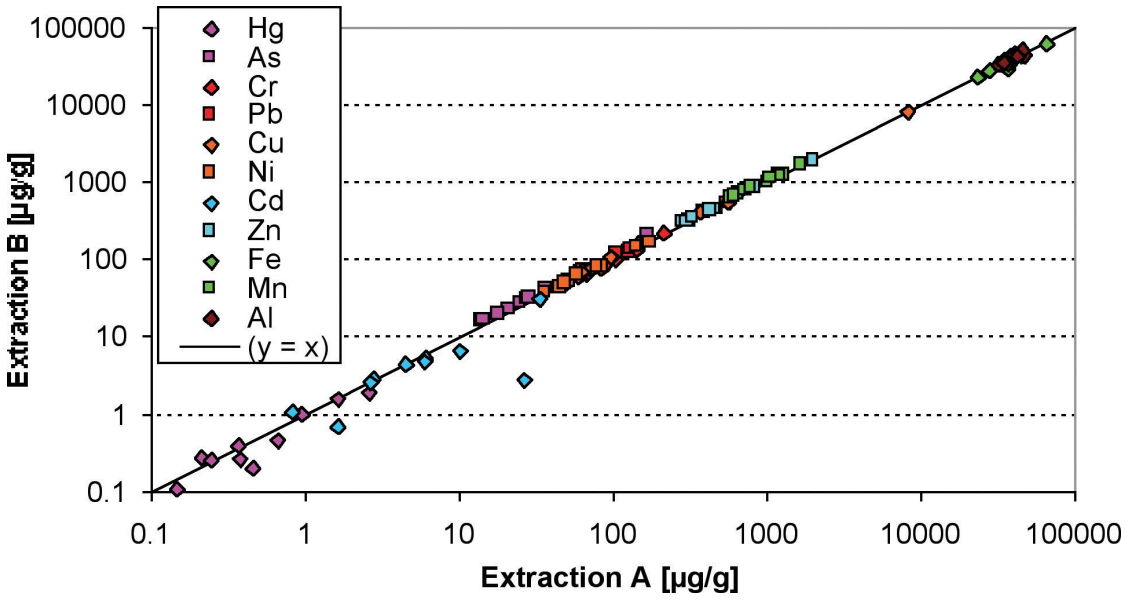

FIGURE C1.4.-1: Comparison of heavy metal concentrations in 10 selected Danube sediment samples extracted twice (A and B) to confirm high element values

References

[1] ASTM D 5258 – 92 (Reapproved 1996): Standard practice for Acid-Extraction of Elements from Sediments Using Closed Vessel Microwave Heating.

[2] ISO 11885 (1996): Water quality - Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy.

[3] Willie S.N.: First order speciation of As using flow injection hydride generation atomic absorption spectrometry with in-situ trapping of the arsine in a graphite furnace. Spectrochim. Acta, Part B (1996) 1781-1790.

[4] Bermejo-Barrera P., Moreda-Piñeiro J., Moreda-Pieiro A., Bermejo-Barrera A.: Use of Flow Injection Cold Vapour Generation and Preconcentration on Coated Graphite Tubes for the Determination of Mercury in Polluted Seawaters by Electrothermal Atomic Absorption. Spectrometry. J. Anal. At. Spectrom. (1997) 317-321.

5.1.5 Analysis of Organic Pollutants

Additional details on the determination of particular organic micropollutants are included in Annex 1 to the chapter on chemical characterisation (CD-ROM).

Aggregate variables

Determination of UV absorbance and humic substances in water

The measurement of UV absorbance at 254 nm wavelength is a simple method that has for a long time been used to estimate dissolved organic matter in water. This method is also applied in automatic water quality monitoring stations and one of the parameters used in several river water quality monitoring, including the Rhein River.

The determination of both UV absorbance and humic substances is very simple; the procedure is summarized in Figure C1.5.-1.

