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Potentially Pathogenic Microorganisms in Water and Bottom Sediments in the Czarna Hańcza River

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Abstract

This work comprises the results of a number of *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Staphylococcus sp.* in water and bottom sediments in the Czarna Hancza River in the region of Suwalki and Wigry National Park from spring to autumn in 1996. All these microorganisms were found in smaller quantities in water, and in larger quantities in the bottom sediments in the Czarna Hancza River. Their number was generally higher than the number of faecal bacteria of *Escherichia coli* group both in water and bottom sediments in this river at site 1 (in Stary Brod above Suwatki) and at sites 7-10 (in Czerwony Folwark, Mackowa Ruda, Buda Ruska, Wysoki Most, to the east of Wigry Lake). Their number was close to or lower than the number of faecal bacteria of *Escherichia coli* group at the sites situated above and below the inflow of treated sewage from the Treatment Sewage Plant in Suwalki (2a and 2b sites), in Sobolewo (3 and 4 sites), in the region of the old river-bed of the Czarna Hancza River (5 site) and its mouth to Wigry Lake (6 site). There were fewer at the sites of sand deposits, more at the sites of clay-argillaceous deposits. In the research period they were more numerous in the second half of summer. The number of *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Staphylococcus* sp. should be taken into account as well as the number of the indicators bacteria of a sanitary state (total coliforms, faecal coliforms and faecal streptococci) while estimating the usefulness of water in the Czarna Hancza River for recreation.

Keywords: river, water, bottom sediments, opportunistic pathogens, *Pseudomonas aeruginosa, Aeromonas hydrophila, Staphylococcus* sp.

Introduction

The estimation of bacteriological quality of surface and underground waters based on classical sanitary indicators (total coliforms, faecal coliforms, faecal streptococci) may not reflect their safety for the health of bathing people and/or using water for drinking and household purposes. Numerous human diseases having bath in rivers, lakes, ponds and coastal sea waters in the area of river and sewage inflow, swimming pools are associated with the presence of opportunistic pathogens from *Pseudomonas, Aeromonas, Staphylococcus* and other microorganisms groups, being able to generate infections by contact with skin, mucous membrane, nosopharyngeal cavity, respiratory ducts, eyes, ears and urogenital passages. Pyogenic infection of injuries, meningitis, urinary system, respiratory system, inflammation of the middle ear and eyes are typical diseases caused by contaminated water where *Pseudomonas aeruginosa* are found [8, 19, 34, 56, 61, 74].

Wound infections, peritonitis, meningitis, endocarditis, septicemia, corneal ulcers, nosocomial infections, urinary tract infections, gastroenteritis of people who bathe and/or use water in other ways are caused by *Aeromonas hydrophila* [25, 36, 38, 41, 42, 44, 45, 68, 69]. Infections of skin, nosopharyngeal cavity, eyes, outer ear among bathing people could be caused by recreational waters polluted by *Staphylococcus aureus* [65, 72, 76]. All the above mentioned species of bacteria survive in water longer than classical indicators of sanitary state and they are not connected with faecal contamination present in water. *Pseudomonas aeruginosa* and *Aeromonas hydrophila* occur in water and bottom sediments of river [16, 33, 40, 48, 50, 57, 63], lake [59, 67, 72], estuaries [21, 23, 39, 64, 75], sea coastal waters in the zone of pollutants effluent from the land [3, 4, 7, 10, 31, 49], sewages [12, 32, 51, 52, 73], soil [13], fish [27, 28], food [58], drinking water [37, 47]. Up till now the maximum number of these bacteria in surface water useful for recreation has not been officially stated, though the above mentioned literature suggests the necessity of including them in the system of bacteriological indicators of water quality. In Poland one cannot find published data on the number of these bacteria in surface waters. The present research presents the results on the number of *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Staphylococcus* sp. in water and bottom sediments of the Czarna Hancza River in the recreational period. The river is utilized, especially in summer, for canoeing by Polish and foreign tourists for its beautiful landscape. Therefore, knowledge of sanitary-bacteriological states of water and bottom sediments of this river and potential sources of bacteriological contamination seem to be significant.

Materials and Methods

The Czarna Hancza River

The Czarna Hancza River is a main river in the basin of the Niemen and the largest one in the area of Wigry National Park. It flows out of a small Lake Jegliszki on Suwalki Northern Lakeland at 230 m a. s. 1. In the upper course it flows across Hancza Lake. In the middle course it meanders stronly, further (in Rygel) it is sewered and is included in the system of the Augustowski Canal. It outflows near Warwiszki Bialostockie. There is a great river gradient, typical for mountain rivers, between springs and Wigry Lake. The river stretch from Wigry Lake to the border of Poland is typical for a lowland river. The Marycha River is main tributary of the Czarna Hancza River. Below Suwalki there is a canal mouth supplying treated sewage from Waste Water Treatment Plant in Suwalki. Wastes are treated with the third degree. Detailed characteristics of the river and the Waste Water Treatment Plant in Suwalki have been presented in other papers [54, 55].

Sampling

Water samples were taken from the Czarna Hancza River directly to sterilized glasses with a grinded cork (300 ml) in the current at 11 sites (every month) from June to November 1996. Sewen sites were situated from Stary Brod above Suwalki up to the mouth of the Czarna Hancza River to Wigry Lake, four sites were found from Czerwony Folwark to Wysoki Most to the east of Wigry Lake. The samples of bottom sediments were taken by a special device to sterille glasses with a twist cover being sterilized by boiling for 30 minutes in distilled water from the same sites as water (except site 2b situated 10 m below the inflow of treated wastes from the Treatment

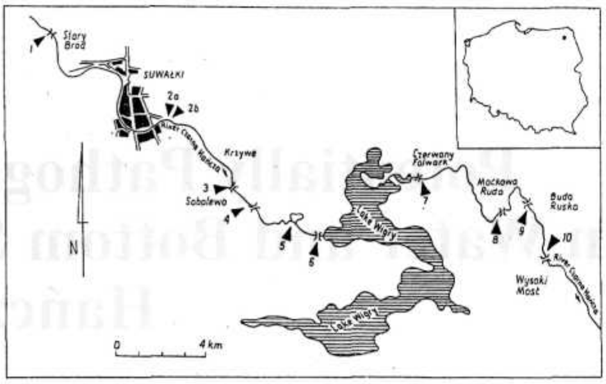


Fig. 1. Situational sketch of Czarna Hancza River in the region of Suwalki and the area of Wigry National Park.
1.2,3 ... 10 sites for collecting water and bottom sediment samples.

