Total Viable Count and Concentration of Enteric Bacteria in Bottom Sediments from the Czarna Hańcza River , Northeast Poland

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Abstract

Studies were carried out to determine counts of TVC 20°C, TVC 37°C, TC, FC, FS and *Clostridium perfringens* in bottom sediments of the Czarna Haricza River, from about 1 cm layer, at 10 stations located in Suwatki region (stations 1 and 2), in the villages Sobolewo (stations 3 and 4), the old river bed of the Czarna Hancza and its inflow to Lake Wigry (stations 5 and 6), and in the villages Czerwony Folwark, Mackowa Ruda, Buda Ruska and Wysoki Most (stations 7-10) east of Lake Wigry. Bottom sediments from stations 1 -4 and 7-10 were mostly sandy, while at stations 5 and 6 they dominated by silty clay. Studies were carried out in 1995 and 1996, at monthly intervals with the exception of winter. Water was examined at the same time. All groups of indicatory bacteria were 100-1000 times more numerous in the bottom sediments than in water. They were usually least numerous in sandy bottom sediments, especially in the villages Buda Ruska and Wysoki Most (stations 9 and 10), and the highest in silty clay sediments in the region of the old Czarna Hancza bed and its inflow to Lake Wigry (stations 5 and 6). Minimal and maximal counts of indicatory bacteria were noted in different months upon particular sampling stations. Only sometimes curves of their numbers corresponded to the respective courves of bacteria counts in water. It is suggested that sanitary and bacteriological studies of water should be supplemented by respective studies of the surface layer of bottom sediments.

Keywords: River, bottom sediments, pollution, sanitary evaluation, indicatory microorganisms, bacteria.

Introduction

Sanitary and bacteriological evaluation of river also comprises bacteria indicatory of water pollution and its sanitary state, and of enteric pathogenic bacteria present in the surface layers of bottom sediment [1, 2, 3]. It has been shown [23, 26, 29, 31, 39, 41] that bottom sediments in rivers and other water bodies contain at least 100-1000 times more of these bacteria than water above these sediments. Hence, they should be taken into account in determining the sources of aquatic epidemics. The presence of these bacteria in bottom sediments reflects faecal pollution even when at the given moment there are no such bacteria in the water [5] they can also cause secondary bacteriological pollution of water. The results of bacteriological examination of river water reflect water quality only at the moment of sample collection. Determination of the numbers of bacteria indicatory of sanitary state and of possible enteric pathogens in the bottom sediments makes it possible to predict possible water pollution in the nearest future, due to heavy rainfall and other causes [1, 2, 3]. The sediment/water phases are not permanently separated. Mineral and organic particles of the bottom sediments, and enteric and other bacteria adsorbed by these particles, can pass to water column as a result of diving, rowing, walking in water, use of motor boats, navigation, sand extraction etc. [17, 29, 30]. Passage of bacteria from bottom sediment to water may also take place as a result of changes in salinity or concentration of organic matter [15].

It is belived [18, 19] that enteric bacteria are able to metabolize eluates of bottom sediments, and develop and multiply in such aquatic ecosystems free of predators [14]. In addition, survival of these micro-organisms in bottom sediments, in this from Salmonella genus, is higher than in water, and bottom sediments may be a reservoir of these bacteria [5, 27, 40].

In view of this, examination of bottom sediments for the presence of bacteria indicatory of sanitary state and of pathogenic enteric bateria may supply information on water quality in the nearest future [1, 2, 3]. So far there is no legislation requiring that sanitary-bacteriological examinations comprised also bottom sediments of rivers and other waters important for recreation and aquaculture. There are also no standard methods of collecting bottom sediments for such studies, although the first steps have already been made in this direction [1, 2, 3].

This paper presents the results of sanitary and bacteriological studies determining the counts of bacteria indicatory of water pollution (TVC 20°C, TVC 37°C) and sanitary state (TC, FC, FS, *Clostridium perfringens)* in the surface (0-1 cm) layer of bottom sediments of the Czarna Haricza River in the region of Suwalki and Wigry National Park. Studies were carried out in 1995 and 1996, together with water examinations.

Material and Methods

Czarna Haricza River

The Czarna Haricza River has its sources at height of 230 m above sea level, in a little Lake Jegliszki in the north part of Suwalki Lake District. This is the major river in the Niemen River catchment basin, and the biggest river of Wigry National Park. It flows through Lake Hancza (over 100 m deep) in its upper course; the middle course meanders considerably, the lower is regulated and included in the system of Augustowski Canal. The river is characterized by considerable slope from its sources to Lake Wigry (1.9%o on the average) and looks like a typical mountain stream. Below the lake, the river flows in a post-glacial deep valley. This is where it gets water from the sources from the post-glacial plateau and valley sander. Just above the border of Wigry National Park, annual river flow is 3.7 m/sec ± 0.8 m. The highest water levels are observed in April and then either September or October, the lowest in January and February, and in August-September. During high spring waters, the river flow at its inlet to the lake is 2-3 times higher than the average annual value. During low water in winter and summer it amounts to some 55% of the annual average. The river section from Lake Wigry to the state border is a typical lowland one. Snow melting in April usually increases water flow in this section 1-2-fold compared to the annual average, which amounts here to 4.7 m/sec. The lowest flow (January and Febuary and August-September) reaches 60% of the annual average. Unit outflow from the catchment in this part of the Czarna Haricza River is 8.9 $m^3/sec/km^2$. Only north part of Lake Wigry participates in the river outflow [6]. The inlet of the Czarna Hancza River to Lake Wigry is classified as a strict nature reserve, with one of the biggest beaver colonies in the whole area of Wigry National Park.

A small Marycha rivulet is the major tributary of the Czarna Haricza. In the section below Suwalki the river is connected with a covered canal discharging effluents (16,000-18,000 m^3/d) from the treatment plant in Suwalki, in which all 3 degrees of purification take place [32].

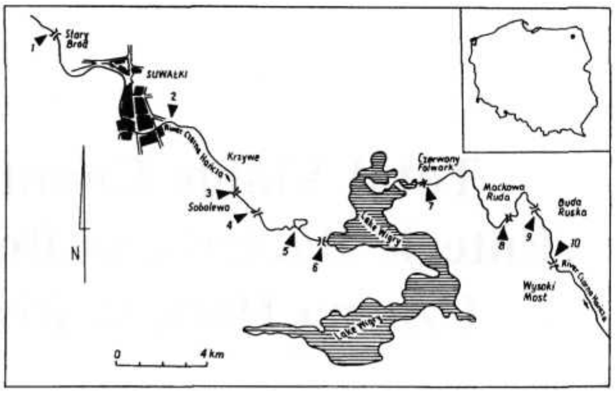

Fig. 1. Situational sketch of Czarna Hancza in the region of Suwalki and the area of the Wigry National Park 1, 2, 3... 10 sites for collecting bottom sediment samples

Sample collection

Samples of bottom sediments from the Czarna Haricza River were collected at monthly intervals from May 1995 till November 1996 except the winter period. Surface sediment layer (0-1 cm) was collected with a sampler of my own design, made of stainless steel, cone-shaped and attached to a stick. It collected both sandy-gravel (stations 1-4 and 7-10) sediments as well as silty-clay (stations 5-6). About 100 g were collected each time, placed into 300 cm^3 sterile glass vessels with a twist-on cap. The samples were collected in the river current: 1) at 6 sites in the upper part of Czarna Haricza, from the village Stary Brod (station 1) to the river outlet into Lake Wigry (station 6), and 2) at 4 sites located in the lower river part, east of Lake Wigry, from the village Czerwony Folwark (station 7) to the village Wysoki Most (station 10) (Fig. 1).

