

# The Sanitary State of Water in the River Vistula between Wyszogrod and Torun

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Received: 18 March, 2002

Accepted: 13 May, 2002

## Abstract

This paper presents the results of microbiological research on the water of the River Vistula between Wyszogrod and Torun, as regards the total number of planktonic bacteria (TNB), the number of heterotrophic bacteria: psychrophilic (TVC 20°C) and mesophilic (TVC 37°C), the occurrence of anaerobic spore-forming bacteria, and the number of coliform bacteria, faecal coliforms and faecal streptococci. The value of the Korsh coefficient and the titre of coli indicate that the waters of the Lower Vistula on the Wyszogrod - Torun section are moderately polluted and in the majority of cases may be classified as class III purity, with the exception of sections of the river near Plock and Torun, which are unclassified. The values of the FC/FS ratio indicate the predominance of faecal contamination of human origin.

**Keywords:** heterotrophic bacteria, psychrophiles, mesophiles, anaerobic spore-forming bacteria, coliform bacteria, faecal coliforms, faecal streptococci.

## Introduction

The rapid economic and industrial development of many countries and negligence of environmental protection have led to tremendous strain being put on surface and ground waters as a result of the enormous quantity of industrial, agricultural and domestic waste. Alongside the increase in water pollution the problem of microbiological contamination is becoming more and more apparent. Through sewage, rainwater or directly through faecal contamination, fungi, viruses, pathogenic bacteria and those whose natural habitat is an animal or human organism find their way into waterbodies. The possibility that humans might become infected through water means that constant hygiene controls must be carried out on drinking water and on surface water used for recreational purposes. The sanitary evaluation is based on indirect inference of the presence of pathogenic microor-

ganisms on the basis of so-called indicator bacteria, which include *Escherichia coli*, *Streptococcus faecalis* and others. These permanently inhabit the alimentary tract of humans and animals as saprophytes.

According to Niewolak [11], bacteria acting as sanitary indicators must fulfill they must certain conditions, e.g. they must occur in water or sewage in the presence of pathogenic bacteria in larger quantities than the latter. They should live longer than pathogenic bacteria in the same conditions, displaying the same sensitivity to disinfection using chlorine or other bactericidal agent, and should not reproduce in water.

In determining the hygiene-sanitary state, it is necessary not only to ascertain the presence of indicatory organisms but also to take into account the quantitative aspect of this phenomenon, since it is necessary to determine the intensity of contamination. Most often the determination of the titre of coli and the coli coefficient