Plant in Suwalki because of stony river bed). Surface layer of bottom sediments 1 cm was collected. The following sites were found:

Site 1 - in Stary Brod above Suwalki, with a sandy bed;
Site 2a - below Suwalki, 10 m above the inflow of the treated wastes from the plant in Suwalki, with a sandy-gravel bed; Site 2b - below Suwalki, 10 m below the inflow of the treated wastes from the plant in Suwalki, with stony bed; Site 3 - in Sobolewo at a bridge on the road Krzywe-Sobolewo, with a sandy bed; Site 4 - in Sobolewo at the second bridge on the road Krzywe-Sobolewo, with a gravel bed; Site 5 - in the region of the old river-bed of the Czarna Hancza on wet and wooded area, with a clayey-argillaceous bed; Site 6 - at the bridge about 100 m before the mouth of the Czarna Hancza to Wigry Lake with a argilloarenaceus bed; Site 7 - in Czerwony Folwark at a bridge near Postaw Lake, with a sandy bed;
Site 8 - in Mackowa Ruda at a bridge, with a sandy bed;
Site 9 - in Buda Ruska at a bridge, with a sandy bed;
Site 10 - in Wysoki Most at a bridge, with a sandy bed;

Sites 1-4 and 10 were situated outside Wigry National Park; sites 5-9 were situated within Wigry National Park (Fig- 1)-

The time from the moment of taking the samples until carrying out analysis did not exceed 12 hours. The water samples and bottom sediments were kept at 4-6°C.

Microbiological Examinations

Microbiological examinations comprised the estimation of the number of the following bacteria: *Pseudomonas aeruginosa, Aeromonas hydrophila and Staphylococcus* sp.

The number of *Pseudomonas aeruginosa* (*CFU/100 ml water or 1 g of fresh mass of bottom sediments) was

* CFU - Colony Forming Unit

determined on Bacto Pseudomonas Agar P with 7.0 pH (20), after 48 h incubation at 41.5°C.50 ml of river water or 1 g of fresh mass of bottom sediments diluted in 50 ml of distilled water was filtered through a membrane filter. Millipore filters, GS Type, 47 mm diameter and 0.22 µm pores were used. The filters were placed on the surface of agar substratum Pseudomonas Agar P on Petri's plates and incubated in an upside down position. The production of blue-green pyocianine *Pseudomonas aeruginosa* pigment was checked in the light of Wood's lamp [20]. The number of *Aeromonas hydrophila* (CFU/100 ml water or 1 g of fresh mass of bottom sediments) was determined on mA agar [50] after 24 h incubation at 37°C [14]. The substratum contained trehalose as a source of carbon and ampicilin and ethanol as inhibitors of bacteria growth. The presence of *Aeromonas hydrophila* was observed in a fermentation sample with mannitol and in a test for cytochromic oxidase. 10 ml water or 1 g of fresh bottom sediment mass in 50 ml distilled water was filtered through a membrane filter as has been mentioned above. The filters were placed on the surface of mA agar substratum on Petri's plates and incubated for 24 h in an upside down position. Typical *Aeromonas hydrophila* colonies were counted (round, convex, 1-3 mm diameter). Taken colonies at random were split up and later examined in a biochemical test to prove the existence of *Aeromonas hydrophila* [59]. The number of *Staphylococcus sp.* (CFU/100 ml water or 1 g fresh mass of bottom sediments) was determined on Chapman's substratum (Bacto Staphylococcus Medium 110) according to Difco [20] after 24 h incubation at 37°C [14]. 10 ml water or 1 g mass of bottom sediments in 50 ml distilled water was filtered through a membrane filter as above. The filters were placed on the surface of Chapman's agar substratum on Petri's plates and incubated in an upside down position. Typical *Staphylococcus* sp. colonies were checked at random in preparates dyed by Gram's method.

All the determinations were carried out in three parallel repetitions from the same sample. The obtained values of the above mentioned bacteria were referred to the frequency of their detection in waters (bottom sediments) with different numbers of indicators of pollution degree (total number of bacteria on broth-agar at 20 and 37°C) and sanitary state (total coliforms, faecal coliforms, faecal streptococci) and yeasts and maximum number of their amount acceptable for recreational waters being presented in literature. The detailed data concerned the above mentioned indicators of pollution degree and sanitary state were given in other papers [54, 55]. The unpublished data concerned yeasts are found in the authors of this paper. Totally 66 water samples, 59 bottom sediment samples taken from the Czarna Haricza River and 6 samples of treated sewage taken from a sewage treatment plant in Suwalki were examined.

Results

Number of Opportunistic Pathogens in the Czarna Hancza River

Pseudomonas aeruginosa. **In** the Czarna Hancza River the number of *Pseudomonas aeruginosa* at site 1 (in Stary Brod above Suwaiki), 2a and 2b (above and below the inflow of treated sewage from a sewage treatment plant in Suwalki) did not exceed 8, 24 and 32 CFU/100 ml, respectively. In the water sample taken at side 1 in

Table 1. Mean (for study period) and range for the numbers of *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Staphylococcus* sp. in the water and bottom sediments of the Czarna Hancza River in 1996.

