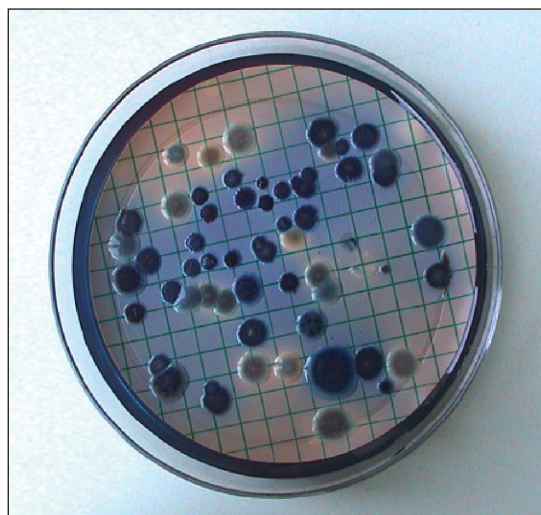


## 4.7 MICROBIOLOGY

### 4.7.1 Introduction

Microbial communities represent a fundamental part of aquatic ecosystems and are of great importance for the matter and energy flux (Kavka et al. 1996). Heterotrophic bacteria play a decisive role in river ecosystems in degrading organic matter that is derived rather from allochthonous than from autochthonous sources (Findlay 1991). Their contribution to self-purification processes of rivers is of great interest within the scope of water quality assessment. Bacteria are ideal sensors because of their fast response to changing environmental conditions.



Bacterial indicators such as total coliforms, faecal coliforms (thermotolerant coliforms), *E. coli*, faecal streptococci (enterococci) and colony counts (plate counts) are widely applied to the assessment of water quality. On the one hand, because of their mainly allochthonous origin, these standard parameters are used as indicators of change in the natural state of rivers. On the other hand, they indicate anthropogenic impacts such as faecal pollution of water. *E. coli* and faecal coliform bacteria are the best indicators for the assessment of faecal pollution (ISO 9308-1, 1990), mainly caused by raw and treated sewage and e. g. diffuse impacts from farmland and pasture. Faecal indicators are excreted by humans and warm-blooded animals, treated to a large extent in sewage treatment plants and ultimately found in aquatic environments where they survive for a relatively long time. *E. coli* and faecal coliforms also indicate the potential presence of pathogenic bacteria, viruses and parasites. The concentrations of heterotrophic bacteria (colony counts) correspond with pollution by organic matter.

For the monitoring of the quality of river water intended for the abstraction of drinking water, irrigation and bathing, the examination of these microbiological standard parameters is obligatory (EU-Surface & Drinking Water Directive 75/440/EEC, WHO - Guidelines for the safe use of wastewater and excreta in agriculture and aquaculture, 1989; OEAWV-Irrigation Water Recommendations 1992; EU-Bathing Water Directive 76/160/EEC). Detailed knowledge of faecal pollution in aquatic environments is crucial for watershed management activities in order to maintain safe waters for recreational and economic purposes (Farnleitner et al. 2001).

It is well known that although biological and chemical water quality may be acceptable, bacteriological parameter might be detected in critical concentrations (Baumann & Popp 1991)

The objectives of the microbiological assessment during JDS were as follows:

- Analysis of the variations of standard bacteriological determinands in the longitudinal stretch of the Danube River and its main tributaries by applying uniform methods in an on-board laboratory as a basis for obtaining comparable results from Neu-Ulm (Germany) to the Black Sea;
- Assessment of the bacteriological water quality by analysing the response of bacteriological parameters to anthropogenic impacts along the entire course of the River as a basis

for identifying hot spots;

- Evaluation of the obtained microbiological results concerning the relevant standard parameters against those cited by National JDS Reports and those previously reported by TNMN as a basis for the harmonization of microbiological analysis methods in the Danube Basin;
- Conclusions and recommendations for future monitoring.

## 4.7.2 Methods

### 4.7.2.1 Sampling and Storage

Water samples were collected from the ship at all sampling sites along the Danube River and selected tributaries, aseptically, in 250 cm<sup>3</sup> sterilised borosilicate glass bottles, from a water depth of 0,2 – 0,3 m. Samples were immediately processed in the on-board laboratory, i.e. within 0,5 hours.

### 4.7.2.2 Microbiological Determinands and Analytical Methods

Before the samples were processed, the bottles were shaken vigorously to guarantee a minimal alteration of bacterial contents in the flasks; aliquot volumes were then examined by membrane filter method. Cellulose nitrate membrane filters (Sartorius) with 50mm diameter and 0.45µm pore size were used for the isolation of total coliforms, faecal coliforms, faecal streptococci and colony counts.

#### **Total coliforms:**

Indicator organisms; arouse suspicion on faecal pollution in the aquatic environment; all types of coliform organisms may occur in faeces; typical coliform bacteria: *Escherichia coli*, *Klebsiella* sp., *Citrobacter* sp., *Enterobacter* sp.

Definition: Aerobic and facultative anaerobic, rod-shaped, gram-negative, nonspore-forming bacteria that develop red colonies with a metallic sheen within 24 hours at 37°C on Endo-Agar containing lactose; coliform bacteria are cytochrome oxidase negative.

Detection method: Membrane filter technique, culture medium: mEndo-Agar LES (Difco), incubation temperature / time : 37 ± 0,5°C / 24 ± 2 hours (ISO 9308-1, 1990).

#### **Faecal coliforms (thermotolerant coliforms):**

Indicator organisms; indicate faecal contamination in the aquatic environment with high probability; typical faecal coliform bacteria (Edberg et al. 1997): *Escherichia coli*, predominant faecal coliform, occurs in faeces, best indicator; *Klebsiella* sp., occurs in faeces and sometimes in other sources like sewage of paper mills.