Location of the sampling stations was as follows:

station 1 - in the village Stary Brod, above Suwalki; sandy bottom;

station 2 - below Suwalki, 10 m before the discharge of treated effluent from the municipal treatment plant in Suwalki; gravel bottom;

station 3 - in Sobolewo, at the first bridge along the Krzywe-Sobolewo road; gravel botoom;

station 4 - in Sobolewo, at the second bridge along the Krzywe-Sobolewo road; sandy bottom;

station 5 - in the region of the old river-bed of the Czarna Haricza River, on a forested and wet area; silty clay bottom;

station 6 - at a bridge about 100 m before the inflow of Czarna Haricza to Lake Wigry, in the region of Haricza Bay; silty-clay bottom;

station 7 - in Czerwony Folwark, at the bridge near Postaw Lake; sandy bottom;

station 8 - in Mackowa Ruda at the bridge; sandy bottom;

station 9 - in Buda Ruska at the bridge; sandy bottom;

station 10 - in Wysoki Most, at the bridge; sandy bottom.

Microbiological studies

Microbiological studies of the collected bottom sediment samples of the Czarna Haricza River comprised the following estimations:

1. the total number (CFU/1 GWW) of bacteria on broth agar after 72 h incubation at 20°C (TVC 20°C);
2. the total number (CFU/1 GWW) of bacteria on broth agar after 24 h incubation at 37°C (TVC 37°C);
3. the total number (MPN/1 GWW) of bacteria from *Escherichia coli* group (TC) on the Eijkman medium after 48 h incubation at 37°C;
4. the number (MPN/1 GWW) of faecal bacteria from *Escherichia coli* group (FC) on the Eijkman medium after 24 h incubation at 44.5°C;
5. the number (MPN/1 GWW) of faecal streptococci (FS) on the Slanetz and Bartley medium with sodium azide and crystal violet after 72h incubation at 37°C;
6. the number (MPN/1 GWW) of anaerobic spore-forming bacteria reducing sulphites *(Clostrifium perfringes)* on the Wilson-Blair medium in pasteurised (80°C/10 min) samples of bottom sediments, after 18 h incubation at 37°C.

Total numbers of TVC 20°C and TVC 37°C were determined according to the usual bacteriological technique for the examination of drinking water. The most probable number of TC, FC and FS was determined according to the Standard Methods [4]. The most probable number of *Clostridium perfringens* was determined with the method of dilution, inoculating high column agar medium in test tubes. All determinations were made in 3 parallel repetitions. The results of examining TC, FC, FS and *Clostridium perfringens* number were read from the MacCrady tables. A physiological solution of NaCL was used for sample dilution. Positive results for the presence of coliforms (TC) and faecal coliforms (FC) in the fermentation test on the Eijkman medium were checked on the Endo medium, on the lau-ryl-tryptone broth, and in biologicals stained with the Gram method. Positive results for the presence of faecal streptococci (FS) obtained on Slanetz and Bartley media were checked on the m-Enterococcus agar. Typical colonies which grew on this medium were dark red; they were grafted into the broth to determine growth ability at 44.5°C, pH 9.6, and in the presence of 6.5% NaCI, as well as on skimmed milk with an addition of 0.01% methylene blue. Positive results for the presence of *Clostridium perfringens* were checked in a fermentation sample on skimmed milk. Difco and Merck media were used.

Results

Numbers of bacteria indicatory of pollution and sanitary state found in the bottom sediments from Czarna Hancza River, along the section between Stary Brod above Suwalki and river inflow to Lake Wigry (Tables 1-5 and Figs ***1-6).***

Bacteria indicatory of pollution. Total numbers of TVC 20°C found in the sediments of this river section ranged from 2.7 thousand CFU/1 GWW in Stary Brod (station 1) to 4.65 milion CFU/1 GWW in the region of river inflow to Lake Wigry (station 6) (Table 1). Their lowest

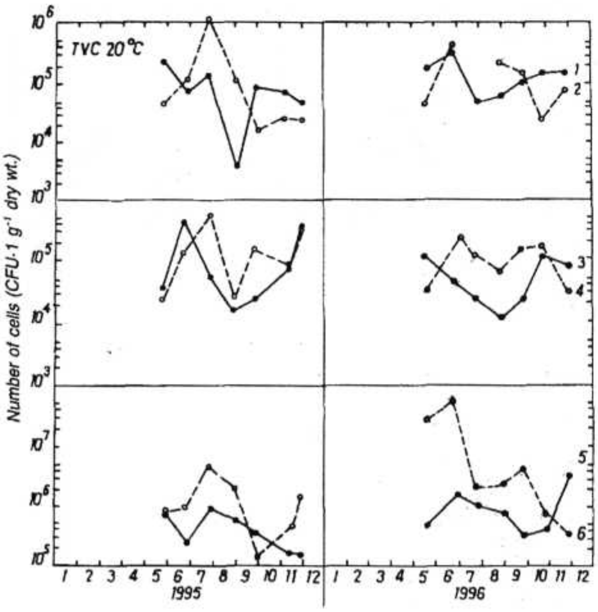

Fig. 2. Seasonal changes of the number TVC 20°C in the bottom sediments of the Czama Hancza from Stary Brod above Suwalki to its inflow to Wigry Lake 1, 2, 3...6 sites for collecting bottom sediment samples

numbers were usually found in the bottom sediments collected in Stary Brod (station 1) above Suwalki and in Sobolewo (station 3), the highest - at river inflow to lake Wigry (station 6). The highest numbers of these bacteria in Stary Br6d above Suwalki (station 1) and in the region of a waste treatment plant, 10 m above the discharge of the effluents from this plant (station 2) were observed at the beginning of September 1995 and 1996; in Sobolewo village the highest numbers of these bacteria were recorded in November 1995 and 1996 (station 3), and in April 1996 (station 4). In the region of the old river-bed, and close to river inflow to Lake Wigry (stations 5 and 6), the highest TVC 20°C numbers were recorded in different periods of spring and summer 1995 and 1996 (Fig. 2).

Total numbers of TVC 37°C ranged from 600 CFU/1 GWW in the region of sewage treatment plant in Suwalki (station 2A) and in Sobolewo (station 3) to 468 thousand CFU/1 GWW at the outlet of Czarna Hancza River to Lake Wigry (station 6) (Table 1). Their mean values for the whole period of studies were the lowest in Sobolewo (station 4) and in the old river-bed of Czarna Hancza (station 5), and the highest at the river outlet to Lake Wigry (station 6). Bottom sediments collected in Stary Brod above Suwalki (station 1), and in the area of the treatment plant, 10 m above the effluent (station 2) contained the highest numbers of these bacteria at the beginning of September 1995 and 1996. High numbers were also recorded in bottom sediments collected in Sobolewo in November 1995 and 1996 (station 3), and in April 1996 (station 4), in the samples collected in the old river-bed and at the river outlet to Lake Wigry (stations 5 and 6) in spring and summer 1995 and 1996 (Fig. 3).