Opportunistic pathogens	[1]Site										
	1	2a	2b	3	4	5	6	7	8	9	10
Water[2] (CFU/100 ml)											
Pseudomonas aeruginosa	1 0-8	4 0-24	7 0-32	0 0	0 0	0 0	0 0	1 0-4	210 0-1060	280 0-770	100 0-340
Aeromonas hydrophila	3980 0-10800	4500 0-15100	55380 0-328000	4420 0-19800	2860 0-13500	3360 0-14800	1800 0-2100	1980 0-9600	2560 0-5200	2610 0-6900	2660 0-6600
Staphylococcus sp.	2600 0-10000	5350 0-17400	29730 0-128000	8660 0-23200	11010 0-35600	8080 0-27800	10300 0-42500	760 0-2600	1000 0-2300	2660 0-5600	3600 0-13200
Bottom sediments[3] (CFU/1 GWW)											
Pseudomonas aeruginosa	0 0	4 0-15	– –	115 0-700	2 0-15	0 0	0 0	0 0	0 0	1 0-10	1 0-10
Aeromonas hydrophila	46830 80-135000	97210 240-477000	– –	9900 835-27000	7950 645-237000	161000 415-394000	101000 1900-256000	83800 300-460000	30600 250-94000	14200 710-34000	39600 120-94000
Staphylococcus sp.	4400 15-12500	4370 120-19200	– –	5350 50-25600	2520 60-13600	955 105-3800	1610 125-5200	2800 170-11300	2440 25-12200	515 35-2050	1890 140-5800

[1] See Fig. 1; [2] CFU Colony Forming Unit; [3] GWW Gram Wet Weight

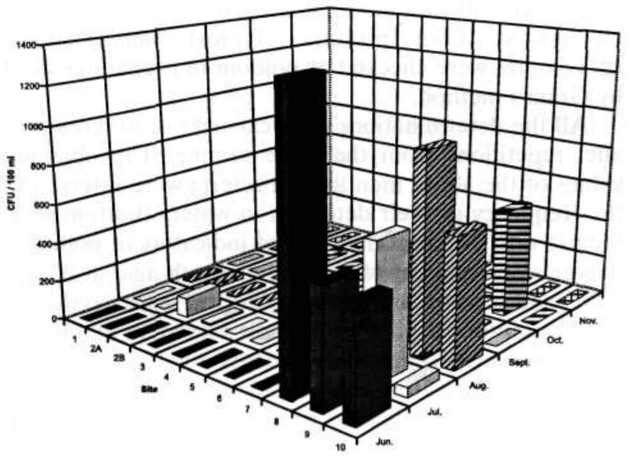


Fig. 2. Number of *Pseudomonas aeruginosa* in the water of the Czarna Haricza River.

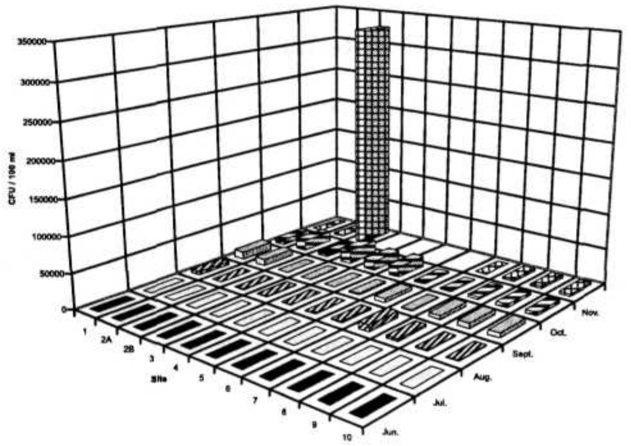


Fig. 3. Number of *Acromonas hydrophila* in the water of the Czarna Hancza River.

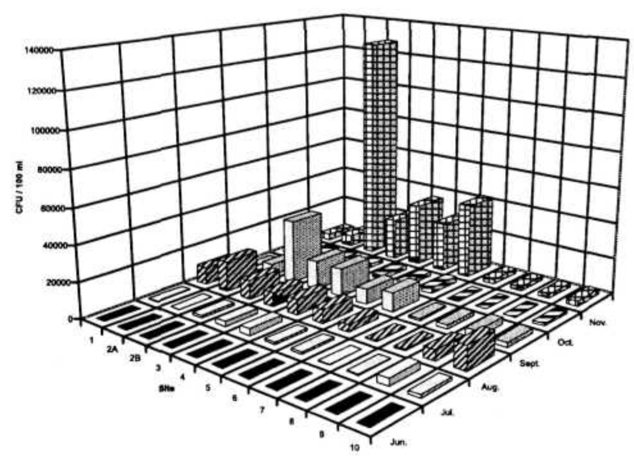


Fig. 4. Number of *Staphylococcus* sp. in the water of the Czarna Hancza River.

Stary Brod above Suwatki and at site 2a, 10 m above the inflow of the treated sewage from a sewage treatment plant in Suwalki they were found only in August 1996, at site 2b, 10 m below the inflow of the treated sewage from a sewage treatment plant in Suwalki they were found in July, August and October. In the water of the Czarna Hancza River taken in Sobolewo the bacteria were found only at site 3 in July 1996. They were not found in the region of the old river bed of the Czarna Hancza River (site 5) and its inflow to Wigry Lake (site 6). The least amount was found at site 7 in Czerwony Folwark (1-4 CFU/100 ml) in the Czarna Hancza River to the east from Wigry Lake. At sites 8, 9 and 10 (in Mackowa Ruda, Buda Ruska and Wysoki Most) their number ranged from 0 to 1060 CFU/100 ml, from 0 to 770 CFU/100 ml and from 0 to 340 CFU/100 ml, respectively. At site 7 they were found only in July, at site 8 in June and August, at site 9 - during the whole period of the experiment, at site 10 in June, July and August 1996 (Fig. 2, Table 1).