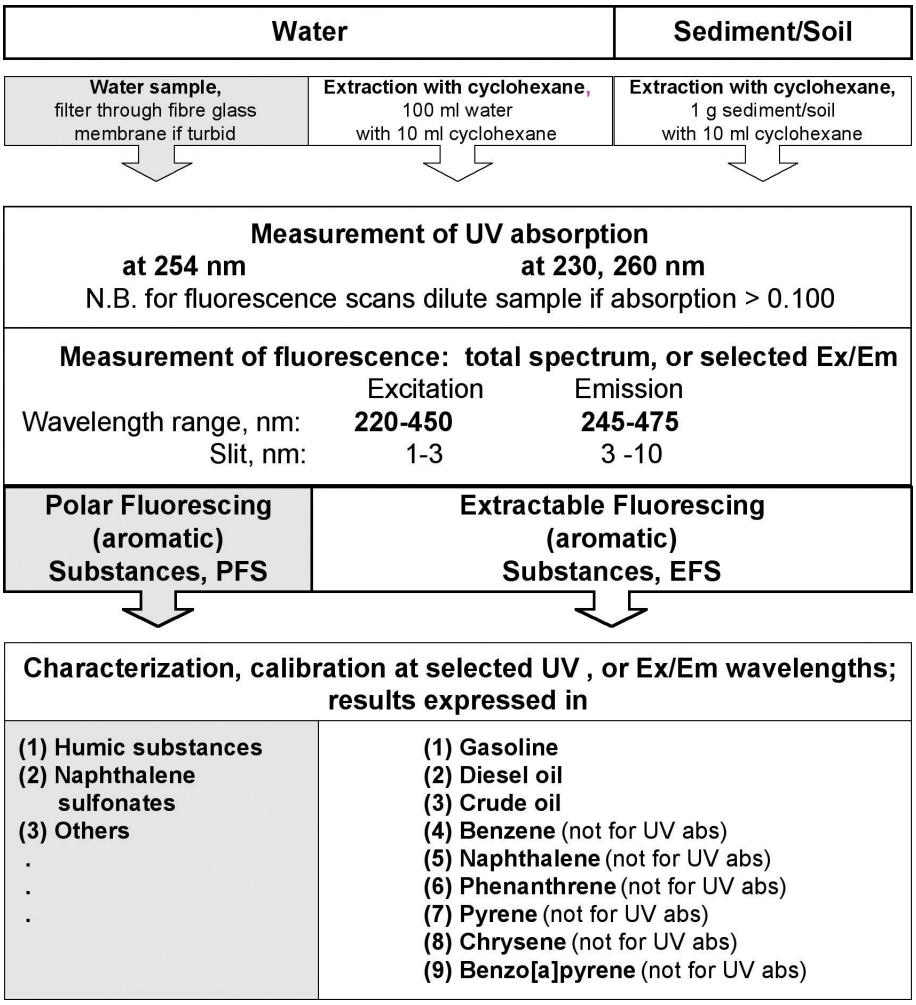

FIGURE C1.5.-1: Analytical scheme of the determination of polar and non-polar organic compounds in water and sediment/soil.

UV absorbance of the filtered water sample was measured in a Cary UV-Vis spectrophotometer at 254 nm in a 1 cm quartz cell.

Total fluorescence spectra were recorded on original water samples as well as on organic solvent extracts of original water and sediments. The procedure has been described in detail elsewhere (Literathy, 2000). A Hitachi F-4500 fluorescence spectrophotometer was used to record total fluorescence spectra in the 220-450 nm excitation wavelength and 245-475 nm emission wavelength range. The excitation slit was at 2.5 nm and the emission slit at 5.0 nm. Scanning was with 5 nm steps with a speed of 12,000 nm/s.

The characteristic total fluorescence spectra of the humic substances are shown in the results of JDS demonstrating polar and non-polar organic pollution in water samples (Fig. C2.4.2.1).

Determination of TOC and TEM in suspended solids and bottom sediment

Samples analysed at ARGE:

Determinand	Unit	Method used	Method used	Limit of quantification [LOQ]
Total Organic Carbon	mg/kg	IR	Equivalent to DIN 38409 Teil 3, ÖN EN13137	100
Total Extractable Matter	mg/kg	Gravimetric	Equivalent to EPA 1664 A	20

Petroleum hydrocarbons

The complexity of the mixture of compounds found in petroleum (crude oils) and most of its refined products is a major challenge to the environmental analysts. Even in the case of a known oil spill into the environment, a rapid loss of volatile fractions, dissolution, dispersion and adsorption onto the particular matter in the water, biodegradation and transformation, as well as photo-oxidation processes start immediately to alter the composition. Complexity of sources, processes and fates must be considered in order to chose the analytical approach for quality/pollution monitoring purposes. There is no single analytical method that can detect and quantify all petroleum components or petroleum-related pollution. The selection of a particular analytical method is always a compromise between the feasibility of the analysis, e.g., instrumentation and available resources, and the degree of chemical detail, selectivity, sensitivity and accuracy. The analytical approaches include determination of: (1) a group of compounds, such as measurement of absorbance due to the C-H streching of aliphatic compounds in the infrared region, or measurement of absorbance or fluorescence of aromatic compounds in the ultraviolet region, or (2) single compounds such as n-alkanes, isoprenoid alkanes, BTEX, PAHs, or benzothiophenes with gas or liquid-chromatography.

Determinands and methods

Petroleum hydrocarbons were analysed during JDS by using different analytical methods, including GC-FID for total petroleum hydrocarbons (TPH), TPH was determined by using UV absorption and fluorescence procedures, as well as GC/MS analysis of Polycyclic Aromatic Hydrocarbons (PAHs).

Determination of TPH by GC-FID

Petroleum hydrocarbons analysed at ARGE:

Determinand	Unit	Method Used	Method	Limit of Quantification [LOQ]
Petroleum hydrocarbons	mg/kg	GC	Equivalent to ÖNORM EN ISO 9377-4	5,0

Determination of TPH by spectrophotometry

Concerning spectrophotometric measurements, the estimation of hydrocarbon contamination in the aquatic environment was conducted using ultraviolet (UV) absorption, or fluorescence spectrophotometry of organic solvent extractable substances recovered from water, suspended solids and bottom sediment samples. An overview of the analytical procedure was given earlier in Fig. C1.5.-1.

Since spectrophotometric determination depends on the organic solvent and the calibration standard used, it is important to specify them.

Both spectrophotometric methods signal the aromatic components of petroleum and its products, but aromatic compounds from another origin that are co-extracted under specified conditions may contribute to the absorption of ultraviolet light, or to the fluorescence intensity.

Cyclohexdane, or n-hexane are the preferred organic solvents for recovering extractable matter from samples.

In the case of the water samples, 10-20 parts of water should be extracted with one part of solvent in a separatory funnel. The solvent extract is dried with anhydrous sodium-sulphate, followed by the measurement of the UV absorption, or fluorescence at the wavelengths as given in Figure C1.5.-1. Suspended solids and sediment samples should preferably be extracted by sonication (0.5-1 g sample, 0.5-1 g anhydrous sodium-sulphate and 10 ml solvent).