Definition: Aerobic and facultative anaerobic, rod-shaped, gram-negative, nonspore-forming bacteria that develops blue colonies within 24 hours at 44 °C on selective mFC-Agar;

Detection method: Membrane filter technique, culture medium: mFC-medium (Difco), incubation temperature / time : 44 ± 0,2 °C (water bath!) / 24 ± 2 hours (ISO 9308-1, 1990).

### Faecal streptococci (enterococci):

Indicator organisms; indicate faecal contamination in the aquatic environment; normal habitat is the gastrointestinal tract of man and warm-blooded animals; FS consists of different species of the genus *Streptococcus*; the enterococcus group belongs as a subgroup to faecal streptococci and includes *Streptococcus faecalis*, *S. faecium*, *S. gallinarum* and *S. avium*, which are more resistant to extreme growing conditions than other streptococci.

Definition: Aerobic, gram-positive, nonspore-forming bacteria that develop pink to dark red colonies within 48 hours at 37°C on selective mEnterococcus-Agar;

Detection method: Membrane filter technique, culture medium: mEnterococcus-Agar (Difco), incubation temperature / time : 37 ± 0,5 °C / 44 ± 4 hours (EN ISO 7899-2, 2000).

### Colony count 22°C (Heterotrophic Plate count):

Indicator organisms; the determinand colony count indicates pollution of water by easily degradable organic matter (Kohl 1975)

Definition: Aerobic and facultative anaerobic heterotrophic bacteria that are cultivable in and on solidified nutrient media at 22°C within 48-72 hours;

Detection method: Membrane filter technique, culture medium: Yeast-extract agar, incubation temperature / time : 22 ± 1°C / 44 ± 4 hours (DIN 38411-5 - 1983, DEV K5 – 1971, EN ISO 6222, 1999).

Comments: Membrane filter technique (48 h) was used instead of pour plate technique (72 h) because of better handling on board! By reducing the incubation time from 72 to 48 hours comparable results are obtained.

## 4.7.2.3 Classification

To facilitate the interpretation of microbiological water quality data and the identification of hot spots, the microbiological results were classified by a new system as presented in Table MB-1.

The classification system of Kohl (1975), the EU-Bathing Water Quality Directive 76/160 EEC, and new EU-expert proposals (verbal information) were taken into consideration.

TABLE MB-1: Class limit values for bacteriological determinands

Microbiological water quality assessment		CLASS				
Determinand	Pollution by organic matter	I low	II moderate	III critical	IV strong	V excessive
Colony Count 22°C	in 1 ml water	< 500	> 500 - 10 000	> 10 000 - 100 000	> 100 000-750 000	> 750 000
Determinand	Faecal pollution	low	moderate	critical	strong	excessive
Total coliforms	in 100ml water	< 500	> 500 -10 000	> 10 000 - 100 000	> 100 000 - 1000 000	>1000 000
Faecal coliforms	in 100ml water	< 100	> 100 - 1 000	> 1 000 - 10 000	> 10 000 - 100 000	> 100 000
Faecal Streptococci	in 100ml water	< 50	> 50 - 100	> 100 - 1 000	> 1 000 - 10 000	> 10 000

#### **4.7.2.4 Quality Control**

Quality control typically includes sample collection, sample storage, personnel training, facilities, equipment and reagents, supplies, culture media, sterilisation techniques, standardised analytical test procedures, and data reporting. Duplicate analyses on 5% of samples and on at least one sample per test run are performed. Known positive and negative reference strains are tested for media control. Interlaboratory quality control was performed with partner labs in Hungary and Austria. The microbiologist on the Core Team had a vast experience gained in laboratories with conditions similar to those found on board. Parallel examinations in the laboratory of the Institute and different field labs indicated no significant differences.

#### **4.7.3 Results and Interpretation**

##### **4.7.3.1 Longitudinal Variations of Microbiological Determinands in the Danube River and Its Tributaries**

Four standard bacteriological determinands were studied in the longitudinal stretch of the Danube River and some tributaries to obtain results processed by the same methodologies in an on-board research laboratory.

The Joint Danube Survey (JDS) included 98 sampling sites from Germany (Neu-Ulm, JDS 01, Danube km 2581) to the Black Sea (Sf. Gheorghe arm, JDS 98, Danube km 64).

Standard microbiological determinands such as colony count (22°C, CC 22), total coliform bacteria (TC), faecal coliform bacteria (FC) and faecal streptococci (FS) were analysed.

Samples were collected from the middle of the stream. For definition and indicator functions see Chapter 4.7.2.

Variations in the microbiological results reported by all sampling sites along the course of the Danube are shown in Figures MB - 1 to 4. Small bars stand for the tributaries. For class limit values see the chapter "Methods for microbiological examinations and quality control".

Colony counts varied from 240 to 54 000 per 1ml in the Danube River. More than 10,000 colonies per 1ml (quality target) were isolated at only about 13% of the sampling sites. Record colony counts (maximum 1 400 000/ml) occurred in the Drava, Russenski Lom and Arges tributaries (Fig. MB-1).

The variations in faecal indicator bacteria (total coliforms, faecal coliforms, faecal streptococci) are demonstrated in Figures MB-2, MB-3 and MB-4. Total coliforms varied from 60 to 75 000, faecal coliforms from 20 to 41 000, faecal streptococci from 5 to 2200 / 100ml. In about 49% of all Danube sampling sites including arms and in 58% of the tributaries, the number of colonies indicating class II (quality target) were exceeded. Record amounts of faecal indicator bacteria were detected in the Moson Danube and Rackeve-Soroksar Danube arms as well as in the Ipoly, Russenski Lom, Arges, Siret and Prut tributaries.