Bacteria indicatory of sanitary state. Total coliforms (TC) and numbers of faecal coliforms (FC) ranged, respectively, from 20 and 7 MPN/1 GWW in bottom sediments of Czarna Hancza River collected in Stary Brod (station 1)

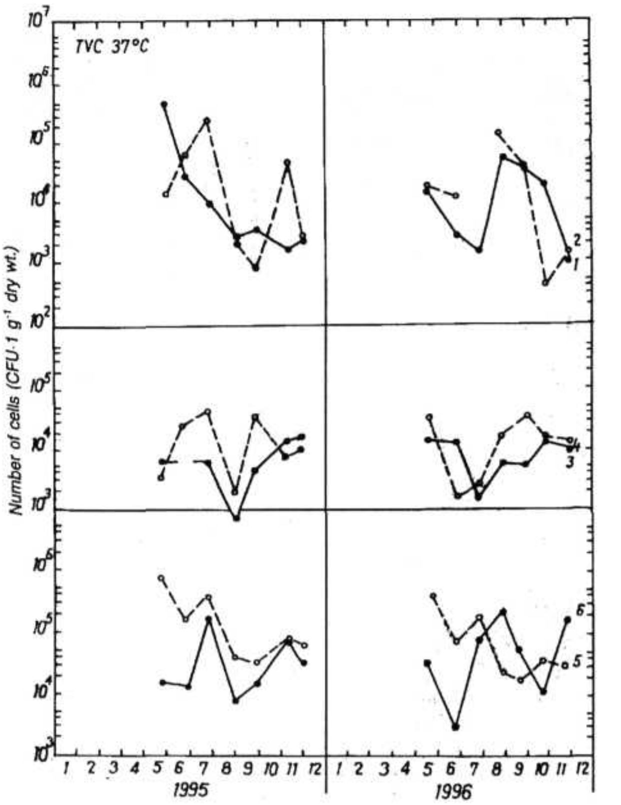

Fig. 3. Seasonal changes of the number TVC 37°C in the bottom sediments of the Czarna Haricza from Stary Brod above Suwalki to its inflow to Wigry Lake. Explanations as in Figure 2.

above Suwalki, to 4500 and 1500 MPN/1 GWW at the river outflow to Lake Wigry (station 6) (Table 2). Average numbers in the study period were the lowest in bottom sediments collected in Stary Brod (station 1), the highest at

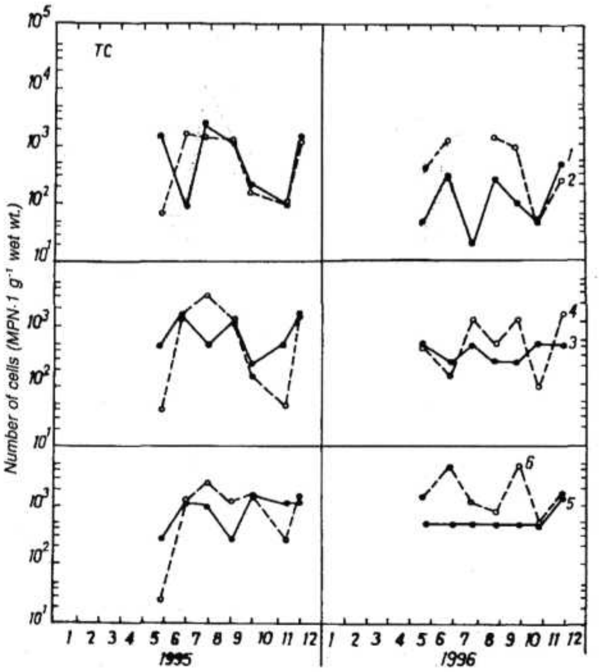

Fig. 4. Seasonal changes of the number TC in the bottom sediments of the Czarna Hancza from Stary Brod above Suwalki to its inflow to Wigry Lake. Explanations as in Figure 2.

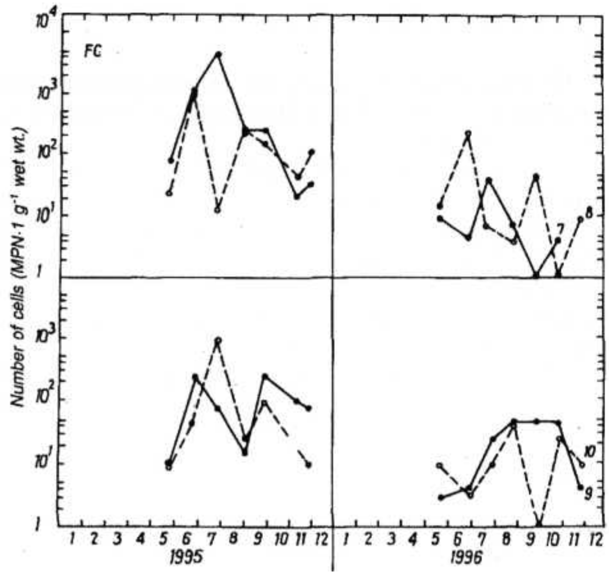

Fig. 5. Seasonal changes of the number FC in the bottom sediments of the Czarna Haricza from Stary Br6d above Suwalki to its inflow to Wigry Lake. Explanations as in Figure 2.

the river inlet to Lake Wigry (station 6). In 1995 the two groups of bacteria were usually more numerous in July, in 1996 - in different months and stations (Figs 4 and 5). Number of faecal streptococci (FS) ranged from 9 MPN/1 GWW in the sediments collected in Stary Br6d (station 1) and in the region of sewage treatment plant in Suwalki (station 2) 10 m above the effluent discharge, to 14,000

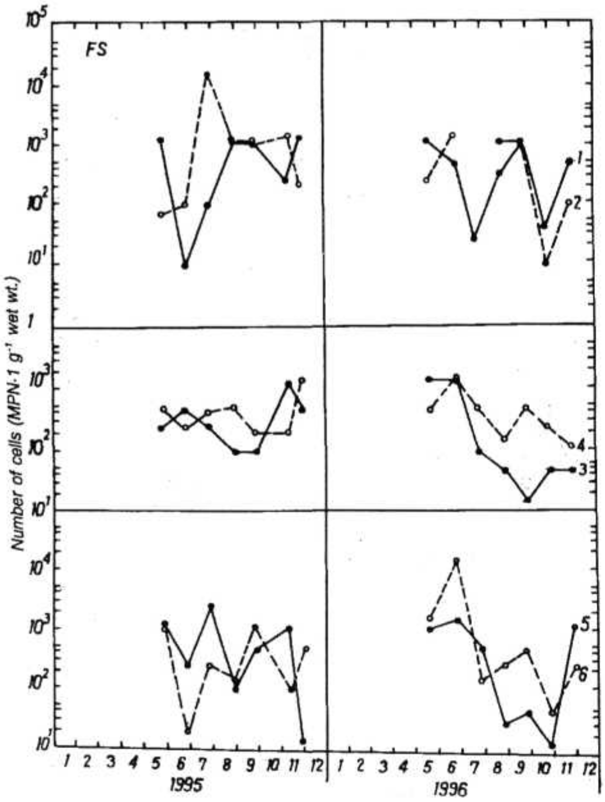

Fig. 6. Seasonal changes of the number FS in the bottom sediments of the Czarna Hancza from Stary Brod above Suwatki to its inflow to Wigry Lake. Explanations as in Figure 2.