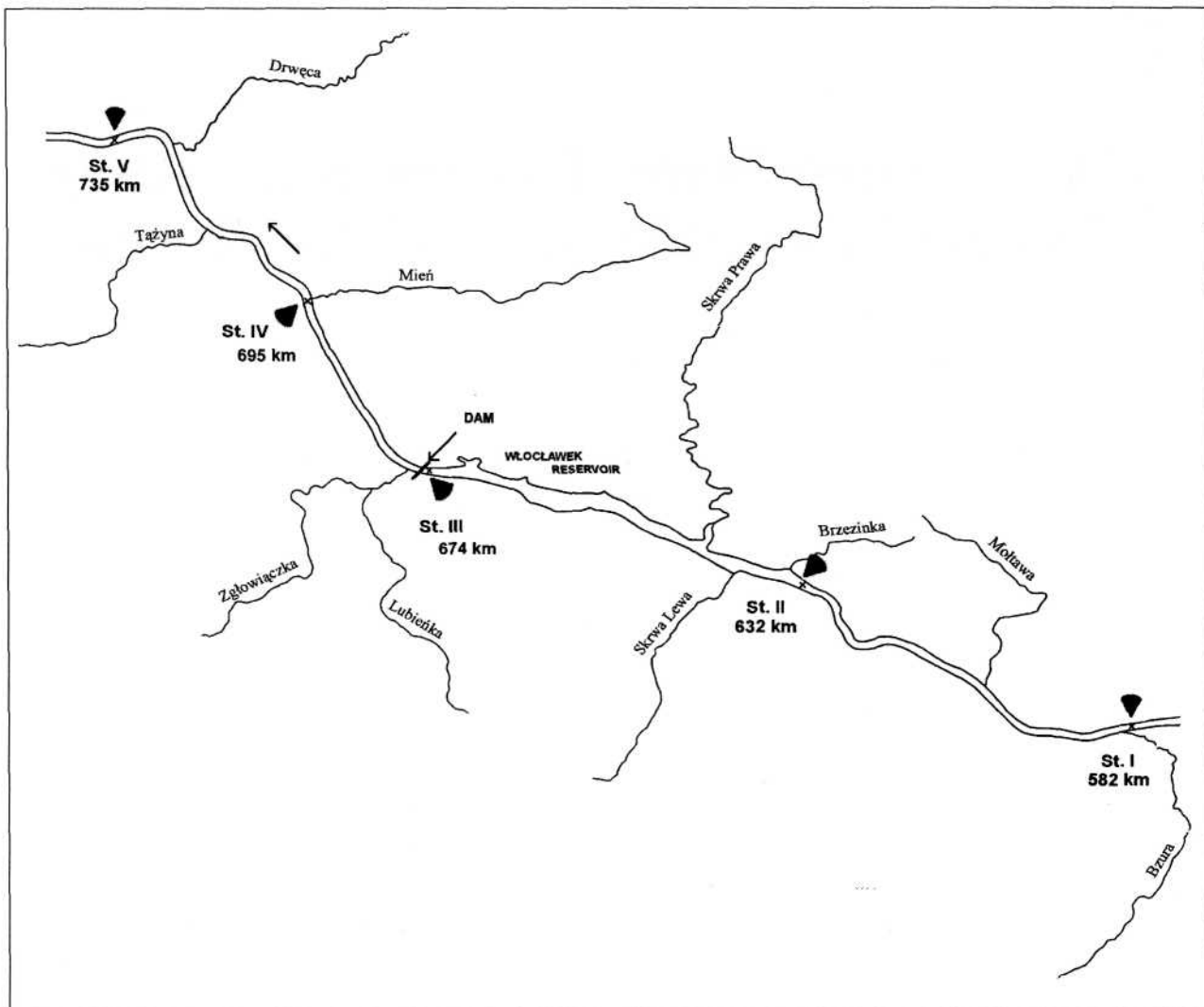


Fig. 1. Standpoint studies on the Lower Vistula on section Wyszogrod - Torun. Explanations: St. I, II - V - sampling sites.

[14] is used for this purpose. At present, the water purity class is established on the basis of the titre of faecal type coli. The principle of classification is regulated by the appropriate rules, which also determine the purpose of exploitation of waters with varying degrees of contamination [3]. The degree of water contamination can also be determined using the Korsh coefficient [7]. This is calculated from the ratio of the total number of planktonic bacteria to the total number of heterotrophic bacteria.

The determination of the titre of faecal streptococci, coliform bacteria and spore-forming bacteria, including *Clostridium perfringens*, is useful. On this basis we can obtain additional information on the source, kind, or recency of the pollution. In determining their epidemiological currency, the most reliable results are obtained by comparing the titre of faecal streptococci with the titre of coli [19].

## Material and Methods

### Study Area

The water of the Lower Vistula was studied on the section between Wyszogrod and Torun over a length of 153 km. On this section the Vistula flows along a proglacial stream valley in a north-westerly direction and reaches the Plock and Torun valleys, taking in more water from the Skrzywa and Drweca rivers. Upstream from Wloclawek a hydroelectric dam is situated on the river, forming the tidal Wloclawek Reservoir with a surface area of 70 km<sup>2</sup>.

### Sampling

Water samples for microbiological studies were taken from five sites (Fig. 1).