Figure C1.5.-2 demonstrates the fluorescence spectra of oil products and selected PAHs, which can be used for the calibration and quantification of oil contamination in solvent (cyclohexane) extracts of environmental samples.

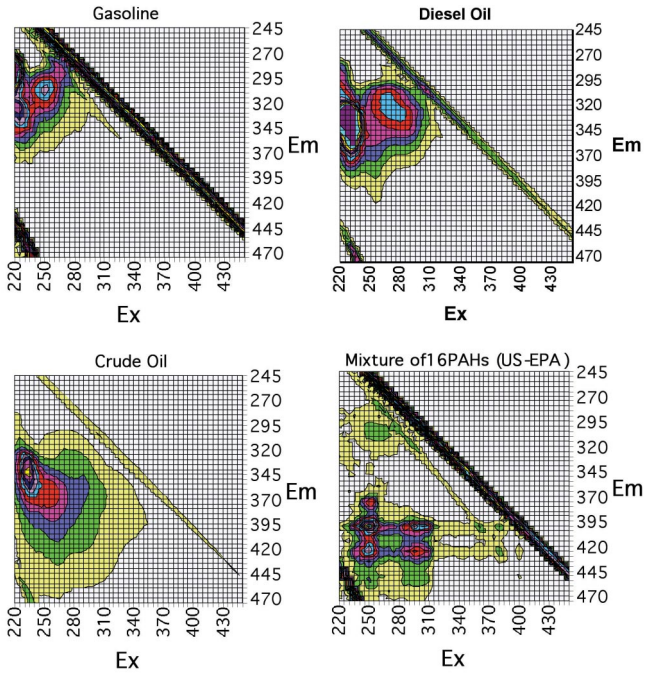

FIGURE C1.5.-2: Fluorescence contour diagrams of selected calibration standards for analysis of petroleum hydrocarbons (Gasoline, 1 μg/ml; Diesel Oil, 1 μg/ml; Crude Oil, 1 μg/ml; and 16 EPA-PAHs, each 3 ng/ml, in cyclohexane).

The shifting of fluorescence to higher wavelengths (both Ex and Em), with increasing ring numbers, is clearly shown in the plots. The level of contamination can be quantified by comparing fluorescence intensities (FI) at characteristic excitation and emission (Ex/Em) wavelengths, calibrated with either pure PAH compound(s) or petroleum products (gas, diesel or crude oil). The fluorescence intensities in the table revealed: (a) the maximum FI of benzene (representing monoaromatic compounds) corresponds to the maximum FI of the gas oil; (b) the maximum FI of naphthalene and phenanthrene corresponds to the two highest FI of diesel oil; and (c) the maximum FI of chrysene corresponds to one of the highest FI of crude oil. The quantification of oil pollution with the standardised fluorescence method uses FI at Ex/EM=310/360 nm. This method can be calibrated with chrysene, diesel or crude oil but not with gas oil. Based on the pattern recognition of the fluorescence spectra, the best matching arbitrary standard (e.g., gas, diesel or crude oil) can be selected and used for estimation of the type and level of oil pollution in environmental sample extracts.

Table C1.5.-1: Relative fluorescence intensity of aromatic hydrocarbons, petroleum products, each 1 µg/ml in cyclohexane, at their characteristic Ex/Em wavelength

Petroleum Product	Characteristic Excitation/Emission Wavelength, nm			
	265/290	275/335	290/390	310/360
Gasoline	18	3.8	0.8	0.4
Diesel Oil	11	44	6.1	9.6
Crude Oil (DRO)	1.1	8.6	54	41

Determination of PAHs

The widely analysed 16 PAH compounds (according to the US-EPA) were analysed in the suspended solids, bottom sediment and mussels samples by using GC/MS analytical procedure.

Volatile organic compounds (VOCs)

Determinands and Methods

In accordance with the EU-WFD, the following VOCs were analyzed (by WRRC VITUKI, Budapest):

1,2,4-Trichlorobenzene
1,2-Dichloroethane
Benzene
Dichloromethane
Trichloromethane

In addition, the values for 1,2,3-trichlorobenzene, 1,3,5-trichlorobenzene and the sum of the three trichlorobenzenes are given.

Sources and effects of VOCs:

- 1,2-Dichloroethane is mainly used as a solvent and an intermediary in the production of vinylchloride,
- Dichloromethane and Trichloromethane are important solvents used in various industries. Because of their toxic effects they are being replaced in most applications,

- Trichlorobenzenes are important intermediaries in chemical industrial processes, and
- Benzene was widely used as a solvent in the past. Because of its high carcinogeneity, in several countries benzene has for many years been banned for industrial use. Benzene is an important component of the gasoline.

The method used was equivalent to the Hungarian Norms (MSz 1484-5) using liquid-liquid extraction followed by GC/MS detection. The compounds were quantified in the SIM-mode. The limit of determination was 0,1 µg/l for each compound.

Analytical quality control

On-site spiking with isotope-labeled internal standards (deutero-benzene and deutero-chloroform) was applied;

In the laboratory, accuracy (< 10 %) and precision (< 10 %) were regularly checked.