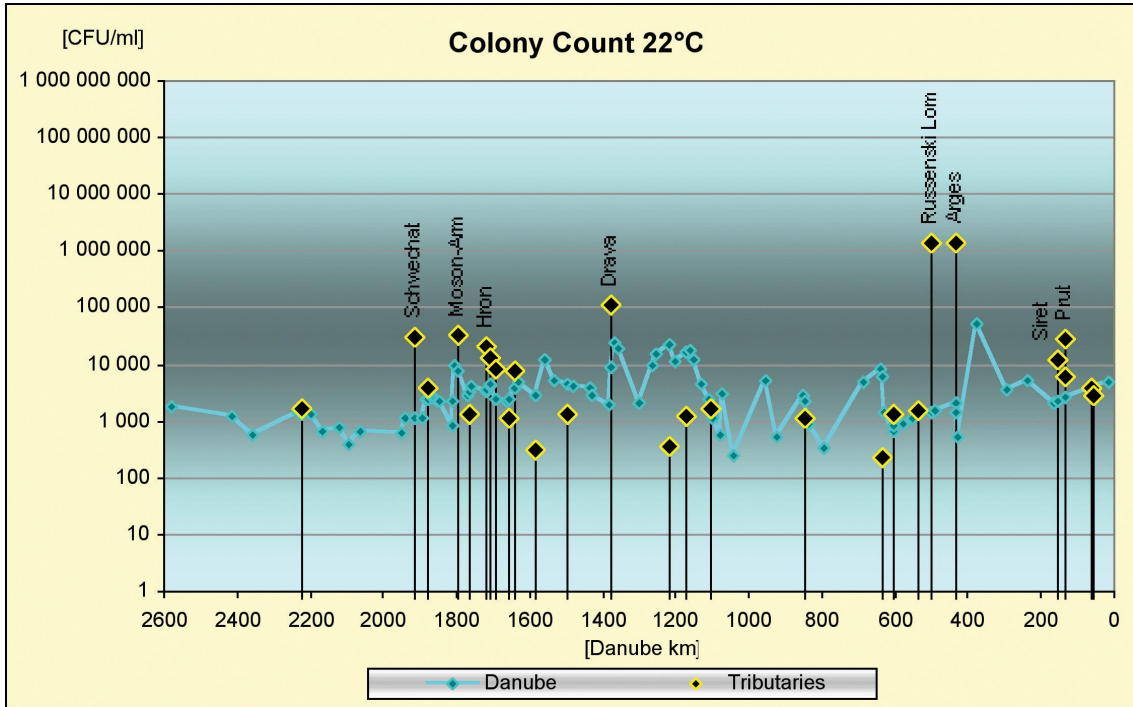


FIGURE MB-1: Variation of colony count I along the course of the Danube River; small columns = tributaries

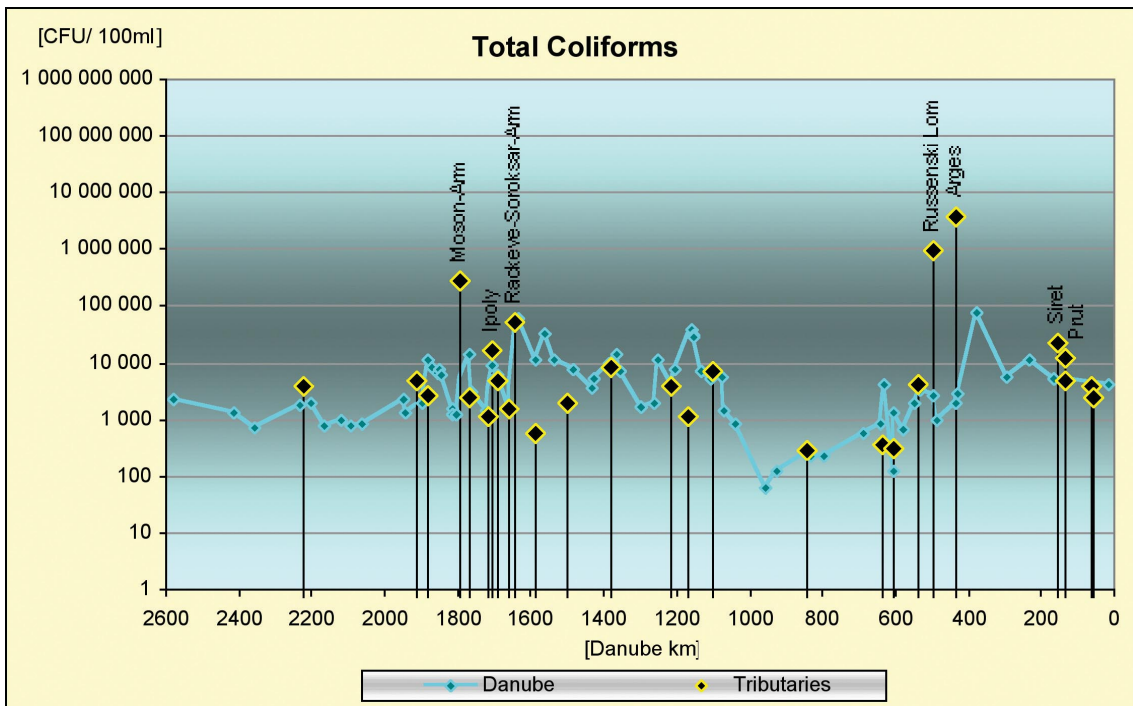


FIGURE MB-2: Variation of total coliforms/100ml along the course of the Danube River; small columns = tributaries;