Site I - situated in Wyszogród, near the old, wooden road bridge (spring and summer sampling - 582 km along the Vistula's course) and near the new road bridge (autumn and winter sampling - 588 km along the Vistula's course),

Site II - in Płock, near the road bridge - 632 km along the Vistula's course,

Site III - in Włocławek, at the Włocławek Reservoir, about 500 km before the dam - 674 km along the Vistula's course,

Site IV - in Nieszawa, near the ferry crossing - 695 km along the Vistula's course,

Site V - in Toruń, near the road bridge - 735 km along the Vistula's course.

Samples were taken in spring (01.04.99), summer (01.07.99), autumn (04.11.99) and winter (22.02.00).

Water to be studied was taken directly into sterilised glass bottles from the central current of the river, from the surface layer (depth of about 20-30 cm). The collected samples were put into a thermoinsulation bag at a temperature of about 7°C and transported to the laboratory in order to carry out analyses. The time delay from the moment of collecting the samples to conducting the analyses did not usually exceed 12 hours.

### Microbiological Study

The studies consisted in determining the following: total number of bacteria (TNB), total number of heterotrophic bacteria: psychrophilic (TVC 20°C) and mesophilic (TVC 37°C), number of spore-forming bacteria, total number of coliform bacteria (TC), number of faecal coliform bacteria (FC), number of faecal streptococci (FS).

Before the studies were carried out, the collected water samples were diluted –  $10^{-1}$ - $10^{-4}$ . A sterile physiological salt solution (0.85% NaCl) was used as the thinning agent.

#### Total Number of Bacteria (TNB)

The total number of bacteria (TNB) in the water of the Lower Vistula was determined by the method of counting bacteria directly after Zimmerman [22], using Nucleopore membrane filters with a pore diameter of 0.22 µm. For this purpose, the water samples were fixed immediately after collection with 37% formaldehyde, whose final concentration was 0.7%.

Membrane filters dyed with Sudan Black III were placed in the filter apparatus and 1 ml of sterile distilled water was applied to their surface, followed by a water sample fixed in formaldehyde. After filtration of the tested sample, 2 ml of acridine orange (100 mg/1000 ml of distilled water) was poured on to the filter and left for 2 minutes. Then, after the filtration of the colouring agent, the filter was rinsed with sterile distilled water and the dyed bacterial cells were counted under an epifluorescence microscope "Jenalumar" (Carl Zeiss, Jena).

#### Number of Psychrophilic (TVC 20°C) and Mesophilic (TVC 37°C) Bacteria

The number of heterotrophic bacteria: psychrophilic (TVC 20°C) and mesophilic (TVC 37°C) was determined by the pour plate method, using a iron-peptone agar medium after Ferrer, Stapert and Sokolski [4]. The inoculations were carried out in six parallel repetitions. Three plates from each dilution (from particular study sites) were incubated at 20°C for 72 hours, after which colonies of psychrophilic heterotrophic bacteria were counted. The remaining three plates from each dilution were incubated at a temperature of 37°C for 24 hours, after which colonies of mesophilic bacteria were counted. The results were calculated for 1 ml of water.

#### Number of Anaerobic Spore-Forming Bacteria Reducing Sulphites

The number of anaerobic spore-forming bacteria in the studied water samples was determined by the two-layer pour plate method. Before inoculation, the water samples were pasteurised at a temperature of 80°C for 20 minutes, after which they were cooled to room temperature and pour inoculation was carried out using Wilson Blair medium from the BTL company. After solidification of the medium, aqueous agar was poured onto its surface in order to cut off the air supply. Plates with the inoculations were incubated at a temperature of 37°C, in anaerostats in the presence of an oxygen absorber (Gas Generating Kits BR 65/60 - Oxoid). The colonies that grew were counted after 18 hours. The results were calculated for 100 ml of water. The presence of spore-forming bacteria was confirmed by carrying out dyed preparations from particular colonies using the Schaeffer-Foulton method [2].