Aeromonas hydrophila. The amount of *Aeromonas hydrophila* in the water of the Czarna Hancza River at site I (in Stary Brod above Suwalki) did not exceed 10,800 CFU/100 ml (in September 1996). In June and July 1996 they were not found. Approximate number of these bacteria was found in the water collected at site 2a 10 m above the inflow of the treated sewage from a sewage treatment plant in Suwalki. In the water collected at site 2b 10 m below the inflow of the treated sewage maximum number of these bacteria was 32,800 CFU/100 ml (in November 1996). In the water collected at sites 3, 4, 5 and 6 (in Sobolewo and in the region of the old river bed and the inflow of the Czarna Hancza River to Wigry Lake) their number did not exceed 19,800, 13,500, 14,800 (in October 1996) and 5100 (in September 1996) CFU/100 ml, respectively. In the water of the Czarna Hancza River from Czerwony Folwark to Wysoki Most (to the east from Wigry Lake) the number *of Aeromonas hydrophila* did not exceed 9600 CFU/100 ml at site 7 in August 1996 and 5200, 6900 and 6600 CFU/100 ml at site 8, 9 and 10 in September 1996, respectively. In June and July they were not found in the samples (Fig. 3, Table 1).

Staphylococcus sp. In the Czarna Hancza River at site 1 (in Stary Brod above Suwalki) the number of *Staphylococcus* sp. did not exceed 10000 CFU/100 ml (in August 1996). They were not found in June 1996. In the Czarna Hancza River from Suwalki to its inflow to Wigry Lake the number of these bacteria sometimes reached several thousand CFU/100 ml (in September 1996 at the site 2b situated 10 m below the inflow of the treated sewage from a sewage treatment plant in Suwalki and in November 1996 at sites 3 and 4 in Sobolewo and 5 and 6 in the region of the old river bed and its inflow to Wigry Lake) and more (12,800 CFU/100 ml in November 1996 at the site 2b). From Czerwony Folwark to Wysoki Most (sites 7-10) the number of these bacteria reached maximum values 2600 CFU/100 ml in Czerwony Folwark, 2300 CFU/100 ml in Mackowa Ruda, 5600 CFU/100 ml in Buda Ruska and 13,200 CFU/100 ml in Wysoki Most. The highest number in Czerwony Folwark and Mackowa Ruda was found in November 1996, in Buda Ruska and Wysoki Most in August 1996 (Fig. 4, Table 1).

Number of Opportunistic Pathogens in Bottom Sediments of the Czarna Hancza

Pseudomonas aeruginosa. These bacteria were not found in 1 g fresh mass of the bottom sediments of the Czarna Hancza in Stary Brod above Suwaiki (site 1) and in the region of the old river bed (site 5) and its inflow to Wigry Lake (site 6). 10 m above the inflow of the treated sewage from a sewage treatment plant in Suwatki (site 2a) they were found only in August and November in amounts not exceeding 15 and 10 CFU/1 GWW, respectively. In the bottom sediments collected in Sobolewo at site 3 they were found only in June 1996 (700 CFU/1 GWW), at site 4 in September 1996 (15 CFU/1 GWW). They were not found in the old river bed and the inflow of the Czarna Hancza to Wigry Lake. In the Czarna Hancza from Czerwony Folwark to Wysoki Most they were only found in June 1996 at the sites 9 and 10 but their amount did not exceed 10 CFU/1 GWW (Fig. 5, Table 1).

Aeromonas hydrophila. These bacteria were found in the bottom sediments of the Czarna Hancza within the whole period of the experiment, the highest amounts were noticed in spring and summer. At site 1 (in Stary Brod above Suwaiki) their number ranged from 80 to 135,000 CFU/1 GWW. From Suwaiki to the river inflow to Wigry Lake their number ranged from 240 CFU/1 GWW to several hundred thousand cells in 1 GWW. On the average the least number of these bacteria in the bottom sediments was found at site 3 in Sobolewo, the highest one at site 5 in the region of the old river bed of the Czarna Hancza. From Czerwony Folwark to Wysoki Most (sites 7-10) to the east from Wigry Lake their number ranged from 120 CFU/1 GWW in November 1996 at the site 10 (in Wysoki Most) to 460,000 CFU/1 GWW in July 1996 at site 7 (in Czerwony Folwark). On the average the lowest amount in bottom sediments at site 9 (in Buda Ruska), the highest amount was found at the site 7 (in Czerwony Folwark) (Fig. 7, Table 1).

Staphylococcus sp. In the bottom sediments of Czarna Hancza at the site 1 (in Stary Brod above Suwaiki) the number of these bacteria ranged from 15 CFU/1 GWW (in October 1996) to 12,500 CFU/1 GWW (in June 1996). In the Czarna Hancza from Suwatki to its inflow to Wigry Lake the number of these bacteria ranged from 50 CFU/1 GWW to 25,600 CFU/1 GWW. On the average the least number was found in the bottom sediments collected at site 5 (in the region of the old river bed of the Czarna Hancza), the highest number was found at site 3 in Sobolewo. They were the lowest at all sites in October and November 1996, the highest number was found in June 1996. In the Czarna Hancza from Czerwony Folwark to Wysoki Most (sites 7-10) to the east from Wigry Lake the number of these bacteria ranged from 25 CFU/1 GWW to 12,500 CFU/1 GWW. On the average the least number was found in the bottom sediments collected at site 9 (in Buda Ruska), the highest number was found at sites 7 and 8 (in Czerwony Folwark and Mackowa Ruda). The highest number of these bacteria in the bottom sediments collected at sites 7 and 8 was found in June 1996, in the bottom sediments collected at sites 9 and 10 in September and July 1996, respectively (Fig. 7, Table 1).