Polar pesticides

Determinands and methods

The JDS list of determinands shows nine "polar pesticides" that are also part of the List of Priority Substances according to Annex X of the EU-WFD:

Compound Name	Pesticide Type
Atrazine	herbicide
Alachlor	herbicide
Chlorfenvinphos	insecticide
Chlorpyriphos	insecticide
Diuron	herbicide
Alpha-Endosulfane	insecticide
Isoproturon	herbicide
Simazine	herbicide
Trifluralin	herbicide

The laboratory (Austrian Research Centers Seibersdorf) analyzed additional 14 polar pesticides or metabolites:

Compound Name	Pesticide Type
Metolachlor	herbicide
Desethylatrazine	metabolite
Desisopropylatrazine	metabolite
Hexachlorobenzene	fungicide
Buturon	herbicide
Chlorobromuron	herbicide
Chlorotoluron	herbicide
Linuron	herbicide

Metobromuron	herbicide
Metoxuron	herbicide
Monolinuron	herbicede
Neburon	herbicide
Hexazinone	herbicide
Lindane	insecticide

The method used was solid-phase extraction of 200 ml-samples on C18-material and elution with ethylacetate. The pesticides were detected with GC-MS and HPLC-UV (after solvent exchange of the extract to the HPLC-mobile phase).

Analytical quality control

Method validation was carried out according to the German DIN 38402 - part 51. Detection limits were in the range of 0,007-0,1 µg/l. In three cases, the uncertainty was higher than the determination limit (diuron, isoproturon, neburon), meaning that the method is not reliable at very low concentrations.

The recovery rates of each pesticide were assessed with control samples in the concentration range between 0,05-0,40 µg/l. The recovery rates were >80% for all compounds except hexachlorobenzene (65%).

Organochlorine compounds

Deteminands and methods

The JDS list of determinands included:

- five organochlorine compounds (lindane [gamma-hexachloro-cyclohexane], hexachlorobenzene, hexachlorobutadiene, pentachlorobenzene, pp'DDT) and
- seven polychlorinated biphenyls (PCB 28, 52, 101, 118, 153, 138, 180).

Lindane, hexachlorobenzene, hexachlorobutadiene and pentachlorobenzene are priority pollutants according to Annex X of the EU-WFD.

Sources and effects of organochlorine compounds:

- ***Hexachlorobutadiene*** (HCBD) is mostly used as a solvent for longer-chain hydrocarbons and elastomers, a hydraulic fluid, a heat transfer liquid and insulating fluid, and as a chemical intermediary in the production of chlorofluorocarbons and lubricants. HCBD is mostly released as a by-product in the production of tetrachloroethylene. It could be found in the fly ash during refuse combustion. Due to its hydrophobic nature, HCBD will not remain in water for long periods and will partition into the atmosphere or be adsorbed to sediments. Its half-life in water is proportional to organic matter and ranges from 4 to 52 weeks. It is expected that HCBD persists in sediments with high organic content. Sediment accumulation factors are estimated at 200-10 000. Its low K_{OC} of 4,9 signifies sorption to suspended particulates, which settle on bottom sediments. Although HCBD bioconcentrates in tissues of freshwater invertebrates and fish, it does not biomagnify to any appreciable extent, possibly because of its fast depuration rate.

• ***Lindane***, the gamma isomer of hexachlorocyclohexane (γ-HCH) is a synthetic organochlorine pesticide that has been used since the early 1950s as a treatment for the control of a variety of insects. Lindane enters aquatic systems mainly as surface runoff from treated lands, and deposition following volatilization and aerial transport. Due to its affinity for organic materials, Lindane in aquatic systems tends to become associated with particulate matter and accumulate in bed sediments. Due to its relatively high water solubility, however, Lindane accumulates in bed sediments to a lesser extent than do many other organochlorines. Because a wide variety of organisms live in bed sediments or are in contact with them, sediments act as an important route of exposure to aquatic organisms. Adverse biological effects of Lindane include decreased benthic invertebrate diversity, reduced abundance, increased mortality, and behavioral changes.

• ***Hexachlorobenzene*** (HCB) is a selective fungicide. In many countries, its production and use as a fungicide have ceased. At present, its main importance appears to be as a by-product of several chemical processes or an impurity in some pesticides. HCB is a widespread contaminant. It has very low solubility in water but it volatilises from water at a significant rate despite its relatively low vapour pressure. The main chemical reaction in water is slow photolysis, whereas hydrolysis and oxidation appear to be unimportant. Biotransformation of HCB in surface water, sludge, or soil suspensions is extremely low because HCB is strongly adsorbed by soil and sediments. Because of its resistance to both abiotic and biotic degradation, and very high octanol-water partition coefficient, it can bioaccumulate in aquatic organisms.

• ***DDT*** (Dichlorodiphenyltrichloroethane) is a chlorinated hydrocarbon compound that exhibits broad-spectrum insecticidal properties. There are several possible configurations of the chlorine atoms on the DDT molecule, resulting in several isomeric forms: p,p'-DDT, o,p'-DDT, and m,p'-DDT. The general term "DDT" is applied to a variety of commercial pesticide formulations that consist predominantly of p,p'-DDT and o,p'-DDT, but may also contain minor amounts of dichloro-diphenyl-dichloroethylene (DDE) and dichloro-diphenyl-dichloroethane (DDD). In the present study, p,p'-DDT was measured as dominant and representative DDT compound. In general, DDTs are chemically stable under ambient environmental conditions. The physicochemical properties of these substances, such as low solubility in water and high solubility in lipids (i.e., high K_{ow}) and high K_{oc}, are such that DDT and its metabolites are preferentially incorporated into bed sediments and accumulate in DDTs. Benthic organisms may be exposed to both particulate and dissolved forms of DDTs in interstitial or overlying waters, as well as to sediment-bound DDTs through surface contact and ingestion of sediment during feeding. Sediments and pore-water are believed to represent the primary routes of exposure for infaunal and epibenthic species. Several properties of DDTs influence their bioavailability to aquatic organisms. Hydrophobicity, as represented by the K_{ow}, and water solubility, have been identified as the two most important factors. Accordingly, substances with high K_{ow} and low water solubilities are considered to be the most readily bioavailable to benthic organisms. Based on their physicochemical properties, p,p'-DDT and o,p'-DDT are likely to be the most readily bioavailable of the six isomers discussed in this fact sheet. Sediment characteristics, including TOC, particle size distribution, and clay content, may affect the bioavailability of DDTs in sediments.