#### Total Number of Coliform Bacteria (TC)

The total number of coliform bacteria (TC) was determined by the most probable number method (MPN). For this, Eijkman medium from the BTL company was used. The three-sample system was used in the tests. The medium was inoculated with 0.1, 1, and 10 ml of the studied water sample. For the inoculation with 10 ml of the water sample, a double concentration of Eijkman medium was used. Incubation was carried out at 37°C for 24 hours. A positive result of the test was taken as being produced by the presence of gas in the Durham test tube, turbidity of the medium and a change of its colour from purple to yellow. The results were interpreted according to the Polish Norm [14].

#### The Number of Faecal Coliform Bacteria (FC)

The number of faecal coliform bacteria (FC) was determined by the most probable number method (MPN). For all the samples which showed a positive or doubtful result on the Eijkman medium, indicating the presence of bacteria from the coli group, inoculation was carried out on a medium with brilliant green [15]. The samples were

then incubated at a temperature of 44°C for 24 hours. A positive result was taken as being given by turbidity of the medium with the simultaneous presence of gas in the Durham test tube or in the whole medium, when slightly shaken. Results were interpreted according to the Polish Norm [14].

### The Number of Faecal Streptococci (FS)

The number of faecal streptococci (FS) was determined using membrane filters for tests. After filtering appropriate volumes of samples through membrane filters (Millipore type HA with a pore diameter of 0.45 µm), they were placed on the surface of SF medium (Difco) and poured into Petri plates. Incubation was carried out at 37°C for 48 hours, after which the colonies of faecal streptococci that had grown were counted and the results calculated according to the Polish Norm [16].

## Results

In this paper, the classification of the quality of river waters and the designation of auxiliary indicator bacteria were carried out on the basis of the method of characteristic concentrations. It is assumed that the characteristic concentration is the average of the two most unfavourable results found during a given observation period [17].

Table 1. Total number of planktonic bacteria (TNB) and the Korsh coefficient in water of the Vistula River.

Place of sampling	TNB	Korsh coefficient
Wyszogród	32.8 x 10 <sup>5</sup>	360
Plock	25.6 x 10 <sup>5</sup>	248
Włocławek	26.6 x 10 <sup>5</sup>	274
Nieszawa	32.0 x 10 <sup>5</sup>	1391
Toruń	30.4 x 10 <sup>5</sup>	453

Table 2. Total number of psychrophilic bacteria (TVC 20°C) and mesophilic bacteria (TVC 37°C) in the water of the Vistula River.

Place of sampling	Number of bacteria		Ratio TVC20°C: TVC37°C
	psychrophilic <sup>1</sup>	mesophilic <sup>2</sup>	
Wyszogród	9.1 x 10 <sup>3</sup>	1.9 x 10 <sup>3</sup>	4.79
Plock	10.3 x 10 <sup>3</sup>	3.7 x 10 <sup>3</sup>	2.78
Włocławek	9.7 x 10 <sup>3</sup>	2.9 x 10 <sup>3</sup>	3.34
Nieszawa	2.3 x 10 <sup>3</sup>	0.5 x 10 <sup>3</sup>	4.60
Toruń	6.7 x 10 <sup>3</sup>	1.0 x 10 <sup>3</sup>	6.70
Average	7.62 x 10 <sup>3</sup>	2.0 x 10 <sup>3</sup>	-

<sup>1</sup> - number of psychrophilic bacteria (TVC 20°C) in 1 ml of water <sup>2</sup> - number of psychrophilic bacteria (TVC 37°C) in 1 ml

