Polish Journal of Environmental Studies Vol. 13, No. 1 (2004), 79-84

Occurrence of Bacteria and Coli Bacteriophages as Potential Indicators of Fecal Pollution of Vistula River and Zegrze Reservoir

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Received: 21 February, 2003
Accepted: 20 May, 2003

Abstract

This paper presents a sanitary state estimation of Zegrze man-made reservoir and Vistula river using typical bacterial factors. The aim of this study was to introduce the possibility of the use of coli bacteriophages as potential indicators of fecal polluted waters.

Zegrze Reservoir, as well as the examined Vistula sector, belong to microbiologically contaminated waters. Especially in the summer period they are rich in coli-type and E.coli bacteria. The changes in E.coli numbers were correlated with bacteria phage numbers.

Keywords: sanitary state, Zegrze Reservoir, Vistula river, coli bacteriophages, indicators of fecal pollution, coliform bacteria

Introduction

Water is one of the most important factors for human life as the main component of food and a necessary element for all metabolic processes. The development of industry, new technologies absorbing and producing enormous amount of chemicals, organic and inorganic compounds, and increasing urban agglomerations have resulted in increasingly sewage-polluted natural waters [28].

The growing deficit of good quality water has spurred the need to utilize not only subterranean waters but also fresh waters at maximal risk of microbiological and chemical pollution.

Viruses and bacteria penetrate sewage effluents with human and animal stools [12, 15]. Considerable amounts of these microorganisms are present in industrial wastewaters from slaughterhouses and tanneries [23, 11].

A significant problem appears to be the survival of various disease-causing bacteria and viruses in water.

It is necessary to underline that the survival period - hence the migration period in water - is higher for viruses than for bacteria. Their participation in total domestic sewage microflora is high. The virus survival period in water or sewage effluents depends to a large extent on physico-chemical conditions and particularly upon temperature [13, 17].

Sewage polluted waters are a potential source of epidemic threat because of the presence of disease-causing bacteria. In Finland, for instance, during 12 years 40% of epidemics were caused by drinkable water [20].

It is difficult to detect microbial pathogenic forms in water because they are in lower numbers than ubiquitous autochthonic bacteria. Moreover; to examine them it is necessary to use more advanced diagnostic methods.

The estimation of water sanitary state bases on indirect assessment of pathogenic bacteria numbers using indicator bacteria are always present in normal intestine human and animal saprophyte micro flora [25].

In general, the number of fecal type coli bacteria is a valuable source of information about sanitary state of water, but we must be aware that some virus epidemics are

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provoked by water free of typical indicator bacteria [27]. According to some publications, intestine viruses show greater environmental resistance than standard bacterial indicators [18, 26]. Some authors indicate than the occurrence of fecal coli or streptococci with intestine viruses is actually evident when previously it was not [5]. Since 1967 a significant presence of enteroviruses (Polio, Coxsackie, ECHO) in domestic wastewaters has been found.

Experiments of [14, 19, 30] have underlined the need for convincing indicators of virus contamination. For this purpose several scientists [10, 32] examined coli phages and *Bacteroides fragilis* [33]. Coli phages were detected as organisms sufficiently representative [34, 22]. In some countries (France and Germany), besides coli group bacteria, phages are also used as fecal pollution indicators [36].

According to some literature data [12, 15] phages attacking E.coli are present in human and animal excrement. Their quantity varies from 10 to 10^8 pfu/g. Besides, coli phages are more frequent in ill persons than in those in good health [8]. Due to the constant presence of coli phages in wastes [11] they have been proposed as potential indicators of fecal-polluted waters [22].

A correlation between phage quantity and degree of fecal pollution has been found [29]. Similar results were obtained by Armon and Knott [1], who found positive correlations of phage number and bacterial pollution indicator factors.

Sogaard [31] detected coli phages in 50% of 340 water samples from Danish beaches. Usually, the phage number was below 10/100 ml, but their detection probability increased simultaneously with E.coli numbers. Dutka *et al*. [4] described the occurrence of various factors and pathogenic organisms in seawater samples from eight seaside resorts in Brazil. In all investigated areas positive and commonly significant correlations between coli phages level and coli faecal forms were chosen. This fact may suggest that the occurrence of phages in water samples indicates a wastewater pollution risk. Coli phage presence tests are proposed as additional criteria for determination of water sanitary state. The main objective of this paper was to estimate the sanitary condition of the Vistula river and Zegrze Reservoir and to test E.coli phages as indicators of fecal-polluted waters.