• ***Polychlorinated biphenyls*** (PCBs) are a class of chlorinated organic compounds, represented by 209 congeners, that can be toxic to aquatic biota. PCB congeners are classified into homologous groups according to the number of chlorine atoms contained in the compound. These groups range from monochlorobiphenyls, which have one chlorine atom, to

decachlorobiphenyl, which has 10 chlorine atoms. Individual congeners within each homologous group have the same molecular formula but different properties because of dissimilar arrangements of chlorine atoms within the molecule. The identities of individual PCB compounds are determined by the number and location of chlorine atoms substituted on the biphenyl molecule. Individual congeners are also referred to with a numbering system established by the International Union of Pure and Applied Chemistry. (N.B. PCB-28, -52, -101, -118, -153, -138 and -180 congeners were measured in JDS samples). Although the use of PCBs has been severely restricted over the last two decades, the main sources to aquatic environments continue to be leaks, spills, municipal and industrial effluents, runoff from contaminated soils, leachates from unsecured landfills, and atmospheric deposition. The fate and behavior of PCBs in aquatic systems are influenced by a number of physical, chemical, and biological processes. While processes such as photooxidation, hydrolysis, and biodegradation result in the transformation of PCBs into other substances (e.g., benzoic acid, p-chlorobenzoic acid, and phenylpyruvic acid), other processes such as remobilization, solubilization, volatilization, adsorption, desorption, resuspension, and bioaccumulation, are responsible for the cycling, long-range transport, and subsequent accumulation of these substances in soils, sediments, and biological tissues. Because of their thermal and chemical stability, the cycling of PCBs among environmental compartments represents the most important process influencing the fate of these compounds in the environment. Furthermore, the majority of PCBs that are introduced into the aquatic environment are eventually incorporated into bed sediments. Therefore, sediments represent an important route of exposure to PCBs for aquatic biota. Adverse biological effects associated with concentrations of PCBs in sediments are manifest in changes in benthic invertebrate species richness and abundance.

Analytical quality control

The identifications were based on the identity of retention time and two characteristic ions for every compound. The quantifications were based on deuterated surrogate and internal standards.

The detection limits were 0,001 mg/kg (for lindane, hexachlorbenzene and pp'DDT), and 0,005 mg/kg (for PCBs, hexachlorobutadiene and pentachlorobenzene).

Method validation was carried out according to the Hungarian validation regulations.

Other WFD priority pollutants

Deteminands and methods

In addition to the 26 WFD priority pollutants described above Octyl- and Nonyl-phenols, Pentachlorophenol, Di-(ethyl-hexyl)-phthalate, Polybrominated-diphenylethers and Tributyltin were analysed in sediment and suspended solid samples. C10-13-chloroalkanes are also on the priority list; however, these groups of substances include a considerable number of individual compounds. At present, the WFD does not provide appropriate indicative parameters. For this reason C10-13-chloroalkanes were not investigated during JDS.

Sources and effects of WFD priority pollutants:

- ***Octylphenols*** (OP) and ***Nonylphenols*** (NP) are mainly used for the production of detergents (alkylphenolethoxylates with different chain length, APEOs) and as anti-oxidants and stabilizers in plastic materials such as polyvinyl chloride pipes. In addition to this, OP as well as NP is a degradation product of the APEOs which is e.g. formed in wastewater treatment plants, especially under anaerobic conditions. Production numbers in Europe are significantly higher for NP than for OP (NP estimated amount 500.000 tons/a). NP and OP are classified as compounds with endocrine disrupting potential (Category II).

- ***Di-(ethylhexyl)-phthalate*** (DEHP) is widely used as plasticizer in polymer products, mainly polyvinyl chloride. Its annual ouput and use in the EU is about 1.000 000 tonnes. It is well known that phthalates are widespread in the environment and that due to their low polarity they tend to adsorb on solid materials in aquatic systems. DEHP is classified as a compound with endocrine disrupting potential (Category I).

- ***Pentabrominated-diphenylethers*** (PBDEs) are used as flame retardants in plastic materials, especially in plastics for electrical and electronic products (E&E products). Worldwide, only three types of polybrominated diphenyl ethers are commercially used: decabromo-diphenyl ether, octabromodiphenyl ether, and pentabromodiphenyl ether. PDBE is not produced in the EU. It is imported by two companies and its total use (especially in polyurethane foams) is estimated to be around 250 tonnes per year. The main polybrominated diphenyl ethers produced and used in Europe are the higher brominated ones (estimated amounts for EU: octabromdiphenyl ether 450 tonnes/a, decabromdiphenyl ether 7500 tonnes/a). Concerning risk assessment, PDBEs were identified to carry unacceptable risks from use in polyurethane foams. Under the proposed Directive on Waste Electrical and Electronic Equipment, it is proposed that PDBEs should be substituted by other substances by 2008. WFD focuses only on 2,2´,4,5,6-pentabromodiphenyl ether (CAS 32534-81-9). Unfortunately, this substance is not available for analysis as a pure compound.