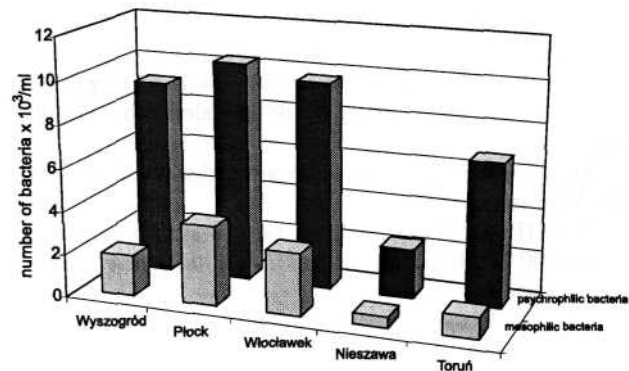


Fig. 2. Number of psychrophilic (TVC 20°C) and mesophilic (TVC 37°C) heterotrophic bacteria in the water of Vistula River on the section Wyszogrod - Torun.

The results of the tests on the total number of planktonic bacteria (TNB) are presented in Table 1. It follows from it that the total number of planktonic bacteria on the studied section of the Lower Vistula river varied from 25.6 x 10<sup>5</sup> to 32.8 x 10<sup>5</sup> cells/ml of water, with the maximum number of bacteria noted in the vicinity of Wyszogrod and Nieszawa, and the minimum in the vicinity of Plock.

From among heterotrophic bacteria (Table 2 and Fig. 2), psychrophilic microorganisms occurred in considerably greater numbers (on average 7.62 x 10<sup>3</sup> cells/ml) than mesophilic ones (on average 2.0 x 10<sup>3</sup> cells/ml). The greatest number of psychrophilic bacteria was found at site II (Plock) - 10.3 x 10<sup>3</sup> cells/ml of water, and only slightly less at site III (Włocławek Reservoir - before the dam) - 9.7 x 10<sup>3</sup> cells/ml of water. A significant decrease in the number of psychrophilic bacteria was noted at site IV in Nieszawa (2.3 x 10<sup>3</sup> cells/ml of water) at a distance of 20 km from the Włocławek Reservoir. A renewed increase in the number of psychrophilic bacteria was noted at site V in Torun (6.7 x 10<sup>3</sup> cells/ml of water).

Mesophilic heterotrophic bacteria displayed a similar tendency to change, with the maximum number in Plock (3.7 x 10<sup>3</sup> cells/ml of water), a slight fall in their numbers at site III - Włocławek Reservoir (2.9 x 10<sup>3</sup> cells/ml of water), and the minimum at site IV in Nieszawa (0.5 x 10<sup>3</sup> cells/ml of water).

The numerical ratio TVC 20°C : TVC 37°C at all the study sites was lower than 10. The greatest numerical difference between psychrophiles and mesophiles was noted in the vicinity of Torun (6.7), the smallest near Plock (2.7).

The results of research on the number of spore-forming bacteria-reducing sulphites (*Clostridium perfringens*) in the water depths of the Lower Vistula are presented in Fig. 3. Their number varied from 10.7 x 10<sup>3</sup> cells/ 100 ml of water in Torun to 50.8 x 10<sup>3</sup> cells/ 100 ml of water in Plock.

It follows from the Korsh coefficient [7] presented in Table 1 that the waters of the Lower Vistula on the section from Wyszogrod to Torun are classified as being moderately polluted, and in the vicinity of Nieszawa are even classified as being clean.

In Table 3 alongside the value of the titre of coli, data concerning the titre of faecal coliforms and faecal streptococci are presented. The values of the titre of coli var-

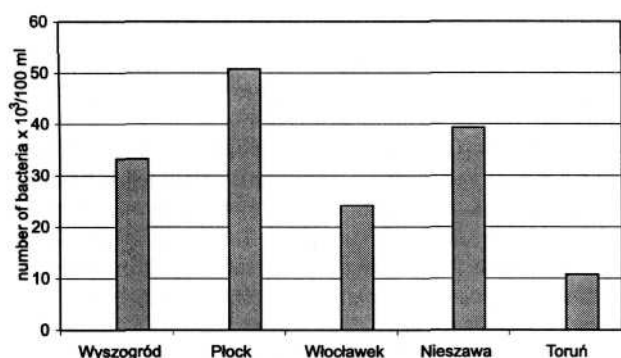


Fig. 3. Number of anaerobic spore-forming bacteria-reducing sulphites (*Clostridium perfringens*) in the water of Vistula River on the section Wyszogrod-Torun.

ied from 0.0014 in Plock and Torun to 0.016 in Wyszogrod and the Wloclawek Reservoir. The titre of faecal coliforms, on the other hand, varied from 0.004 in Plock and Torun to 0.08 in Wyszogrod and Wloclawek.