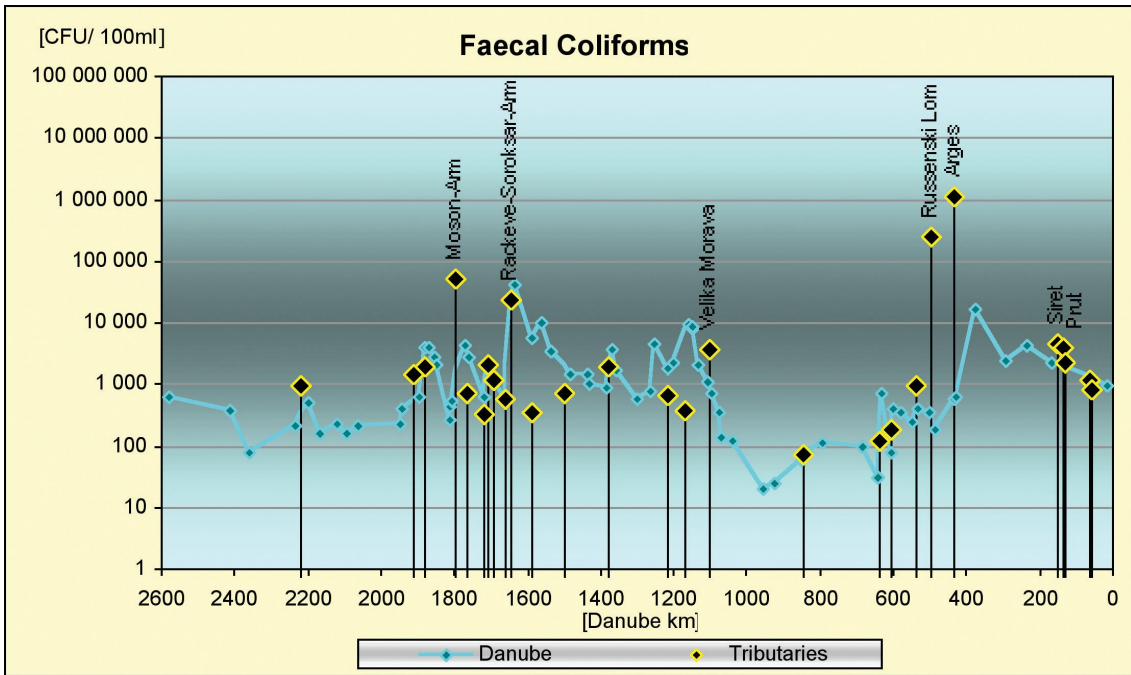


FIGURE MB-3: Variation of faecal coliforms / 100ml along the course of the Danube River; small columns = tributaries

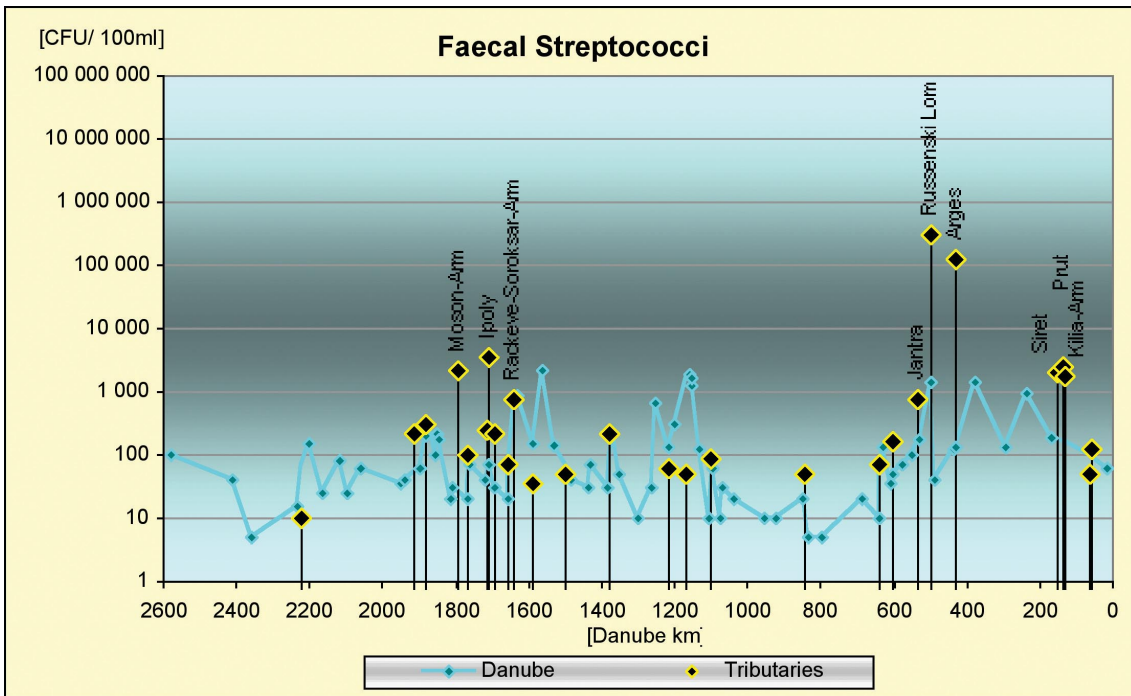


FIGURE MB-4: Variation of faecal streptococci / 100ml along the course of the Danube River; small columns = tributaries

### 4.7.3.2 Assessment of Microbiological Water Quality

For a better overview of the bacteriological situation observed in the Danube and its most important tributaries during JDS, results of the analysis of the four microbiological determinands are presented in Figure MB-5 by using the classification system already mentioned in Chapter 4.7.2.3. Data on the variable faecal coliforms, an excellent indicator of anthropogenic impact such as faecal pollution, are combined with geographical information in a water quality map (Fig. MB-6).

The microbiological situation in the nine geomorphological Reaches of the Danube (see Chapter 3) can be described as follows:

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#### **Reach 1: 2581 – 2225 river km**

Neu-Ulm, D (JDS 1) – Confluence with the Inn River, D-A (JDS 5)

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Microbiological determinands indicated a good bacteriological water quality at the four German sampling sites in Reach 1 of the upper Danube and in the Inn tributary. Colony counts and concentrations of faecal indicator bacteria reflect moderate organic pollution (class II) and low to moderate faecal contamination of water (classes I and II) (Figure MB-5, MB-6).