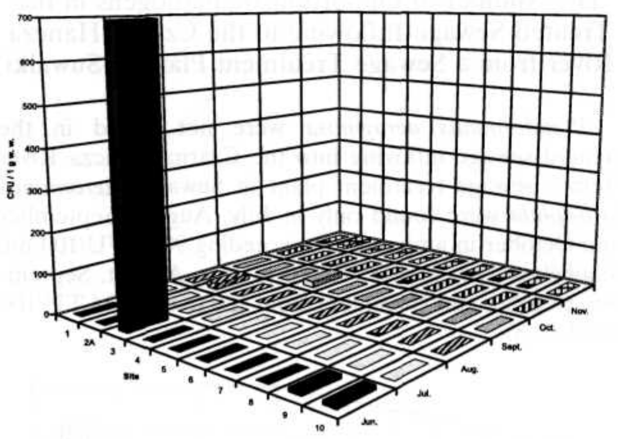


Fig. 5. Number of *Pseudomonas aeruginosa* in the bottom sediments of the Czarna Hancza River.

Fig. 6. Number of *Aeromonas hydrophila* in the bottom sediments of the Czarna Hancza River.

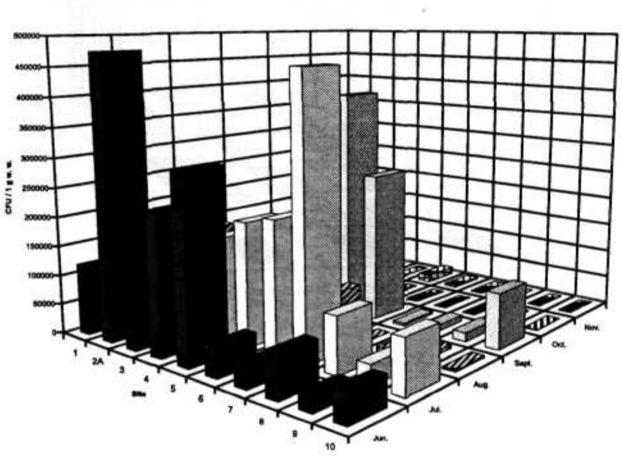
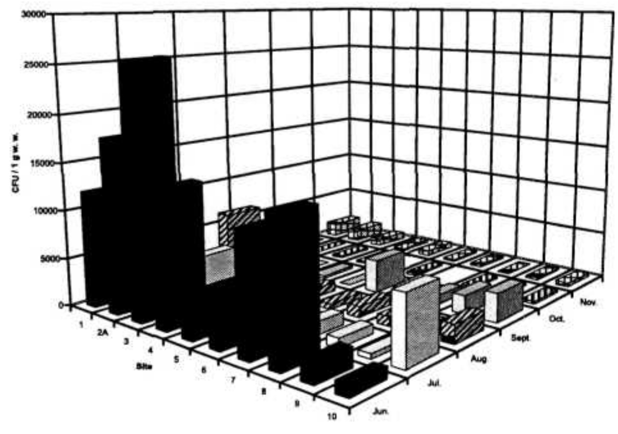


Fig. 7. Number of *Staphylococcus* sp. in the bottom sediments of the Czarna Hancza River.



The Number of Opportunistic Pathogens in the Treated Sewage Inflowing to the Czarna Hancza River from a Sewage Treatment Plant in Suwalki

Pseudomonas aeruginosa were not found in the treated sewage infloving into the Czarna Hancza River from a sewage treatment plant in Suwatki. *Aeromonas hydrophila* were found only in July, August, September and October in amounts not exceeding 900 CFU/100 ml. *Staphylococcus* sp. were found in July, August, September, October and November from 180 to 62,700 CFU/100 ml (Fig. 8).

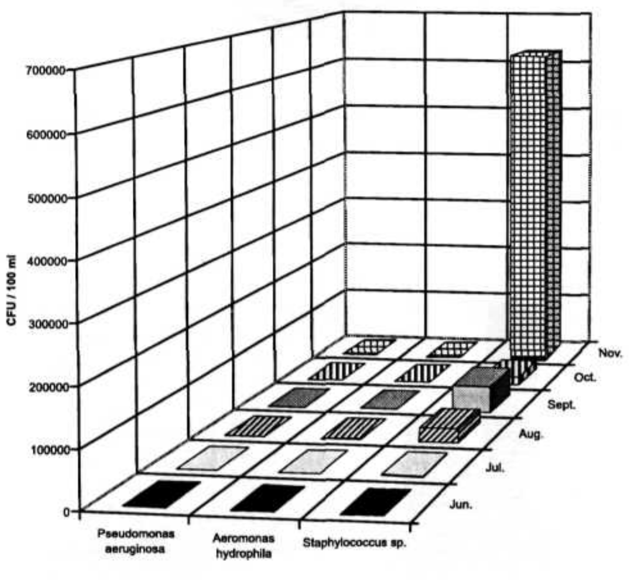


Fig. 8. Number of *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Staphylococcus* sp. in treated sewage infloving into the Czarna Hancza River from a sewage treatment plant in Suwatki.

Table 2. Frequency (%) distribution of *Pseudomonas aeruginosa, Aeromonas hydrophila* and *Staphylococcus* sp. in the water and bottom sediments of the Czarna Hancza River with different level of faecal coliforms.

Faecal coliforms	*Pseudomonas aeruginosa*	*Aeromonas hydrophila*	*Staphylococcus* sp.
[1](MPN/100 ml)	Water		
< 100	68	52	41
100-1000	0	13	7
>1000	32	35	52
[2](MPN/1 GWW)	Bottom sediments		
< 100	50	47	47
100-1000	27	29	29
> 1000	17	24	24