The sites in Plock and Torun had the lowest and, at the same time, a similar, numerical value of both of these indices (0.0014; 0.004). This indicates the greatest pollution of the water of the Lower Vistula of all the studied sites in this region with sewage containing faecal substances. At the study site in Nieszawa, both the titre of coli (0.008) and faecal coliforms (0.04) had average values.

Table 3. Titre of coliform bacteria, faecal coliform and faecal streptococci in the water of the Vistula River.

Place of sampling	Titre		
	coliform bacteria	faecal coliform	faecal streptococci
Wyszogrod	0.016	0.08	0.02
Plock	0.0014	0.004	0.09
Wloclawek	0.016	0.08	0.45
Nieszawa	0.008	0.04	8.30
Torun	0.0014	0.004	0.50

The titre of faecal streptococci varied from 0.02 in Wyszogrod to 8.3 in Nieszawa. The sites in the Wloclawek Reservoir and Torun had similar numerical values of the titre (0.45 and 0.5 respectively). Near Plock it came to 0.09. Comparing the titre of coli with the titre of faecal streptococci, a decided predominance of the number of bacteria from the coli group is visible at all the study sites.

On the basis of regulations currently in force concerning surface waters [3], the value of the titre of faecal type coli allows the water of the Lower Vistula on the studied section to be classified in most cases as class III purity (Table 3); only the water in the vicinity of Plock and Torun is unclassified.

It follows from the ratio of faecal coliform bacteria (FC) and faecal streptococci (FS) that pollution of the water of the Lower Vistula on the studied section occurs through the deposition of faecal substances mainly of hu-

man origin (Table 4, Fig. 4). The highest index of faecal coliforms was noted at sites in Plock and Torun (24000 cells/100 ml of water), while the lowest value was noted in Wyszogrod and the Wloclawek Reservoir (1300 cells/100 ml of water). At these sites, about 18.5 times fewer faecal coliform bacteria were found than in Torun and Plock. At the site in Nieszawa the faecal coliforms index was 2400 cells/100 ml of water. In Wyszogrod, however, the highest number of faecal streptococci was noted (4200 cells/100 ml of water). The least was found in Nieszawa (12 cells/100 ml of water). It follows from the values of the FC/FS ratio presented in Table 4 that at most study sites it was greater than 4, which would indicate the human origin of the contamination. Its highest value was noted in Nieszawa (200), and the lowest in Wyszogrod (0.31), which indicates the predominance of animal faecal contamination at that site.

Table 4. Number of faecal coliform bacteria, faecal streptococci and the ratio FC/FS in the water of the Vistula River.

Place of sampling	FC <sup>1</sup>	FS <sup>2</sup>	FC/FS
Wyszogrod	1300	4200	0.31
Plock	24000	1050	22.8
Wloclawek	1300	220	5.90
Nieszawa	2400	12	200
Torun	24000	200	120