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#### **Reach 2: 2225 – 1880 river km**

The Inn River, D-A (JDS 5) – Confluence with the Morava River, A-SK (JDS 16)

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Nine sampling stations were situated in Reach 2 in the Austrian section of the Danube River. Standard parameters indicated low to moderate pollution of the Danube (classes I and II). Only at one station in Hainburg, Danube km 1881, concentrations of faecal indicator bacteria were relative high (critical faecal pollution, class III; figure MB-5, MB-6). Influences from local contamination by the settlement Hainburg, from tributary Schwechat river, receiving treated waste water, and probably from the sewage treatment plant of Vienna, are supposed to be responsible (Kavka 2001). It is emphasised that a realistic assessment of microbiological water quality by only one determined value is extremely limited. Additional investigations from both river banks allow better detecting the impact of tributaries, raw sewage or of waste water treatment plants. Tributaries Schwechat river and Morava river were critical polluted by indicator bacteria. Results are corresponding with data received during Danube survey "From river Rhine to the Hungarian stretch of the Danube River (1998)".

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#### **Reach 3: 1880 – 1846 river km**

The Morava River, A-SK (JDS 16) – Gabčíkovo Reservoir, SK-H (JDS 20)

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Four sampling sites along the Slovakian Danube, one at Bratislava and three in Gabčíkovo Reservoir reported a critical level of faecal contamination (class III). Colony counts reflected moderate organic pollution (class II). This is probably due to the influence of the Morava River and the city of Bratislava (Figure MB-5, MB-6).

Tributary right bank	CC/ml	TC /100ml	Danube sampling site	FC /100ml	FS /100ml	Tributary left bank
	1800	2300	JDS 1 Neu Ulm; km 2581	610	100	
	1200	1300	JDS 2 Kelheim; km 2412	380	40	
	580	700	JDS 3 us dam Geisling (Rb); km 2358	80	5	
	1400	1800	JDS 4 us dam Kachlet (P); km 2233	210	15	
JDS 5 Inn; km 2221	1700	3800	Tributary	920	10	
	1300	2000	JDS 6 Jochenstein; km 2200	500	150	
	660	750	JDS 7 us. dam Aschach; km 2165	160	25	
	750	1000	JDS 8 us dam Abwinden-A; km 2120	230	80	
	380	750	JDS 9 Wallsee; km 2095	160	25	
	680	850	JDS 10 us dam Ybbs-P.; km 2061	210	60	
	620	2300	JDS 11 us. dam Greifenst.; km 1950	220	35	
	1100	1350	JDS 12 Klosterneuburg; km 1942	400	40	
JDS 13 Schwechat; km 1913	31000	4900	Tributary	1500	210	
	1100	2000	JDS 14 Wildungsmauer; km 1895	620	60	
	2500	11000	JDS 15 us Morava (Hainb); km 1881	4000	195	
	3800	142700	Tributary	2000	310	JDS 16 Morava; km 1880
	2600	8100	JDS 17 Bratislava; km 1869	3900	300	
	2500	8800	JDS 18 Gabčíkovo.res.entr; km 1856	2800	100	
	2200	7700	JDS 19 Gabčíkovo rezerv1; km 1852	2800	210	
	2200	5900	JDS 20 Gabčíkovo rezerv2; km 1846	2100	170	
	820	1500	JDS 21 Aevanyraro; km 1812	260	20	
	2200	1200	JDS 22 Sap.(Outlet-ch.); km 1812	420	20	
	9800	1200	JDS 23 Medvedov; km 1806	550	30	
JDS 24 Moson Arm-end; km 1794	34000	280000	Arm	51000	2200	
	2900	14000	JDS 25 Komarno / K. km 1768	4200	20	
	1300	2400	Tributary	730	100	JDS 26 Vah; km 1766
	4300	3000	JDS 27 Iza / Szony; km 1761	2700	70	
	3400	1400	JDS 28 St. / Esztergom; km 1719	620	40	
	21000	1100	Tributary	330	250	JDS 29 Hron; km 1716
	13000	16000	tributary	2100	3500	JDS 30 Ipoly; km 1708
	4400	8800	JDS 31 Szob; km 1707	2300	70	
	2400	6000	JDS 32 us Szentendre I.; km 1692	1300	30	
JDS 33 Szent I.Arm-start; km 1692	8300	4800	Arm	1200	220	
	2300	1600	JDS 34 D71 us. Budapest; km 1659	520	20	
JDS 35 Szent I.Arm-end; km 1658	1100	1500	Arm	580	70	
	7600	54000	Arm	23000	770	JDS 36 Rack. Arm-start; km 1642
	4900	61000	JDS 37 ds. Budapest; km 1632	41000	900	
	310	590	Arm	350	34	JDS 38 Rack.-S.Arm-end; km 1586
	2800	11000	JDS 39 Tass; km 1586	5600	150	
	12000	33000	JDS 40 Dunafoldvar; km 1560	9800	2200	
	5200	11000	JDS 41 Paks; km 1533	3400	140	
JDS 42 Sio; km 1497	1300	2000	Tributary	730	50	
	4100	7900	JDS 43 Baja; km 1481	1500	40	
	3900	3700	JDS 44 Hercegszanto; km 1434	1500	30	
	2800	5300	JDS 45 Batina; km 1429	1000	70	
	1900	9500	JDS 46 us. Drava; km 1384	600	30	
JDS 47 Drava; km 1379	110000	8200	Tributary	2000	220	
	25000	14000	JDS 48 ds. Drava (Erdut/B); km 1367	3800	220	
	19000	7300	JDS 49 Daj; km 1355	1700	50	
	2100	1700	JDS 50 Ilok/Backa Palanka; km 1300	560	10	
	9500	1900	JDS 51 us. Novi Sad; km 1262	740	30	
	15000	11000	JDS 52 ds. Novi Sad; km 1252	4400	650	
	23000	4100	JDS 53 us Tisa (Stari S.); km 1216	1800	120	
	350	4000	Tributary	680	60	JDS 54 Tisa; km 1215
	11000	7600	JDS 55 ds Tisa/us Sava; km 1202	2200	310	
JDS 56 Sava; km 1170	1200	1100	Tributary	380	50	
	18000	39000	JDS 57us Pancevo/ds Sava;km1159	9200	1900	
	12000	31000	JDS 58 ds. Pancevo; km 1151	8000	1200	
	4600	6900	JDS 59 rocka; km 1132	2100	120	
	2400	5200	JDS 60 us. Veliko Morava; km 1107	1100	10	
JDS 61 Velika Morava; km 1103	1700	7200	Tributary	3800	90	
	1100	6600	JDS 62 ds. Veliko Morava; km 1097	700	60	
	550	5800	JDS 63 Starapalanka-Ram; km 1077	340	10	
	3000	1400	JDS 64 Banatska P. / B.; km 1071	140	30	
	240	850	JDS 65 Irongate res.(G./K.); km 1040	120	20	
	5300	60	JDS 66 Irongate res.(T./O.); km 954	20	10	
	520	120	JDS 67 Vrbaica / Simjan; km 924	25	10	
	2800	240	JDS 68 us Timok (R. / G.); km 849	60	20	
JDS 69 Timok; km 845	1100	280	Tributary	70	50	
	920	230	JDS 70 Pristol/ Novo Selo H.; km 834	80	5	
	340	230	JDS 71 Calafat; km 795	110	5	
	4700	550	JDS 72 ds. Kozloduy; km 685	100	20	
	8600	850	JDS 73 us. Iskar (Bajkal); km 641	30	10	
JDS 74 Iskar; km 637	220	360	Tributary	120	70	
	1400	4200	JDS 75 ds. Iskar; km 630	700	130	
	670	120	JDS 76 us. Olt; km 606	80	35	
	1300	320	Tributary	180	160	JDS 77 Olt; km 605
	1200	1300	JDS 78 ds. Olt; km 603	390	50	
	880	650	Jds 79 ds Turmu-M.Nikopol; km 579	350	70	
	1100	1900	JDS 80 ds. Zimnicea / S.; km 550	240	100	
JDS 81 A119Jantra; km 537	1500	4200	Tributary	980	780	
	1500	3300	JDS 82 ds. Jantra; km 532	400	180	
	1400	2800	JDS 83 us. Ruse; km 499	360	1400	
JDS 84 Russenski Lom; km 498	1400000	960000	Tributary	240000	310000	
	1500	1000	JDS 85 ds. Ruse / Giurgiu; km 488	190	40	
	2100	1900	JDS 86 us. Arges; km 434	560	120	
	1400000	3800000	Tributary	1100000	120000	JDS 87Arges; km 432
	520	2800	JDS 88 ds. Arges (Otenita); km 429	640	130	
	54000	75000	Jds 89 Chiciu / Silistra; km 375	17000	1400	
	3500	6500	JDS 90 ds. Cernavoda; km 293	2400	130	
	5400	11000	JDS 91 Giurgeni; km 235	4300	950	
	2100	5300	JDS 92 Braila; km 167	2200	190	
	12000	22000	Tributary	4400	2000	JDS 93 Siret; km 154
	29000	12000	Tributary	3900	2500	JDS 94 Prut; km 135
	6200	4800	JDS 95 Reni; km 132	2300	1800	
JDS 96 Sf. Gheorge Arm; km 64	3800	4000	Arm	1200	50	
	2800	2500	Arm	810	120	JDS 97 Vikova - Kilia Arm; km 56
	4800	4200	JDS 98 Sulina - Sulina Arm; km 15	950	60	
	1	1	class I	1	1	
	500	500		100	50	
	501	501	class II	101	51	
	10000	10000		1000	100	
	10001	10001	class III	1001	101	
	100000	100000		10000	1000	
	100001	100001	class IV	10001	1001	
	750000	1000000		100000	10000	
	750001	1000001	class V	100001	10001	