Table 1. Mean (for study period) and range for the numbers of total viable counts at 20°C and 37°C in bottom sediments of particular sites in the Czarna Hancza River, 1995-1996.

Site[1]	Number of samples		Total viable count at 20°C		Total viable count at 37°C	
			CFU·10³/1 g			
			wet wt.	dry wt.	wet wt.	dry wt.
1	14	A B	95 2.5–258	120 3.0–325	38 0.8–400	48 1–500
2	13	A B	130 13–835	160 16–1020	37 0.6–217	45 0.7–267
3	14[a] 13[b]	A B	85 10.3–388	102 12.3–465	36 0.6–12.2	9 0.7–14.4
4	14	A B	110 19–405	143 23.7–506	14 1.4–30	17 1.7–35.7
5	14	A B	135 27.2–590	850 170–3530	19 1–72.3	140 6.2–452
6	14	A B	653 37.5–4650	7317 139–51600	62 1.8–468	690 20–5200
7	14	A B	95 10–385	172 12.9–493	85 0.5–890	108 0.7–1140
8	14	A B	126 4–430	175 5.5–592	26 0.8–100	36 1.1–117
9	14	A B	348 16.5–3220	387 20.6–4025	80 0.2–900	87 0.2–1125
10	14[a] 13[b]	A B	80 5.1–264	105 6.6–340	13 0.3–41.6	17 0.4–53.6

[1] – See Figure 1, A – mean, B – range, [a] – TVC 20°C, [b] – TVC 37°C

Table 2. Mean (for study period) and range for the number of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) in bottom sediments of particular sites in the Czarna Hancza River, 1995-1996.

Site[1]	Number of samples		Total coliforms		Fecal coliforms		Fecal streptococci	
			MPN/1 g					
			wet wt.	dry wt.	wet wt.	dry wt.	wet wt.	dry wt.
1	14	A B	567 20–2500	708 25–3120	177 7–1400	225 8–1750	607 9–1400	785 11–1750
2	13	A B	686 45–1400	840 55–1720	402 25–1400	490 32–1722	1612 9–14000	1985 11–17220
3	14	A B	574 250–1400	688 300–1670	360 25–1400	433 30–1678	410 15–1400	490 18–1678
4	14	A B	850 40–3000	1060 50–3750	373 15–1400	457 19–1750	472 95–1400	390 118–1750
5	14	A B	730 250–1400	4575 1560–8760	5585 25–1400	3655 155–8760	690 15–2500	4305 93–15620
6	14	A B	1562 25–4500	17350 277–50000	810 25–1500	8975 277–16600	1405 20–14000	15630 222–155000
7	14	A B	468 0.7–1500	605 0.9–1920	445 3–4500	575 4–5760	305 3–1100	395 14–1410
8	14	A B	285 4–1400	390 5.5–1928	145 3–1100	195 4–1510	365 4–1400	490 5–1928
9	14	A B	234 7–1400	295 8.7–1750	60 4–250	75 5–312	285 3–1400	355 4–1760
10	14[ac] 13[b]	A B	375 4.5–2500	480 5.8–3200	100 0.3–950	130 0.4–1225	215 3–1400	270 4–1800

[1] – See Figure 1, A – mean, B – range, [a] – total coliforms, [b] – faecal coliforms, [c] – faecal streptococci

Table 3. Percentage distribution of the values of ratio FC:FS in the bottom sediments of particular sites in the Czarna Haricza River (1995-1996).

Ratio FC:FS	Site[1]									
	1	2	3	4	5	6	7	8	9	10
< 0.7	64	69	36	29	36	28	54	47	47	64
0.7 – 4.0	28	16	30	64	50	36	27	53	47	27
> 4.0	8	15	14	7	14	36	18	0	6	9
	(14)[2]	(13)	(14)	(14)	(14)	(14)	(11)	(13)	(13)	(11)

[1] – See Figure 1; [2] – number of samples investigated

Table 4. Number of anaerobic spore-forming, sulphite reducing bacteria *(Clostridium perfringens)* in bottom sediments of particular sites in the Czarna Haricza River (1996).

Site[1]	1996											
	25 June		24 July		27 Aug.		16 Sept.		25 Oct.		29 Nov.	
	MPN/1 g											
	A	B	A	B	A	B	A	B	A	B	A	B
1	< 3	–	< 3	–	25	31	4	5	< 3	–	< 3	–
2	4	5	< 3	–	25	31	25	31	4	5	20	25
3	95	114	3	–	25	31	9	11	250	300	250	300
4	< 3	–	250	312	45	56	95	118	45	56	9	11
5	25	154	95	593	250	1560	250	1560	3	19	250	1560
6	250	2780	95	1055	95	1055	95	1055	45	500	250	2770
7	< 3	–	< 3	–	< 3	–	< 3	–	< 3	–	4	5
8	< 3	–	< 3	–	< 3	–	< 3	–	< 3	–	< 3	–
9	4	5	< 3	–	< 3	–	< 3	–	4	5	< 3	–
10	< 3	–	< 3	–	< 3	–	< 3	–	4	5	< 3	–

[1] – See Figure 1, A – wet wt., B – dry wt.

Table 5. Analysis of sediment quality of the Czarna Hancza River using criteria given by Kavka [24] and Kohl [25], after Albinger [2], Percent distribution of samples relevant to the given class.

Bottom sediments quality criteria		Sediment quality level[5]	Site[1]									
Microorganisms	Number of bacteria		1	2	3	4	5	6	7	8	9	10
TVC 20°C[2]	– < 500,000	1	100	92	100	100	93	78	100	100	85	100
	> 500,000 – 1,000,000	2	0	8	0	0	7	7	0	0	8	0
	> 1,000,000 – 10,000,000	3	0	0	0	0	0	15	0	0	7	0
	> 10,000,000 – 50,000,000	4	0	0	0	0	0	0	0	0	0	0
	> 50,000,000 – 100,000,000	5	0	0	0	0	0	0	0	0	0	0
	> 100,000,000 – 750,000,000	6	0	0	0	0	0	0	0	0	0	0
	> 750,000,000	7	0	0	0	0	0	0	0	0	0	0
			(14)[4]	(13)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
FC[3]	1 – 10	1	14	0	0	0	0	0	21	28	29	54
	> 10 – 100	2	50	60	43	14	35	15	36	43	57	38
	> 100 – 1,000	3	28	15	50	78	36	35	8	21	14	8
	> 1,000 – 5,000	4	8	25	7	8	29	50	35	8	0	0
	> 5,000 – 10,000	5	0	0	0	0	0	0	0	0	0	0
	> 10,000 – 100,000	6	0	0	0	0	0	0	0	0	0	0
	> 100,000	7	0	0	0	0	0	0	0	0	0	0
			(14)	(13)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(13)

[1] – See Figure 1, [2] – total viable count at 20°C (saprophytic bacteria as CFU/1 g wet wt. sediments), [3] – number of faecal coliforms (MPN/1 g wet wt. sediments), [4] – number of samples investigated, [5] – degree of loading with organic substances which can be well decomposed by bacteria (TVC 20°C) and degree of loading with faecal substances (FC): 1 – very little, 2 – little, 3 – moderate, 4 – moderate high, 5 – high, 6 – very high, 7 – extreme high.