<sup>1</sup> - number of faecal coliforms in 100 ml of water

<sup>2</sup> - number of faecal streptococci in 100 ml of water

Ratio - FC/FS > 4 - human origin,  
FC/FS < 0.7 - animal origin

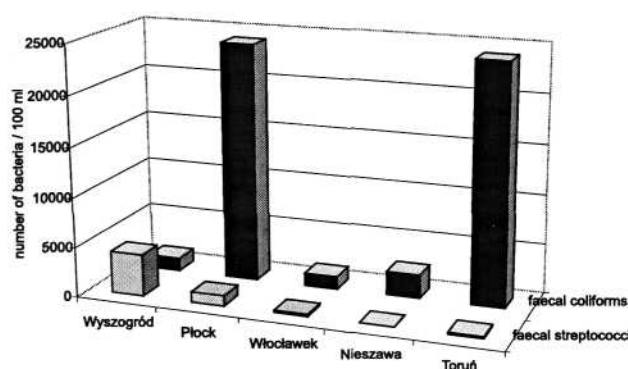


Fig. 4. Number of faecal coliform and faecal streptococci in the water of Vistula River on the section Wyszogrod - Torun.

## Discussion

Surface waterbodies like lakes or rivers are often the main source of usable and drinking water. They are mainly exploited for economic and recreational purposes. They should therefore be protected against excessive pollution and far-reaching degradation in order not to allow their natural biological balance to be upset or destroyed.

At present, a growing battle is under way to preserve the purity of our surface waters. Since 1978, systematic

tests have been carried out on the main waters in Poland. These also include the River Vistula. They are regularly carried out by the State Inspectorate of Environmental Protection as part of basic environmental monitoring. Based on the regulations currently in force [3], three classes of purity and unclassified waters are distinguished. The classification of waters is based above all on 54 physico-chemical indices, determination of saprophytic, and on only one fundamental bacteriological index, this being the titre of faecal-type coli [20].

In order to obtain a full picture of the state of pollution, its sources and remoteness in time, wide-ranging bacteriological and sanitary studies of the Lower Vistula on the section between Wyszogrod and Torun have been carried out. Apart from the titre of faecal coliform, this involved determining the total number of planktonic bacteria (TNB), psychrophilic heterotrophic bacteria (TVC 20°C) and mesophilic bacteria (TVC 37°C), anaerobic spore-forming bacteria, and bacteria from the coli group (TC) and faecal streptococci (FS).

The results of research on the total number of planktonic bacteria, including heterotrophic bacteria, indicate the significant predominance of the first group of microorganisms. According to Niewolak [12], heterotrophic bacteria can constitute up to 5% of the total number of bacterioplankton in badly contaminated waters. The results of the present research on the number of heterotrophic bacteria in the water of the Lower Vistula are comparable to the results obtained by Niewolak [13] for the river Czarna Hancza, especially for study sites situated downstream from sewage treatment plants in Suwalki. It follows from the present research that the site with the lowest number of planktonic bacteria, and at the same time the highest number of heterotrophic bacteria is Plock. The high number of heterotrophic bacteria at this site may be evidence of the greater amount of sewage deposited in the river and the presence of easily absorbed organic matter in the water [20]. A confirmation of this fact is also the highest number of mesophilic bacteria at this site, this type being allochthonous. A significant decrease in the number of psychro- and mesophilic bacteria at site IV in Nieszawa, at a distance of 20 km from the Wloclawek Reservoir, indicates the great ability of the river to clean itself both within the reservoir itself and downstream, and also indicates the absence of further large sources of pollution downstream from the dam.

Taking into account the number of heterotrophic bacteria whose incubation was carried out at a temperature of 20 and 37°C, it should be stated that psychrophilic bacteria dominate in the water of the Vistula on the studied section. From a health point of view, it is more dangerous if the number of mesophilic bacteria exceeds the norm because they may contain pathogenic microorganisms. At the lower temperature (20°C), it is above all non-pathogenic aquatic microorganisms that grow. However, according to Tyski and Krogulska [20], Gram negative aquatic bacteria produce lipopolysaccharides of the cell wall, which can act toxically, like endotoxins of pathogenic bacteria. Monitoring the number of both groups of bacteria (psychro- and mesophilic) seems to be justified.

The degree of river water pollution can also be indicated by the numerical ratio of TVC 20°C : TVC 37°C

("differential temperature ratio test"), which in polluted waters is lower than 10 [9]. On the studied section of the Lower Vistula, its value varied from 6.7 in Torun to 2.78 in Plock.