FIGURE MB-5: Assessment of microbiological results (see Chapter 4.7.2); CC=Colony count TC=Total coliforms, FC=Faecal coliforms, FS= Faecal streptococci



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**Reach 4: 1846 – 1659 river km**

Gabcikovo Reservoir, SK-H (JDS 20) – Budapest end of arm, H (JDS 35)

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In this geo-morphological Reach, nine sampling sites were located along the Danube and five along its tributaries and arms. Three stations upstream of the Moson Danube arm (end) were moderately contaminated by bacteria (class II). Colony count in the Moson Danube arm (end) indicated critical pollution (class III) and indicator bacteria reflected strong faecal contamination (class IV, Fig. MB-5, MB-6). This arm can be defined as a bacteriological hot spot. The main impact source is probably the city of Győr. Downstream at Komarno/Komarom, critical concentrations of faecal bacteria could be observed. The Vah tributary contained only small amounts of bacterial indicators. In the Hron tributary, the bacteria occurred in bigger numbers, indicating critical pollution (class III); the Ipoly River reflected critical organic (class III) and strong faecal (class IV) pollution. The sampling stations downstream of Budapest reported a critical level of pollution; upstream of Budapest, the Danube water was found to be of a good bacteriological quality.

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**Reach 5: 1659 – 1202 river km**

Budapest, H (JDS 35) – Confluence with the Sava, YU (Belgrade) JDS 55

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In Reach 5, the impact of the city of Budapest was significant from the bacteriological point of view. A marked increase of bacterial load was observed downstream of Budapest (strong faecal contamination, class IV). Similar water quality (class IV) was assessed in the Rackeve-Soroksar arm (start). The influence of the bacteria- contaminated Drava River seemed remarkable (class III). Downstream Novi Sad Critical bacterial concentrations (class III) occurred downstream of Novi Sad. In the Sio and Tisza rivers, a good bacteriological water quality was observed during JDS (Fig. MB-5, MB-6).

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**Reach 6: 1202 – 956 river km**

The Sava, YU (Belgrade) JDS 55 – Iron Gate Reservoir, YU-RO (JDS 66)

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The Sava tributary was found to be moderately contaminated by indicator bacteria (class II). It was probably the city of Belgrade that caused a marked increase in pollution indicated by standard bacteriological determinands (classes III, IV, Fig. MB-5, MB-6). At Pancevo, the Danube was found to be critically contaminated by bacteria. The Velika Morava River contained increased concentrations of faecal coliforms. Downstream of the Velika Morava, all sampling sites reported a significant decrease in the number of indicator bacteria. Microbiological water quality was good (class II, I). Low pollution and sedimentation effects are probably responsible for the elimination of allochthonic bacteria.