[1] MPN - Most Probable Number
[2] GWW - Gram Wet Weight

Discussion

Pseudomonas aeruginosa regarded as organisms of a faecal origin [8, 19, 24, 26, 73, 74] was found in the Czarna Hancza River seldom in the amount exceeding 100 MPN/100 ml. They are values above which inflammatory states of ears and eyes of swimmers in such water may be observed [35]. Maximum numbers of these bacteria were most frequently found in the waters of the Czarna Hancza River from Czerwony Folwark to Wysoki Most (sites 7-10) to the east from Wigry Lake were close to the given ones [65, 67, 72] for some waters of the lakes and rivers in Canada and in the United States. It is interesting that this part of the Czarna Hancza River is most often used for recreational purposes (canoeing) by Polish and foreign tourists in the summer season. Especially higher amounts of *Pseudomonas aeruginosa* were found once in the bottom sediments of the Czarna Hancza River at site 3 in Sobolewo but they were associated with a higher number of FC [55]. In other seasons at the same and other sites such dependence was not found. On the contrary higher amounts of *Pseudomonas aeruginosa* were rather associated with a lower number of FC (Table 2). These bacteria were not even found in the above mentioned quantities in the treated sewage inflowing into the Czarna Hancza River from a waste treatment plant in Suwalki containing 14,000 - 1,100,000 MPN/100 ml FC [54]. However, Guimares et al. [26] isolated these bacteria from coastal sea waters at different degrees of faecal contamination but in higher quantities from water samples containing numerous FC and FS. These authors emphasize that in non-polluted waters frequency of isolating *Pseudomonas aeruginosa* was still quite high. Foster et al. [24] quote Costerton's data from 1979 according to which *Pseudomonas aeruginosa* is a penetrating organism. It is found in soil, water, sewage, homoiothermic animal excrement, on plants from which it gets to surface waters during rainfalls. Marsalek et al. [59] found them in the water of the St. Clair River in Sarnia in the amount of 361-2137 CFU/100 ml in "dry" season and 1570-173,000 CFU/100 ml during "wet" season. Pellet et al. [56] examining the occurrence of *Pseudomonas aeruginosa* in different ecosystems of the Mississippi River in the USA (water, bottom sediments, plants, "Aufwuchs", surface layer of mucus and gastric contents of fish) state that *Pseudomonas aeruginosa* is an autochtonic organism of biological biocenosis of waters and algal blooms may stimulate their development. Regardless of the auto- or allochtonous origin of *Pseudomonas aeruginosa* it is an epiphyte bacteria and it occurs in surface water at the boundary of phases "stable parts-water" and in bottom sediments, and due to bottom deeping or swimming activities [65, 66] it may get into the water.

Mean number and the range of fluctuation of *Aeromonas hydrophila* in the Czarna Hancza River did not differ from the given ones in literature (Table 3) for rivers, lakes, estuaries, coastal sea waters in the region of bathing places. Their higher number was found at site 2b situated 10 m below the inflow of treated sewage from a waste treatment plant in Suwalki (on the average 55,300 CFU/100 ml) than at other sites on the river (on the average 1100-4500 CFU/100 ml) that may be associated with a higher number of organic substance easily decomposed by these bacteria. Examinations of the number of

Table 3. Number of *Aeromonas* in water and bottom sediments of some water reservoirs according to literature data.

Aeromonas Type	Water/sediments	Number of bacteria	No. ref.
1	2	3	4
1. *Motile Aeromonas*	Sea water at Spain (North of Valencia): in Puebla de Farnals site in Port Saplaya site in Puig site in Puzol site	 $2.2\text{x}10^4 - 9\text{x}10^6$ [a]CFU/100 ml $2.0\text{x}10^3 - 4.1\text{x}10^5$ CFU/100 ml $5.0\text{x}10^2 - 2.0\text{x}10^4$ CFU/100 ml $1.0\text{x}10^2 - 2.4\text{x}10^4$ CFU/100 ml	3
2. *Aeromonas* spp.	Freshwater with low FC number Freshwater with high FC number	$1.0\text{x}10^5$ CFU/100 ml $1.0\text{x}10^7$ CFU/100 ml	5
3. *Aeromonas* spp.	Sea water at Spain coast in Casteldefels River water: River Duero next Soria Rivers Besos and Llobregat 3 km from the mouth to the sea Sewage canals in Barcelona	$4.0\text{x}10^3 - 1.2\text{x}10^4$ CFU/100 ml $9.7\text{x}10^2 - 4.5\text{x}10^3$ CFU/100 ml $5.0\text{x}10^5 - 7.2\text{x}10^7$ CFU/100 ml $1.6 - 5.8\text{x}10^3$CFU/100 ml	6
4. *Aeromonas* spp. (60% A. hydrophila)	Fresh and brackish water	$120 - 1.2\text{x}10^6$ CFU/100 ml	9
5. *Aeromonas hydrophila*	Lake water Par Pond (thermally enriched monimictic south-eastern lake which receives heated effluent from a production nuclear reactor) – a coling reservoir Savannah River Plant near Aiken, South Carolina	[b]ND-300 CFU/100 ml	22
6. *Aeromonads*	Estuarine waters of the Italian coast of the Adriatic sea (along North-Central coast of the Adriatic sea) – samples from the mouth of the Metauro River – samples from the mouth of the Foglia River	 $10^2 - 10^5$ CFU/100 ml $10^1 - 10^6$ CFU/100 ml	23
7. *Aeromonas hydrophila*	Albemarle Sound (in northeast corner of North Carolina – a natural estuary with 2 major tributaries: the Hoanoke River and Chowan River)	ND – 10^3 CFU/1 ml	31
8. *Aeromonas hydrophila*	Hypersaline lakes: Badwater Lake and Great Salt Lake Extremally polluted rivers: Wabash, Mississippi, San Antonio Geothermal springs: White Done Geyser, Black Sand Basin	746 CFU/1 ml (average) 161 CFU/1 ml (average) 20 CFU/1 ml (average)	33
9. *Aeromonas hydrophila*	Chesapeake Bay (water) – at Jones Falls (station 4) water: sediment: – in Chester River (station 3) water: sediment:	ND – $5.0\text{x}10^5$ [c]MPN/100 ml ND – $5.0\text{x}10^4$ MPN/100 ml $9.0\times10^2 - 4.6\times10^4$MPN/100cc ND – 15 MPN/100 ml ND – 3.3 MPN/100 cc	39
10. *Aeromonas* spp.	Rivers: Ohta and Osa (Japan)	10^3/1 ml at 27°C 10^1/1 ml at 20°C	40
11. *Aeromonas* spp.	Open storage reservoirs from surface water	$1\times10^4 - 1\times10^6$ CFU/1 l	43
12. *Aeromonas* organisms	Raw water: surface water ground water artificial ground water	$250 - 1\times10^6$ CFU/100 ml 0 – 6 CFU/100 ml 5 – 900 CFU/100 ml	45
13. *Aeromonas* spp.	Resources of drinking water in a mountainous region North-East Italy (the Dolomites)	1 – 240 CFU/100 ml	48
14. *Aeromonas hydrophila*	Beaches of the Mediterranean littoral Malaga, Spain in a bathing season: Santa Ana Misericordia	11 – 405 CFU/100 ml 3297 – 40366 CFU/100 ml	49
15. *Aeromonas* spp.	Metauro and Foglia Rivers in the region of small urban area and areas which are mostly used for agriculture/animal breeding (Pesario-Urbino Province, Italy)	$0 - 1\times10^4$ CFU/100 ml	57
16. *Aeromonas hydrophila*	Water from Rhode Island ponds, lakes and reservoirs	1.3 – 19049 CFU/100 ml	60