- ***Pentachlorophenol*** (PCP) and its salts have been used as bactericides and fungicides with a variety of applications in the industrial, agricultural, and domestic fields (e.g. as wood preservative). It has been banned in Germany since 1987 and most developed countries have in recent years restricted the use of PCP, especially for agricultural and domestic applications. It is reported in literature that the partition coefficient of PCP between suspended matter and the aqueous phase at natural pH values is small and, hence, less than 1% of the total amount of PCP in a river is adsorbed onto the solid material.

- ***Tributyltin*** (TBT) is mainly used in antifouling paints (especially on vessels) and as antifouling agent in connection with different materials (e.g. paper). Its annual output in the EU was 3.000 tonnes in 1996 with exports of 1.700 tonnes. In the EU, there is a ban on its use for vessels below 25 meters long (Directive 1999/51/EC). This decision should have had a positive effect on inland waters (lakes and rivers). The International Maritime Organisation has prohibited globally the use of all TBT antifouling systems from 2008 onwards. TBT is classified as a compound with endocrine disrupting potential (Category I).

During the preparation of JDS it was decided to analyze the above mentioned compounds in sediments and suspended solids but not in the water phase.

Analysis of OP, NP, DEHP, PCP and PBDEs

OP, NP, DEHP, PCP and PBDEs were analyzed by DVGW-Technologiezentrum Wasser (TZW), Karlsruhe (Germany).

The dried solid material was stored in glass containers at 4 °C in the dark until extraction started. The extraction was performed by sonication using cyclohexane and acetone solvent mixture. Centrifugation of the extraction solutions was done in a temperature-controlled centrifuge concentrated in a TurboVap II concentrator. Derivatization reactions took place in a drying oven.

After extraction of the analytes from the solid samples, determination was done by GC/MS after silylation of the phenolic compounds. Different systems were used for the analysis of sediments and suspended solids (Annex 1).

Quantification was done using an external calibration with 4-nonylphenol as internal standard. The other internal standards (17α-methyltestosterone and chrysene-d12) were only used for quality control. Limits of quantification (LOQ) were 0.010 mg/kg for para-nonylphenol, di(2-ethylhexyl)phthalate, and the pentabromodiphenyl ethers and 0.005 mg/kg for pentachlorophenol and para-tert.-octylphenol.

Analysis of TBT

TBT was analyzed by Umweltbundesamt Wien (Austria).

The analysis was performed according to ISO/CD 17353. The detection limit was 0,003 mg/kg.

Analytical quality control

Two independent measures were used for quality control of the analytical procedure for the determination of para-tert.-octylphenol, para-nonylphenol, di(2-ethylhexyl)phthalate, pentachlorophenol, and pentabromobiphenyl-ether in suspended matters and sediments of the Danube River:

- The ratios of peak areas of the different internal standards used were calculated and controlled in each sample. Internal standard 1 (17α-methyltestosterone) was added during the extraction procedure, while internal standards 2 and 3 (4-n-nonylphenol and chrysene-d12) were added after extraction but before derivatisation. Hence, the peak area ratio of 4-n-nonylphenol and 17α-methyltestosterone provides information on the recovery of the analytes during the extraction procedure and the separation step of solid and liquid phase. Additionally, as nonylphenol is transferred into its trimethylsilyl derivative while chrysene-d12 remains unchanged, the peak area ratio of 4-n-nonylphenol and chrysene-d12 provides information on the yield of the derivatisation reaction. Out of 15 samples (suspended matter as well as sediment; after elimination of obvious outliers) the "theoretical" ratios were determined and deviations of +/- 25% to the specified value were accepted. If deviations exceeded these limits, the whole analysis of the solid sample was repeated. If the results indicated that strong matrix effects might have negative effects either on the extraction yield or on the derivatisation procedure, the analysis was repeated with a smaller amount of solid material.

- As no certified reference material is available for the analytes under investigation, a sediment sample from the Rhine River was analysed once a working day. As the concentra-

tions of octylphenol, para-nonylphenol and di(2-ethylhexyl)phthalate in this sediment are well known (all other compounds under investigation are not present in this sample), a comparison of the measured concentration to the specified value again provides information on the efficiency of sample preparation as well as on the performance of the analytical system. Deviations of +/- 25% against the predicted value were accepted. If the concentrations exceeded these limits, the injection system of the gas chromatograph was carefully cleaned (and sometimes the separation column cut) and the Rhine River sample was measured a second time. If then the concentration of the analytes proved to be within the defined limits, the samples were also measured a second time (without a second extraction procedure). If this was not the case, the whole analytical procedure was repeated for the total set of samples treated during this working day.

Concerning TBT, 35 samples were spiked in the range from 5 to 100 µg/kg. The mean recovery rate was 88.4% and standard deviation was 10.5%. 20 samples were analyzed in duplicates.

At JDS18-L sampling site, two samples were taken in parallel for comparison. The analytical results obtained for these two samples were as follows:

Compound	Concentration, mg/kg	
	JDS-L	JDS-LB
OP	< 0.005	<0.005
NP	0.027	0.028
DEHP	0.65	0.64
PBDE	< 0.010	< 0.010
PCP	< 0.005	< 0.005
TBT	0.008	0.005

The high correspondence of the results reflects on the homogeneity of the samples as well as on the performance of the analytical methods.