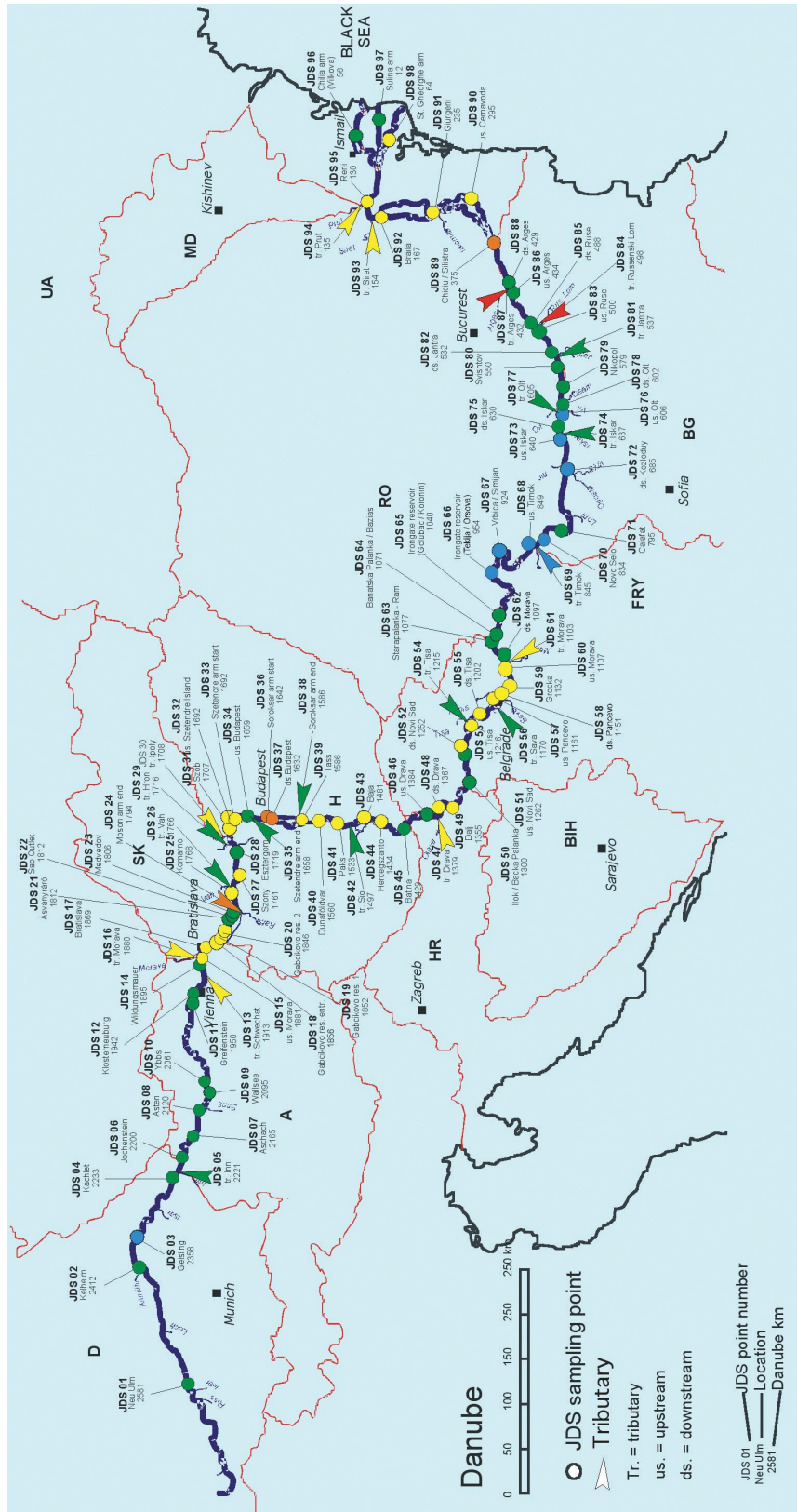
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**Reach 7: 956 – 537 river km**

Iron Gate Reservoir, YU-RO (JDS 66) – Confluence with the Jantra, BG (JDS 81)

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Reach 7 was found during JDS to have the best bacteriological water quality (Fig. MB-5, MB-6). Colony counts and faecal bacteria indicated little to moderate pollution by organic matter and faeces respectively (classes I and II). Low contamination was observed in the Timok and Jantra tributaries. The Olt River contained relatively small amounts of indicator bacteria; only faecal streptococci were a little increased.



**FIGURE MB-6:** Faecal coliforms indicate five classes of faecal pollution: blue: low, green: moderate, yellow: critical, orange: strong, red: excessive (for more details see Table MB-1). Geomorphological Reach 1: JDS 1-6, Reach 2: JDS 6-16, Reach 3: JDS 16-20, Reach 4: JDS 20-35, Reach 5: JDS 35-55, Reach 6: JDS 55-66, Reach 7: JDS 66-81, Reach 8: JDS 81-95, Reach 9: JDS 85-98

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### Reach 8: 537 – 135 river km

The Jantra, BG (JDS 81) – Reni, RO-UA (JDS 95)

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In Reach 8, two tributaries stood out as being excessively polluted (hot spots). The Russenski Lom and the Arges were the most contaminated tributaries investigated during JDS (Fig. MB-5, MB-6). Standard bacteria indicated excessive organic and faecal pollution (class V). In the Danube downstream of the confluences, the impact was not really detectable - perhaps the sampling sites in the middle of the Danube River were not suitable for observing the influence of the polluted tributaries. At Chiciu/Silistra, km375, strong faecal pollution of the Danube River (class IV) was assessed. All sampling stations downstream to Reni, km 132, reported critical contamination by faecal bacteria (class III). The Siret and the Prut rivers were identified as a significant pollution source for the Danube (classes III and IV).