MPN/1 GWW at the same station 2 and at the river inlet to Lake Wigry (station 6) (Table 2). The mean bacteria numbers for the whole period of studies were the lowest in the bottom sediments collected in Sobolewo (stations 3 and 4), and the highest in the sediments collected at the outflow of Czarna Hancza to Lake Wigry (station 6). In 1995 there were usually more bacteria in July, September, October and November, in 1996 - in May and/or June; in Stary Brod above Suwalki (station 1) and in the region of sewage treatment plant in Suwalki, 10 m above the discharge of treated effluents (station 2) - also in August and September (Fig. 6). Ratio of FC:FS numbers ranged from 0.01 in the bottom sediment collected in Stary Brod above Suwalki (station 1) to 55.0 in the sediments collected at the outlet of Czarna Hancza River to Lake Wigry (station 6). In 22-69% of the examined sediment samples, FC:FS ratio was lower than 0.7; in 16-64% of the samples it ranged from 0.7 to 4.0, and in 7-36% of the examined samples it was higher than 4.0 (Table 3). At the subsequent stations the percentage of bottom sediment samples with FC:FS ratio lower than 0.7 decreased, while the per cent of samples with FC:FS ratio of 0.7-4.0 (stations 2-4) and more (stations 4-6) - increased.

Numbers of anaerobic sporeforms reducing sulphites *(Clostridium perfringens)* ranged in 1996 from <3 to 250 MPN/1 GWW. Their maximum numbers were usually recorded in the sediments collected from the old bed of Czarna Hancza River and at its inflow to Lake Wigry (stations 5 and 6) (Table 4). Numbers of bacteria indicatory of pollution (TVC 20°C, TVC 37°C) and of the sanitary state (TC, FC, FS, *Clostridium perfringens),* recalculated to 1 GDW of the bottom sediments, were about 20% higher in Stary Brod above Suwalki (station 1), in the region of Suwalki sewage treatment plant, 10 m above effluent discharge (station 2), and in Sobolewo (stations 3 and 4), while they were, respectively, 6 and 11 times higher in the region the of the old river bed and the Czarna Hancza outlet to Lake Wigry (stations 5 and 6).

Numbers of bacteria indicatory of pollution and sanitary state, found in bottom sediments from the Czarna Hancza River along the section between Czerwony Folwark and Wysoki Most, east of Lake Wigry (Tables 1-5 and Figures 7-12).

Bacteria indicatory of pollution. Total numbers of TVC 20°C in the bottom sediments of the Czarna Hancza River along the section from Czerwony Folwark to Wysoki Most ranged from 4 thousand CFU/1 GWW in Mackowa Ruda village (station 8) to 3.2 million/1 GWW in Buda Ruska village (station 9) (Table 1). Mean values for the study period were the lowest in the sediment samples collected in Czerwony Folwark and Wysoki Most (stations 7 and 10), and the highest in Buda Ruska (station 9). Maximal numbers in particular years were recorded at different stations and months (Fig. 8).

Total TVC 37°C numbers ranged from 200 CFU/1 GWW in the sediments collected in Buda Ruska (station 9) to 890 thousand/1 GWW in the sediments collected in Czerwony Folwark (station 7) and 900 thousand/1 GWW in Buda Ruska (station 9). Mean values for the study period were the lowest in the sediments collected in Mackowa Ruda and Wysoki Most (stations 8 and 10), and the highest in the villages Czerwony Folwark and Buda Ruska (stations 7 and 9) (Table 1). In 1995 the highest numbers were recorded in the sediments collected in June or July, and in September and/or October; in 1996 they were recorded from August to November (Fig. 7).

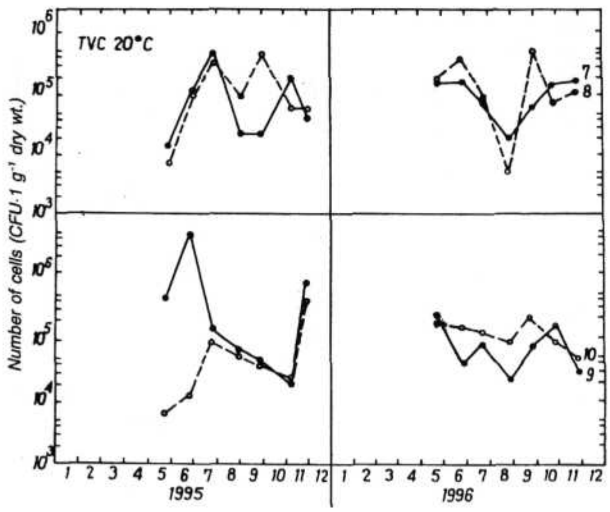

Fig. 7. Seasonal changes of the number TVC 20°C in the bottom sediments of the Czarna Hancza from Czerwony Folwark to Wysoki Most towards the east from Wigry Lake 7, 8, 9, 10 sites for collecting bottom sediment samples.

Bacteria indicatory of the sanitary state. Numbers of total coliforms (TC) ranged from 0.7 MPN/1 GWW in the sediments collected in Czerwony Folwark (station 7) to 2500 MPN/1 GWW in those collected in the village Wysoki Most (station 10) (Table 2). The lowest mean values for the study period were recorded in sediments collected in the villages Mackowa Ruda and Buda Ruska (stations 8 and 9), the highest in the sediments in Czerwony Folwark (station 7). Seasonal changes of TC numbers were characterized by maximal values in different months at particular stations (Fig. 9). In the case of samples collected in Czer-

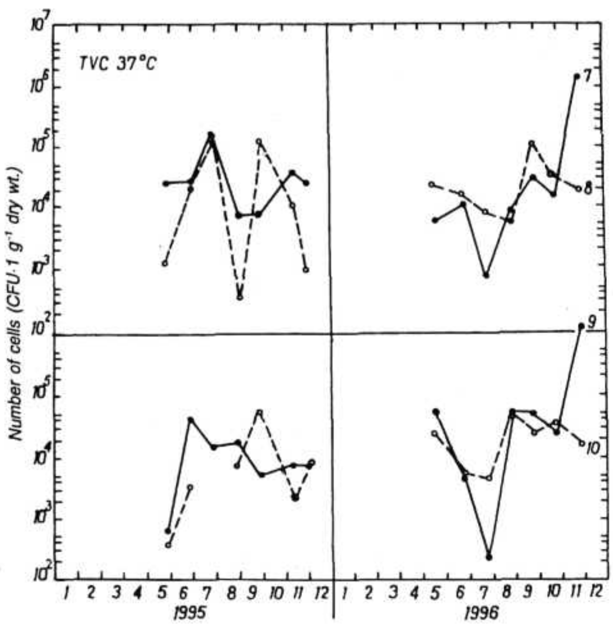

Fig. 8. Seasonal changes of the number TVC 37°C in the bottom sediments of the Czarna Hancza from Czerwony Folwark to Wysoki Most towards the east from Wigry Lake. Explanations as in Figure 7.