References:

1. Water Quality Enhancement in the Danube River Basin, Final Report, Phare 98-0399.00, February 2000

2. Council Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community, Official Journal, L129, May 18th 1976

Pharmaceuticals

Determinands and methods

Two separate analyses were carried out on the delivered samples, with different extraction and derivatization procedures and different instrumentation. One half of each sample was extracted at neutral pH to analyse neutral and slightly basic substances, and the other half was extracted at pH 2.0 to analyse the acidic substances.

After extraction of the analytes from the samples, determination was done by GC/MS:

- The analysis of neutral extracts was carried out using an HP 5890 Series II gas chromatograph equipped with a Gerstel KAS3 temperature programmable injector. Detection was done with an MSD (HP 5970) quadrupole mass selective detector.
- The analysis of acidic extracts was done with a HP 6890 Series gas chromatograph equipped with a split/splitless injector. Detection was done with a MSD (HP 5973) quadruple mass selective detector.

Analytical Quality Control

For quality control (QC) of the analytical procedure, two independent measures were taken:

- To control recovery rates and matrix effects, a mixture of surrogate analytes was added to every sample. For the analytes extracted at neutral pH, five surrogates were used. For the analytes extracted at acidic pH, two surrogates were used.
- To control laboratory contaminations, recovery rates at low concentrations and limits of detection and quantitation, for every 20 real samples, two unspiked and four spiked surface water samples were analysed in conjunction.

Because of a limited sample volume it was not possible to do a duplicate analysis on every sample.

Screening for Organic Micropollutants

Determination and Methods

Water samples collected during the survey were analysed at the Water Research Institute in Bratislava. Before GC-MS analysis, the organic compounds were extracted from the samples. In the extraction procedure 1.5 l of the water sample was shaken 5 minutes with dichloromethane in a 2-litre separatory funnel. Two-step extraction was applied using 40 ml and 10 ml of dichloromethane respectively. The combined extract was dried over anhydrous sodium sulphate and after concentration to a final volume of 150 μl and addition of internal standard (4 μl of 250 ppm solution of 4-bromofluorobenzene in dichloromethane) it was analysed by GC-MS.

The analyses were performed by using a Hewlett-Packard Model 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a split-splitless injector and a Model 5972 mass-selective detector. A 25 m, 0.20 mm I.D. fused-silica HP-5MS capillary column with a film thickness of 0.33 μm connected with a 1.6 m HP deactivated retention gap (0.53 I.D.) was used for the separation of analytes. Helium (purity 4.6, Linde, Bratislava, Slovak Republic) was used as a carrier gas at a constant flow of 1.5 ml/min. The analyses were run at the following temperatures: injection port: 250°C; transfer line: 280°C; MS source: 176°C; furnace: 40°C for 5 min, 8°C/min up to 280°C, 280°C for 15 min. Extract aliquots of 2 μl were injected into the column by means of an autosampler using a splitless injection mode.

The mass-selective detector was working in the scan mode and the mass range was scanned from m/z 10 to 500 every 1.56 sec. Identification of chromatographic peaks was done using Wiley275 mass spectral library. Area of internal standard peak (4-bromofluorobenzene) was integrated for the ion m/z 176.

Determination method for suspended solids and sediments at ARGE:

Determinand	Unit	Method used	Method used	Limit of Quantification [LOQ]
GC/MS screening	mg/kg	GC/MS	Soxhlett extraction; two solvents (Hexane and acetone); MS scan 80–700; database WILEY 138	Approx. 0,1 mg/kg TS depending on substance

Analytical Quality Control

Quality Assurance at ARGE:

Fluoranthene (d10) was used as an internal standard because it is a synthetic substance and the method has high detection sensitivity.

The standard additions of different PAHs were carried out in very low concentrations, in order to take into consideration the losses caused by sample preparation. The recovery rate was calculated with peak height, which is a rather inexact method of evaluation in comparison with the recovery calculation using peak area. However, evaluation of peak areas is also not the perfect way because of the high variation of signals concerning the small or "indented" peaks. In general, most of the standards were redetected. The quality assurance can be assessed as sufficient enough for the required semi-quantitative analyses.

5.2 RESULTS AND DISCUSSION

The analytical results obtained during the chemical analysis of the water, sediment and mussel samples are presented and discussed in groups of determinands as described earlier. Because of the different types of samples and better orientation in the interpretation of the results, an overview of the sampling sites and locations is given in Figure C2.-1.