Table 3 continued

1	2	3	4
17. *Aeromonas* sp.	Crude water from a reservoir of drinking water (Trinkwasserversorgung Talsperrenwasser aus der Feilebachtalsperre)	15 – 4200 CFU/100 ml	70
18. *Aeromonas hydrophila*	Lakes, rivers and marine beaches in the Pacific Northwest region of the U. S. suitable for swimming, skiing and other recreational uses	0 – 9300 CFU/100 ml	72
19. *Aeromonas hydrophila*	Marine (estuarine) sediments of the Apalachicola Bay, Florida in the region of shellfish production: Water: sediments: (0 – 2.5 cm layer):	11 – 24000 CFU/1 ml 1 – 24000/[d]GWW	75

[a] CFU - Colony Forming Unit; [b] ND - Not Detected; [c] MPN - Most Probable Number; [d] GWW - Gram Wet Weight

bacteria determined on broth-agar at 20°C (TVC 20°C) and 37°C (TVC 37°C), total coliforms (TC), faecal coliforms (FS) and faecal streptococci (FS) in the Czarna Haficza River water [54] snowed respectively higher or lower numbers only for TVC 20°C and TVC 37°C. Both groups of heterotrophic bacteria are regarded as indicators of pollution degree of water and bottom sediments by organic substance easily assimilated [2,11, 46,96]. The dependence among the number of *Aeromonas hydrophila* and the number TVC 20°C and TVC 37°C in waters was found by Kersters et al. [41, 42, 43] and LeChevallier et al. [46, 47]. They think that *Aeromonas hydrophila* are a specific part of a general number of heterotrophic bacteria in waters. However, the examination of the number of *Aeromonas hydrophila* and heterotrophic bacteria (heterotrophic plate count, in brief HPC) in the resources of drinking water carried out by Havelaar et al. [29] did not show dependence between these bacteria groups. The data of the present paper presented in Tables 4 and 5 suggest an increase of frequency detection of higher number of *Aeromonas hydrophila* in water and bottom sediments samples in the Czarna Hancza River containing higher numbers of TVC 20°C and TVC 37°C. It is known from literature that *Aeromonas* are widely spread in water reservoirs [71] but the most numerous are found close to the discharge of wastes [10, 11, 39, 46, 53, 57, 60, 62, 69] where the content of nutrients is the highest although their faecal origin is discursive [5, 6]. Many examinations showed that fewer than 1% of healthy people is a carrier of these pathogens [39]. This can explain the lack of a connection, as well as in other research carried out abroad [1-5, 9, 15, 25] between the number of *Aeromonas hydrophila* and the number of FC in water and in the bottom sediments of the Czarna Hancza River. For example, at site 1 (in Stary Brod above Suwalki) and at sites 7-10 (on the Czarna Hancza River from Czerwony Folwark to Wysoki Most) to the east from Wigry Lake a number ratio of *Aeromonas hydrophila* to FC was 3.5-3000 in water samples and 0.03-4200 in bottom sediment samples. At sites 2a-6 situated in the region of inflow of treated sewage from a waste treatment plant in Suwaiki, in Sobolewo and in

Table 4. Frequency (%) occurrence of *Aeromonas hydrophila* in the water of the Czarna Hancza River with different levels of TVC 20°C, TVC 37"C, yeasts, TC, FC and FS counts.

Aeromonas hydrophila (CFU/50 ml)	[1]TVC 20°C			[2]TVC 37°C			[3] Yeasts		
	[7] CFU/1 ml								
	< 100	100-1000	> 1000	< 100	100-1000	> 1000	< 100	100-1000	> 1000
< 100	2	2	30	13	16	5	10	8	15
100-1000	0	5	13	3	5	9	3	12	3
> 1000	0	9	39	2	29	18	27	20	2
Aeromonas hydrophila (CFU/50 ml)	[4] TC			[5] FC			[6] FS		
	[8] MPN/100 ml								
	< 100	100-1000	> 1000	< 100	100-1000	> 1000	< 100	100-1000	> 1000
< 100	2	8	24	9	3	24	4	5	27
100-1000	3	2	14	5	3	9	4	4	7
> 1000	5	15	27	28	6	13	14	18	17

[1] - Total viable count at 20°C; [2] - Total viable count at 37°C; [3] - Number of yeasts; [4] - Number of total coliforms; [5] - Number of faecal coliforms [6] - Number of faecal streptococci; [7] - Colony Forming Unit; [8] - Most Probable Number

Table 5. Frequency (%) occurrence of *Aeromonas hydrophila* in the bottom sediments of the Czarna Haricza River with different levels of TVC 20°C, TVC 37°C, yeasts, TC, FC and FS counts.