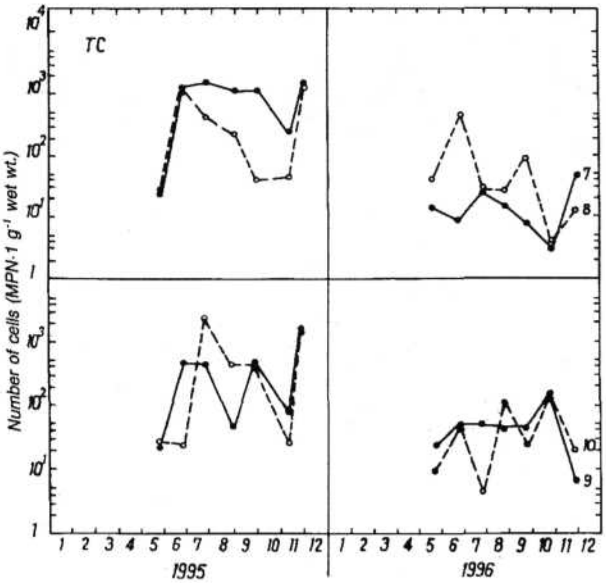

Fig. 9. Seasonal changes of the number TC in the bottom sediments of the Czarna Haricza from Czerwony Folwark to Wysoki Most towards the east from Wigry Lake. Explanations as in Figure 7.

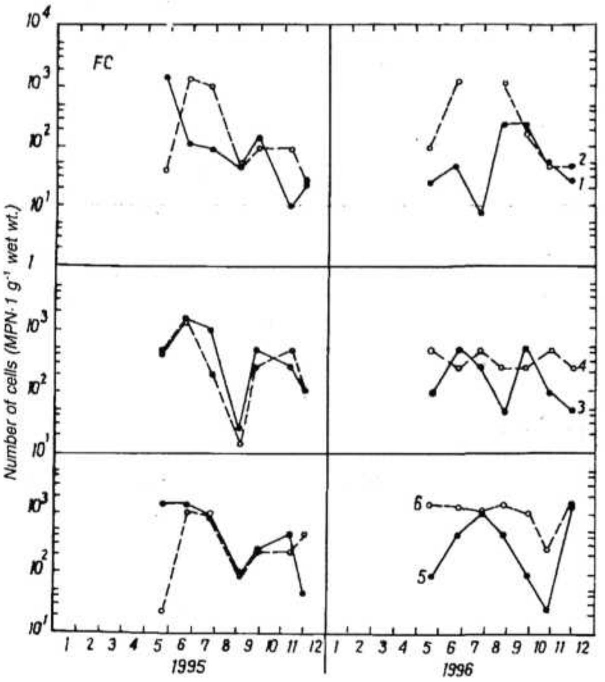

Fig. 10. Seasonal changes of the number FC in the bottom sediments of the Czarna Haricza from Czerwony Folwark to Wysoki Most towards the east from Wigry Lake. Explanations as in Figure 7.

wony Folwark and Mackowa Ruda, their seasonal changes resembles those of TVC 37°C numbers, while there was no such similarity in the case of sediments collected in Buda Ruska and Wysoki Most (stations 9 and 10). Numbers of faecal coliforms (FC) ranged from <3 MPN/1 GWW in the sediments collected in Wysoki Most (station 10) to 4500 MPN/1 GWW in those collected in Czerwony Folwark (station 7) (Table 2). The lowest mean values in the whole

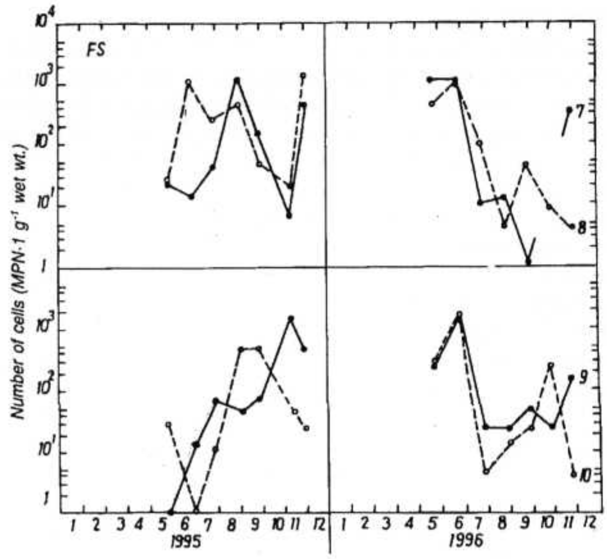

Fig. 11. Seasonal changes of the number FS in the bottom sediments of the Czarna Haricza from Czerwony Folwark to Wysoki Most towards the east from Wigry Lake. Explanations as in Figure 7.

period of studies were observed in the sediments collected in Buda Ruska (station 9), the highest in the sediments collected in Czerwony Folwark (station 7). Seasonal changes of FC numbers were characterized in 1995 by the maximal values in June and/or July and in September, while in 1996 maxima were recorded in different summer months, rarely in spring and autumn (Fig. 10). Numbers of faecal streptococci (FS) ranged from 3 or 4 MPN/1 GWW in the sediments collected in all four villages (stations 7-10) to 1100 MPN/1 GWW in the sediments collected in Czerwony Folwark (station 7) and 1400 MPN/1 GWW in those collected in Mackowa Ruda, Buda Ruska and Wysoki Most (stations 8-10) (Table 2). Mean values were the lowest in the sediments collected in Wysoki Most (station 10), and the highest in the sediments collected in Buda Ruska (station 9). Higher levels of these bacteria were observed in 1995 in the sediments collected in August and/or September and October, in 1996 - in those collected in June (Fig. 11). FC:FS ratio ranged from 0.002 or 0.003 in the sediments collected in different villages, to 112.5 in the sediments collected in Czerwony Folwark (station 7). This ratio was lower than 0.7 in 47-64% of the samples; in 27-53% it ranged from 0.7 to 4.0, and in 0-18% it was higher than 4.0. The highest percentages of sediment samples in which FC:FS ratio was lower than 0.7 were recorded in the village Wysoki Most (station 10), while FC:FS ratio higher than 4.0 was most frequently recorded in Czerwony Folwark (station 7) (Table 3).

Anaerobic spore-forming bacteria-reducing sulfites *(Clostridium perfringens)* were very rare in the bottom sediments of this section of the Czarna Hancza River, and their numbers did not exceed 4 MPN/1 GWW (Table 4). Numbers of bacteria indicatory of pollution (TVC 20°C, TVC 37°C) and of sanitary state (TC, FC, FS), when recalculated to 1 GWW, was about 20% higher than per 1 GWW.