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### Reach 9: 135 – 12 river km

Reni, RO-UA (JDS 95) – The Black Sea / Danube Delta arms, RO -UA, RO, RO (JDS 96-98)

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Reach 9 is characterized by the three Danube Delta arms discharging into the Black Sea. The Sulina and Vilkova-Kilia arms were mainly moderately contaminated by bacteria. In Vilkova-Kilia arm, a little increased number of faecal streptococci could be isolated. In Sf. Gheorgh arm, critical concentrations of faecal coliforms were observed (class III). Colony counts indicated moderate pollution by organic matter (class II) (Fig. MB-5, MB-6).

#### 4.7.4 Comparison with National Results and TNMN data

Unfortunately, microbiological water samples were nationally analysed at only 11 sampling sites in the Slovakian stretch of the Danube (JDS No. 17-31). Therefore, data from the JDS on-board laboratory which were meant to be used as an intercomparison exercise, could only be directly compared to the results from the National Laboratory in Slovakia.

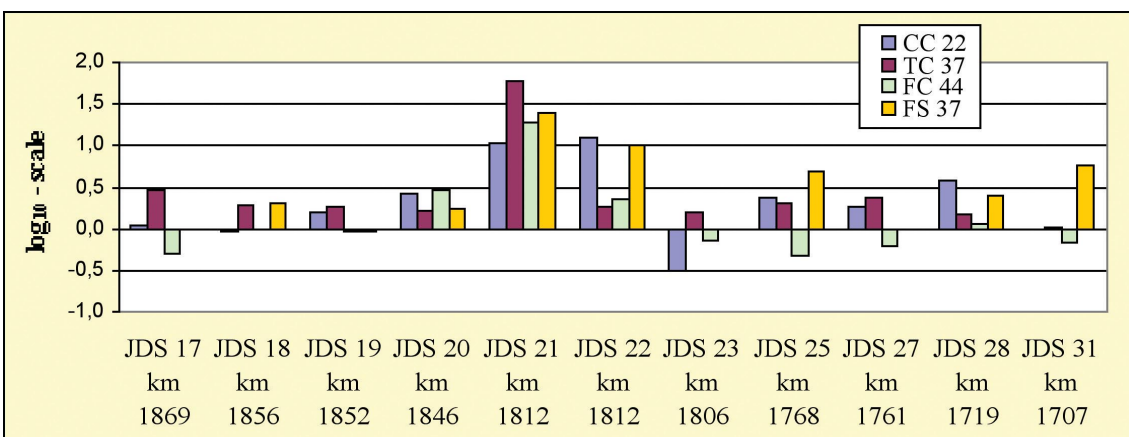


FIGURE MB-7: Comparison of JDS data 2001 with parallelly processed national data from Slovakia; differences in log<sub>10</sub>-levels; JDS data = 0,0; CC=colony count, TC=total coliforms, FC=faecal coliforms, FS=faecal streptococci

The standard determinands such as colony count (22°C), total coliforms, faecal coliforms and faecal streptococci were examined by standardised methods described in Chapter 4.7.2.

The results of this very simple but informative comparison are presented in Fig. MB-7. Logarithm was taken from all data and the differences between Slovakian data and data obtained on-board are calculated in  $\pm \log_{10}$ -levels. At most sampling stations, the data for all determinands vary within  $\pm 1,0$ , many of them within  $\pm 0,5$  logs. This can be interpreted as a good result. Only the results reported by two stations (JDS 21, 22) show relatively big differences.

For other sections of the Danube Basin, only data from TNMN were available, with many gaps (only from 13 stations). An attempt was made to compare the mean values reported in the third quarter of the the last five years (2000–1996) with those reported by JDS. Comparison of on-board data and data from the Austrian laboratory (two sampling sites) and from Slovakia (four stations) brought positive results as mentioned above. At six sampling sites downstream, the differences were enormously high (often more than two logs<sub>10</sub>). The reasons cannot be explained due to the very poor data base. Besides methodological problems, differences in natural conditions and changing impacts might also be responsible.

#### 4.7.5 Conclusions and Recommendations for Future Microbiological Monitoring

- The longitudinal study of the entire course of the Danube River by applying uniform methods in an on-board laboratory produced a reliable overview of the variation of microbiological determinands;
- Microbiological water quality was described by one proposed assessment method and supported the detection of anthropogenic impacts;
- Microbiological results support ecological and physico-chemical assessment of the natural state and water quality of the Danube River and its tributaries;
- Microorganisms are very sensitive indicators for the detection of faecal and organic pollution caused by raw sewage, municipal waste water treatment plants and diffuse impacts from farmland; quantitative results from indicator bacteria facilitate the detection of hot spots;
- Microbial investigations are obligatory for testing compliance with the requirements for the utilisations of river water for drinking and bathing;
- The evaluation of microbiological results showed that - compared to the Danube itself - record pollution levels were found in the tributaries (the Russenski Lom, the Arges, the Siret and the Prut in particular) and in the side arms (the Moson arm, the Soroksar arm);
- Lower bacterial values were observed in the upper section of the Danube and downstream of the Iron Gate Reservoir. Higher levels of faecal pollution were found in the middle part of the Danube down to km 1100 and again downstream of km 500;
- The interlaboratory comparison of results indicated some differences and probably quality problems in analytical methods;
- Monitoring program and methods should be upgraded especially with reference to the updated EN ISO Standards for isolating indicator bacteria; sampling sites should include river banks to increase the chance of detecting focal points of pollution;
- Intensive information exchange (know-how transfer, training programmes) is needed; step by step implementation of AQC is necessary;
- More final control of data in the field of microbiology is required; development of joint assessment methods is urgent;
- Continuation of JDS is recommended: e.g., hot spots, the Black Sea, evaluation aspects.

#### 4.7.6 References

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