Aeromonas hydrophila (CFU/1 GWW)	TVC 20°C			TVC 37°C			Yeasts		
	CFU/1 GWW								
	< 1000	1000-10000	> 10000	< 1000	1000-10000	> 10000	< 1000	1000-10000	> 10000
< 1000	0	0	21	0	9	12	2	15	2
1000-10000	0	2	25	0	5	22	3	16	9
> 10000	0	1	59	4	20	35	6	23	29
Aeromonas hydrophila (CFU/1 GWW)	TC			FC			FS		
	MPN/1 GWW								
	< 1000	1000-10000	> 10000	< 1000	1000-10000	> 10000	< 1000	1000-10000	> 10000
< 1000	10	30	24	38	8	6	34	18	0
1000-10000	12	6	6	24	14	0	38	2	0
> 10000	0	10	2	6	2	2	8	0	0

TVC 20°C, TVC 37°C, yeasts, TC, FC, FS, CFU, MPN – as in Table 4; GWW – Gram Wet Weight

the region of the old river bed of the Czarna Haricza River and its inflow to Lake Wigry the most polluted ones [54, 55] this ratio was 0.03-162 in water samples and 0.02-9.5 in bottom sediment samples. The fluctuation of *Aeromonas hydrophila* number in the research season might be caused by rainfalls and pollution leakage together with adsorbed cells of these bacteria from the catchment of the Czarna Haricza [57]. These bacteria may survive for a long time in soil [13], where they are washed out to the river and may undergo proliferation if the water temperature is adequate [64]. The decrease of their number in water observed in November (in bottom sediments even in October) except the samples taken at site 2b situated 10 m below the inflow of the treated sewage from the waste treatment plant in Suwaiki may be connected with a low temperature. It is known from literature [17, 43] that at water temperature below 15°C, besides the ability of using trace amounts of organic carbon [71], the speed of taking nutrients by *Aeromonas hydrophila* does not stand the competition with psychrophilic heterotrophic bacteria.

The more polluted water of the Czarna Hancza the higher number of staphylococci from Suwaiki to the inflow to Wigry Lake [54] than in water of the river from Czerwony Folwark to Wysoki Most (to the east from Wigry Lake). The number of *Staphylococcus* sp. exceeded 100 CFU/100 ml, maximum accepted [18] number for water used for recreational purposes. Similar numbers of these bacteria were found in literature for coastal recreational waters in the north-west areas of the Pacific Ocean in the USA. Lower numbers were found [1, 53] in waters of streams, rivers and little lakes in Finland, in less populated areas. The number of *Staphylococcus* sp. in water and bottom sediments exceeded FC number (the ratio of *Staphylococcus* sp. to FC was 0.3-2850 at the site 1 in Stary Brod above Suwatki and at the site 7 from Czerwony Folwark to Wysoki Most to the east from Wigry Lake and 0.1-56.8 at the sites 2a-6 from Suwaiki to the mouth of the river to Wigry Lake. Numerical superiority of *Staphylococcus* sp. over FC was found in literature [1, 53, 76] for different types of water.

The frequency of detecting *Staphylococcus* sp. in recreational waters as well as illnesses of bathing people depending on the number of these bacteria suggests [66] the necessity of monitoring these pathogens in surface waters used for recreational purposes. Higher numbers of *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Staphylococcus* sp. in the bottom sediments of the Czarna Hancza River than in water is a known phenomenon in literature [16, 17, 30, 64, 65]. The number of *Pseudomonas aeruginosa* reached the values approximate to the given ones [65] for bottom sediments of some Canadian lakes. The number of *Aeromonas hydrophila* was about 10 times higher than that found [75] in the bottom sediments of the Apalachicola Bay, Florida in the USA. Probably the presence of a higher number of organic substance, phytoplankton, PO_4, NO_3, adsorbed to sand grains, clays etc. is a factor adventageous for proliferation and survival of these bacteria in the bottom sediments in the Czarna Haricza River. At least twice higher numbers of *Aeromonas hydrophila* in the bottom sediments of the Czarna Haricza River in the region of the old river bed and its mouth to Wigry Lake (sites 5 and 6) than in the bottom sediments at other sites of this river (sites 1, 2a, 3, 4, 7-10) is explained by clay-argillaceous-sandy character of the bottom and higher amount of organic substance [55] and probably their longer survival [21] than in the bottom sediments at the sites representing more sandy or gravely deposits.

Conclusions

1. *Pseudomonas aeruginosa* in the water of the Czarna Haricza River were found more often at sites situated 10 m below the inflow of the treated sewage from a waste treatment plant in Suwaiki (site 2b) and at sites situated in Mackowa Ruda, Buda Ruska, Wysoki Most (sites 8-10) to the east from Wigry Lake. Pseudomonas aeruginosa were seldom found in the bottom sediments of this river.

2. *Aeromonas hydrophila* and *Staphylococcus* sp. in

water and bottom sediments of the Czarna Hancza River taken in Stary Brod above Suwalki (site 1) and in Czerwony Folwark, Mackowa Ruda, Buda Ruska and Wysoki Most (sites 7-10) to the east from Wigry Lake were generally found in amounts much higher than FC number. Their number was much closer to FC number in water and bottom sediments of the Czarna Hancza River taken from Suwalki to its mouth to Wigry Lake (sites 2a-6).

3. The number of *Aeromonas hydrophila* and *Staphylococcus* sp. in the bottom sediments of the Czarna Hancza River was higher at the sites with the majority of clay-argillaceous sandy deposits (sites 5 and 6 in the re gion of the old river bed of the Czarna Hancza River and its mouth to Wigry Lake) than at the sites with the major ity of sandy and/or gravely deposits (the sites 1-4 in the region of Suwalki and 7-10 to the east of Wigry Lake).

4. As there are more numerous pathogenic bacteria (especially *Aeromonas hydrophila* and *Staphylococcus* sp.) than FC in water and bottom sediments of the Czar na Hancza River the examinations of not only traditional indicatory bacteria of sanitary state but pathogenic bac teria of *Pseudomonas*, *Aeromonas* and *Staphylococcus* are necessary as well while estimating the usefulness of this river for recreation.

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