Numbers of bacteria indicatory of pollution and the sanitary state versus the degree of loading of the bottom *sediments in the Czarna Haiicza River with easily decomposing organic matter and human and animal faeces.*

Taking into account the numbers of TVC 20°C and FC in the bottom sediments of the two sections of the Czarna Hancza River (from Stary Brod above Suwalki to the outflow of Czarna Hancza to Lake Wigry, and from Czerwony Folwark to Wysoki Most, east of Lake Wigry), as well as the bacteriological criteria for bottom sediment loading given by Kavka [24] and Kohl [25] and modified by Albinger [2] (Table 5), the degree of sediment loading with organic matter and human and animal faeces in the Czarna Hancza River may be assessed as follows:

1. Bottom sediments of the river in the village Stary Brod (station 1) above Suwalki were characterized by very low loading with organic matter easily decomposed by heterotrophic bacteria, and by very low, moderate, moderately high or high loading with human and animal faeces.
2. Bottom sediments of Czarna Hancza River in the region of the sewage treatment plant in Suwalki (station 2), 10 m above the effluent discharge, were characterised by low or exceptionally low loading with organic matter decomposed by heterotrophic bacteria, and by low, moderate or moderately high loading with human and animal faeces.
3. Bottom sediments of the river in the village Sobolewo (stations 3 and 4) were characterized by very low loading with organic matter easily decomposed by heterotrophic bacteria, and by usually low or moderate loading with human and animal faeces.
4. Bottom sediments of the Czarna Hancza River in the region of the old river bed and its inflow to Lake Wigry (stations 5 and 6) were usually characterized by very low, rarely low or moderate loading with organic matter easily decomposed by heterotrophic bacteria, and by low, moderate or moderately high loading with human and animal faeces.
5. Bottom sediments of Czarna Hancza, in the river section from Czerwony Folwark to Wysoki Most (stations 7-10), were characterized by very low, exceptionally low (in the village Buda Ruska) and moderate loading with organic matter easily decomposed by heterotrophic bacteria, and by very low, low and moderate (in the villages Buda Ruska and Wysoki Most, stations 9 and 10), and by moderately high (in the villages Czerwony Folwark and Mackowa Ruda, stations 7 and 8) loading with human and animal faeces (Table 5).

Discussion

Numbers of indicatory bacteria in the bottom sediments of the Czarna Hancza River

Diversified bottom character in the examined sections of the Czarna Hancza River (sandy, gravel, loamy) as well as varying degree of water pollution [31] were reflected in different numbers of TVC 20°C, TVC 37°C, TC, FC, FS and *Clostridium perfringens* at particular sampling stations and in different villages. Lower numbers of all these bacteria indicatory of pollution and sanitary state were found in the sediment samples collected in Stary Brod above Suwalki (station 1), in the area of sewage treatment plant, 10 m above the effluent discharge in Suwalki (station 2), in Sobolewo village (stations 3 and 4) and in the river section from Czerwony Folwark to Wysoki Most (stations 7-10) compared to samples collected in the region of the old river bed and river inflow to Lake Wigry (stations 5 and 6). This was probably due to sandy or gravel bottom at the first stations. Contrary to this, higher numbers of the respective indicatory bacteria were found in the bottom sediments of Czarna Hancza River collected at river inflow to Lake Wigry and in the old river bed (stations 5 and 6), where the bottom was muddy.

Sandy and/or gravel bottom of the Czarna Hancza River at the majority of stations, with low organic matter content (dry weight after 24 h heating in 105°C amounted to about 80% of wet weight), does not favour nutrient absorption or colonization and survival of bacteria. Smaller or greater differences in the numbers of indicatory bacteria, found in the samples collected at Stary Brod, in the region of Suwalki sewage treatment plant, in Sobolewo (stations 1-4), and in the river section from Czerwony Folwark to Wysoki Most (stations 7-10), might have been due to a variety of reasons. Slightly higher numbers of these bacteria were found in the sediments collected in the vicinity of the sewage treatment plant in Suwalki (station 2), 10 m above effluent discharge to the river compared to the samples collected in Stary Brod above Suwalki (station 1); this was probably due to higher numbers of these bacteria in the river water [3]. On the other hand, low numbers of the respective indicatory bacteria in the sediments of Czarna Hancza River in Sobolewo (especially at station 3) notwithstanding their high numbers in the river water [33] may be explained by specific hydrologic conditions. Station 3 was located 2-3 m below a waterfall which had once been constructed at a water mill sluice. The waterfall prevented sedimentation and absorption of nutrients and organic matter by sand and gravel particles, thereby preventing absorption of indicatory bacteria from the water. Quite the opposite: turbulence and relocation of the bottom sediments caused by the waterfall must have enhanced liberation of these bacteria from the sediments. It is known from literatue [18, 19] that sand grains adsorb bacteria in a very loose way. Consequently, cascade water flow at this station might have played a similar role as river bed dredging, or high and low tides [8, 17, 28, 30, 34, 35]. Higher numbers of indicatory bacteria in the sediments of the Czarna Hancza River in the region of the old river bed and its inflow to Lake Wigry (stations 5 and 6) are explained by their higher numbers in the river water, and by bottom sediment, where clay-muddy sediments dominated (dry weight of the sediments after 24 h heating in 105°C was, respectively, 1.6 and 0.95% of their wet weight), favoring bacteria survival [3, 10, 11, 42] and ensuring physical protection against grazing [9, 21, 38], In addition to this, nutrient composition in such sediments is different than in water [14], probably more assimilable by the enteric bacteria. The discussed changes of the numbers of indicatory bacteria are presented in Fig. 12. This figure presents mean numbers of TVC 20°C, TVC 37°C, TC, FC and FS for the whole period of studies and consecutively for each of the 10 stations.

Seasonal changes of the numbers of indicatory bacteria in bottom sediments of the Czarna Hancza River.

Seasonal changes of indicatory bacteria in the bottom sediments of the Czarna Hancza River could have been connected with atmospheric conditions, precipitation, thun-