On the basis of the Korsh coefficient [7] obtained from the ratio of the total number of planktonic bacteria (TNB) to the total number of heterotrophic bacteria (TVC), the waters on the studied section of the River Vistula can be classified as moderately polluted (values of the coefficient ranging from 100-1000); only near Nieszawa was the coefficient higher than 1000, indicating clean waters. In turn, the values of the titre of faecal coliform indicate class III purity, with the exception of the sections of the river near Plock and Torun, which must be categorised as unclassified. It follows from results carried out by WIOS (Provincial Inspectorate of Environmental Protection) as part of regional monitoring [17] that the River Vistula is unclassified on the territory of the entire province. According to this report, from the physico-chemical point of view, the water at individual study sites belonged to class III purity, with a visible tendency towards improving its purity in this regard. Its unclassified designation was decided, however, by hydrobiological (concentration of chlorophyll "a") and bacteriological (titre of faecal type coli) indices. The divergence of the research results presented by WIOS and those contained in the present paper, with the application of the same system for calculation, may be the result of the different frequency and time of sample collection.

The best index determining the degree of sanitary contamination of water is the microflora of the alimentary tract of humans and warm-blooded animals. These include bacteria from the coli group, faecal type coli and faecal streptococci [10], whose number testifies not only to the direct faecal contamination of a given waterbody, but also indicates the character and type of deposited sewage. According to Tec [19], the low titre of faecal streptococci and the high titre of coli testifies to the moderately recent contamination of the water with faecal substances. Many authors [6, 13, 21] believe that the quantitative ratio of bacteria from the faecal type coli group (FC) to faecal streptococci (FS) may be of fundamental importance in evaluating the type of pollution. At most study sites on the Lower Vistula, the ratio of faecal pollution FC/FS had a value higher than 4, which indicates the predominance of contamination of human origin.

The maximum survival time of pathogenic bacteria in water varies [1, 8]. The survival rate of *Salmonella* in water in comparison to faecal coliform is more or less of the same order [10]. According to Geldreich [5], a value of the faecal coli index higher than 2000 gives an almost one hundred percent probability of the occurrence of bacteria from the *Salmonella* group in the environment. The fact that this index considerably exceeded this threshold at study sites in Torun and Plock, reaching a value of 24000, is therefore unsettling.

It seems that the accepted system for the classification of rivers in Poland should be verified. At present, from among the 54 tested indices, the evaluation of water quality is based on only one bacteriological index [20]. It is evident from many studies that it is precisely this index that most often determines the lowest class of water qual-

ity. It is the index that exceeds the norm by the greatest extent. The Polish system also deviates significantly from the systems in force in the states of the European Union, where heterogeneous methods for evaluating waters are applied depending on the way they plan to be exploited. However, the majority of unclassified Polish waters do not differ significantly as concerns their parameters from the rivers in other European countries [18].

## Conclusions

1. Among heterotrophic bacteria in the water of the Lower Vistula on the section Wyszogrod-Torun, psychrophilic microorganisms occurred considerably more often than mesophilic ones.

2. The numerical ratio of TVC 20°C : TVC 37°C ("differential temperature ratio test") at all the test sites had a value lower than 10, which indicates considerable allochthonous contamination of waters on the studied section of the Lower Vistula.

3. On the basis of the bacteriological Korsh coefficient, the water on the studied section of the river should be classified as moderately contaminated.

4. The high titre of faecal type coli indicates class III water purity in most cases; only near Torun and Plock do unclassified waters occur.

5. The value of the ratio of faecal contamination FC/FS at most study sites being higher than 4 indicates the predominance of faecal contamination of human origin.

6. The high value of the faecal coli index (24000) at sites in Plock and Torun gives an almost one hundred percent probability of the presence in the water of pathogenic bacteria of the *Salmonella* type.

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