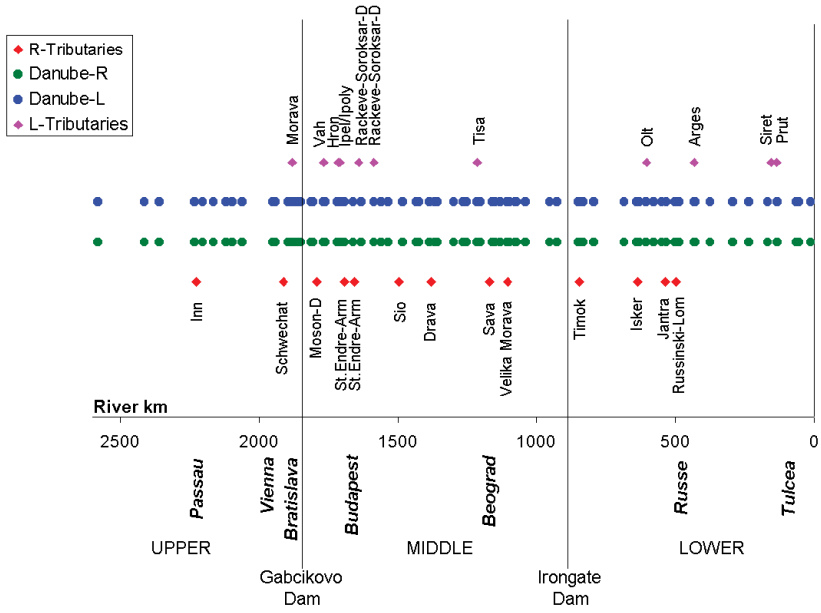

FIGURE C2.-1: Color designation of JDS sampling sites with the name of the tributaries, major cities and indication of the three major Danube Reaches. (N.B.: In the case of water and suspended solid samples, the results for the Danube are indicated in blue colour only)

It is worth mentioning that the interpretation of the results in the different determinand groups will follow the characterization of the three major sections of the Danube, i.e., the upper, middle and lower Danube Reaches, or the geomorphological Reaches as described in Chapter 3.

The interpretation of the physical and chemical characteristics of a river depends on the flow regime at the time the samples are collected. Figure C2.-2. shows the discharge rates in the Danube and the surveyed tributaries during JDS.

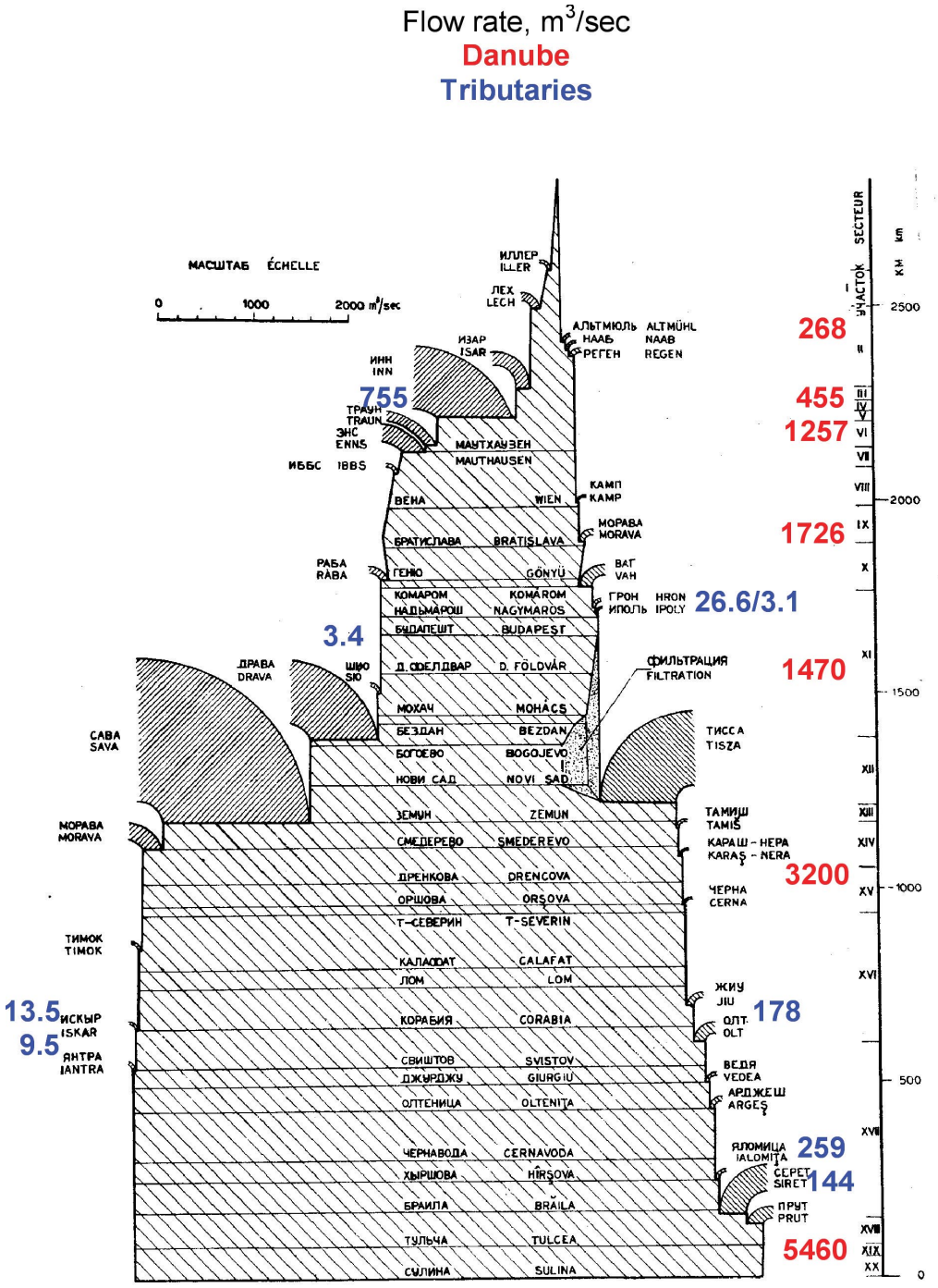

FIGURE C2.-2: Discharge rates of the Danube and its tributaries: average flow rates as provided by the Danube Commission and the actual flow rates at the time of sampling, i.e. between August 13 and September 19, 2001.