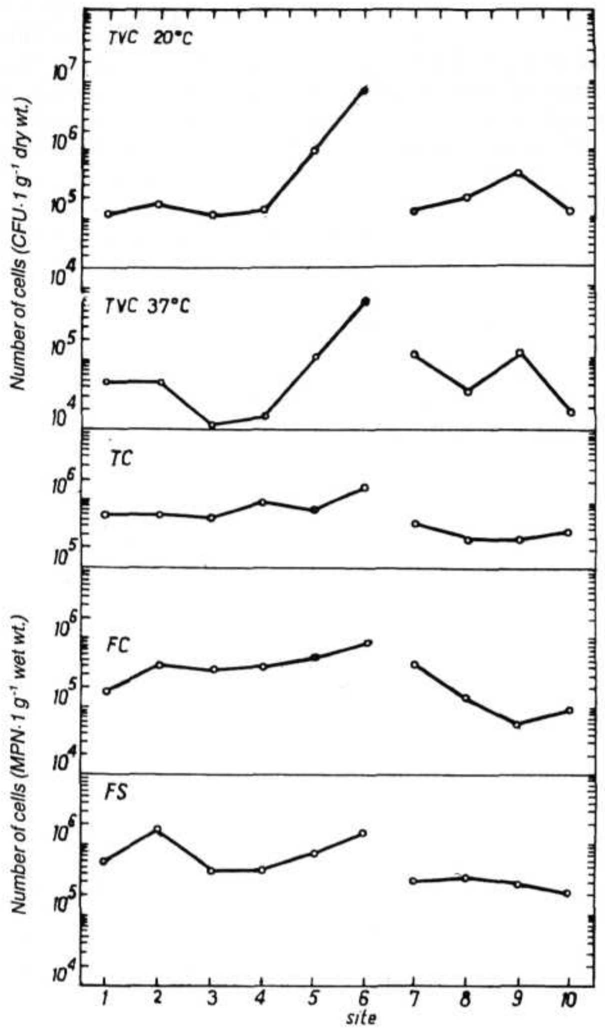

Fig. 12. Average number TVC 20°C, TVC 37°C, TC, FC, FS in the bottom sediments of investigated parts of the Czarna Hancza in 1994-1996.

der storms, seasonal temperature changes, salinity, and bacteria numbers in the river water, and in the section below Suwalki to its inflow to Lake Wigry (stations 3-6) - also with bacteria numbers discharged with the effluents from sewage treatment plant in Suwalki. Water fowl, especially ducks, might have also had a modifying effect. These birds excrete unproportionally high levels of coliforms and other bacteria [36]. It has been reported that FC numbers increased 100-fold in the sediments of water bodies following their colonization by water fowl (cit. 32) due to sedimentation of the bird faeces. It is also possible that bacteria proliferated during the first 24-48 hours after their release to water [18, 19, 41]. This increase in numbers may compensate for continuous bacteria mortalities under the effect of different factors. As a result, bacteria numbers (contrarily to their content in water) may remain at a more or less stable level for fairly long periods [31]. Hence, curves of bacteria numbers in the bottom sediments of the Czarna Hancza River rarely corresponded to the respective curves of their numbers in water. Lack of a relationship between TC, FC an FS numbers in water and in bottom sediments has often been mentioned [8, 16]. Mutual relations between numbers of these bacteria in water and their numbers in the bottom sediments are the function of continuous bacteria precipitation and resuspension [41], as well as their survival in the two biotopes.

FC:FS ratio in bottom sediments of Czarna Hancza River

Data on absolute values of FC:FS ratio in the bottom sediments of the Czarna Hancza River may not fully reflect the character of sediment contamination (by humans and/or animals). The effect of pollutants on river sediments depends on many factors. It is commonly known that in bottom sediments of water bodies, FS survive for a longer time than FC. Contrary to FC [14, 18, 19, 20] they do not proliferate outside human or animal food tracts [23]. Hence, their numbers cannot increase, but can only decrease. FC (but not FS) survival is significantly affected by the type of bottom sediments. Clay and loamy sediments favour FC survival compared to sandy and/or gravel bottom. Although FS survival is also higher in loamy and clay sediments, but in both types of sediments the differences in their survival were lower than in the case of FC [37]. High value of FC:FS ratio in the bottom sediments of the Czarna Hancza River in the region of the old river bed and river inflow to Lake Wigry (stations 5 and 6), where clay-loamy sediments dominated, and lower in sandy and/or gravel sediments collected in Stary Brod above Suwalki (station 1) and in the region of Suwalki treatment plant, 10 m above the effluent discharge (station 2), as well as in the sediments collected along the river section from Czerwony Folwark to Wysoki Most, east of Lake Wigry (stations 7-10), might have resulted from the differences in FC and FS absorption [7], and in different survival of these two groups of indicatory bacteria. In addition to this, FS are characterized by higher hydrophobicity than *Escherichia coli* [22], and by an ability to adhere to clay bottom sediments. Consequently, fewer FS are extracted from such sediments during sample preparation for microbiological studies [1]. This may effect the results in determining the numbers of the two groups of bacteria, and - thus - also in the value of FC:FS ratio. Low temperature of bottom sediments in spring and autumn (there were no bacteriological studies in winter) may hamper regrowth of FC and cause mortalities of *Streptococcus bovis*, thereby causing the FC:FS ratio to remain at a level suggesting pollution of animal origin [13]. FC:FS ratios given in literature [12] were within the range from to 315 during 40 hours, at water temperature of 21°C, in surface runoffs polluted with animal faeces. This was caused by an almost 330-fold increase of FC numbers, while FS numbers did not change.

Final remarks

It is known from literature that bottom sediments represent a most stable habitat for enteric bacteria from the genera *Escherichia, Salmonella, Shigella, Streptococcus* and other (cit. 31). Bacteriological examination of bottom sediments may be taken advantage of to conclude on river water quality. Bacteriological analysis of bottom sediments can also be used to predict the degree of water contamination by bacteria remaining at the river bottom due to a variety of reasons (spring and autumn high waters, bottom dredging, turbulence caused by motor boats, navigation, rowing). Bacteriological examination of water reflects water pollution only at the moment of sampling. Sampling and analyses of water along the whole river length, in the middle of current and close to the banks, above and below pollution discharge seems impractical due to high costs and considerable time demand. If bacteriological analyses of bottom sediments were included in the examination of river quality, this problem would be solved [3]. Time needed to perform bacteriological analysis of water is an important factor that must be taken into consideration in establishing sampling sites. So far there is no legislation requiring bacteriological examination of bottom sediments for the content of bacteria indicatory of pollution and sanitary state. Moreover, there are no standard methods of sample collection ensuring representative results of analyses. As numbers of bacteria indicatory of sanitary state and of enteric bacteria decrease with depth; it has been suggested [1] that the surface sediment layer (0-1 cm) be sampled. Examination of deeper layers may yield underestimated results. This is especially important in determining FC numbers.

Conclusions

1. Bottom sediments of the Czarna Hancza River differed with respect to the numbers of bacteria indicatory of pollution (TVC 20°C, TVC 37°C) and sanitary state (TC, FC, FS, *Clostridium perfringens).* The lowest nu mbers were found in the case of sandy and/or gravel bottom, the highest in clay-loamy sediments.
2. Lower numbers of indicatory bacteria found in the san dy and gravel bottom of the Czarna Hancza River in Sobolewo (station 3) compared to other villages in which river bottom was of the same character (stations 1, 2a, 4, 7-10) were due to cascade water flow and bacteria and nutrient washing out from sand and gravel particles.
3. Seasonal changes in the numbers of indicatory bacteria in different types of bottom sediments of the Czarna Hancza River may be caused by thunderstorms, changes in bacteria numbers in the effluents discharged from the local sewage treatment plant, temperature in water and sediments, or survival of bacteria.
4. Sandy and/or gravel bottom of the Czarna Hancza River at the majority of sampling stations, and loose attach ment of indicatory bacteria to sand grains results in the fact that even small movements (dredging, motor boats, rowing boats) may liberate to water bacteria indicatory of sanitary state and enteric bacteria.

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