Materials and Methods

Sampling Sites

This study was carried out in the Vistula river and Zegrze Reservoir (see Table 1).

Water samples were collected under non-sterile conditions into polypropylene bottles (previously rinsed with a sampled water) from the surface layer (1 m) from May to January.

Strains

The following strains were used:

1. Strain E.coli ATCC 35218
2. E.coli strains isolated from the natural environment (Vistula river). Strains were stored at -70° C.

Table 1.Vistula River and Zegrze Reservoir sampling sites.

Sampling sites	
Vistula river	Zegrze reservoir
1.Situated about 50 m before the collector (Vistula's 513 kilometer)	1.Estuary of Żerań Canal
2.Situated about 50 m after the collector	2.Żerań Canal-neighborhood of automobile market
3.Situated in the neighborhood of Princess Anne Gynecological Hospital	3.Nieporęt Bridge
4.Situated about 100 m below Śląsko-Dąbrowski Bridge	4.Zegrze Bridge

Media

To isolate bacteria from the natural environment the following media were used:

1. Plate Count Agar (Difco)-isolation medium for autochthonic bacteria (psychrophilic) incubated at 20° C was used. Suitable sampling from Vistula water and Zegrze reservoir were diluted [9], plated and incubated for 48h at 20°C.
2. Plate Count Agar + MUG - isolation medium for coli type bacteria and E.coli incubated at 37° C was used. Suitable sampling from Vistula water and Zegrze reservoir were diluted [9] plated and incubated for 24h at 37°C. From among the Enterobacteriaceae only E.coli, Shigella and Salmonella produce constitutive enzyme ß-D-glucuronidase. 94% of all E.coli strains possess this enzyme [6]. It splits MUG (4-methylumbeliferyl-ß-D-glukuronide) to 4-methylumbelliferone, which can be identified because it fluoresces in long wave UV light.
3. Liquid LB medium (Peptone tryptone-10g/l, yeast extract-5g/l, NaCl-5g/l, distilled water-1000 ml, pH=7.0) for multiplying E.coli strain ATCC 35218 and E.coli isolated from natural environment (Vistula river and Zegrze reservoir) were used. The strains were incubated for 12h at 37°C (bath water shaker).
4. Semi-liquid LB medium (Peptone tryptone-10g/l, yeast extract-5g/l, NaCl-5g/l, Agar-agar – 7.0 g/l, distilled water –1000 ml, pH=7.0).
5. Solid LB medium - as above (+ agar-agar – 15.0 g/l).

Isolation of E.coli Bacteriophages

For isolation the plaque assay technique was used. Water samples from Vistula river and Zegrze reservoir were filtrated through membrane filter 0.2 µm (MINISPART PLUS) to receive water free of bacteria. 0.2 ml of 12h strain cultures E.coli ATCC 35218 or E.coli isolated from the natural environment were added to 2 ml of that water.

The samples were stored for 20 min at room temperature, which was necessary for phage absorption to host cells. After that 3 ml portions of semi-liquid LB medium (46°C) were

added to the above-mentioned samples and the entire volume was poured into Petri dishes with previously prepared solid LB medium. Petri dishes were incubated for 24 h at 37° C; plaques were counted on the lawn of host cells.

At the same time that the water samples were taken for microbiological research, water temperature in situ was determined.

Results

Vistula River

The number of bacteria incubated at 20°C and 37°C and the number of coli-type bacteria were determined (Fig. 1). At sampling sites 1, 2, 3, 4 the highest number of psychrophilic bacteria (incubated at 20°C) were observed in summer. The quantity of bacteria varied from 3.8 to 4.5 x 10^4/ml.

The number of mesophilic bacteria incubated at 37°C changed similarly to those incubated at 20°C. In the summer a high level of this kind of bacteria at all 4 sampling sites was observed. The highest number of bacteria occurred at sampling sites 2 (after the collector) and 3 (hospital). Among the bacteria incubated at 37°C, a significant part consisted of allochtonic bacteria and in the their frame, coli-type bacteria.

At all sampling sites a higher number of E. coli in comparison to bacteriophages was observed (usually about two orders of magnitude, Fig.3).

The changes in E. coli numbers are correlated with bacteriophage changes (Fig.3).

Zegrze Reservoir

The number of bacteria incubated at 20° C and 37°C, and the number of coli-type bacteria were determined. At sampling sites the highest number of psychrophilic bacteria (incubated at 20°C) and mesophilic bacteria incubated at 37°C was found in summer. This was especially evident in July.

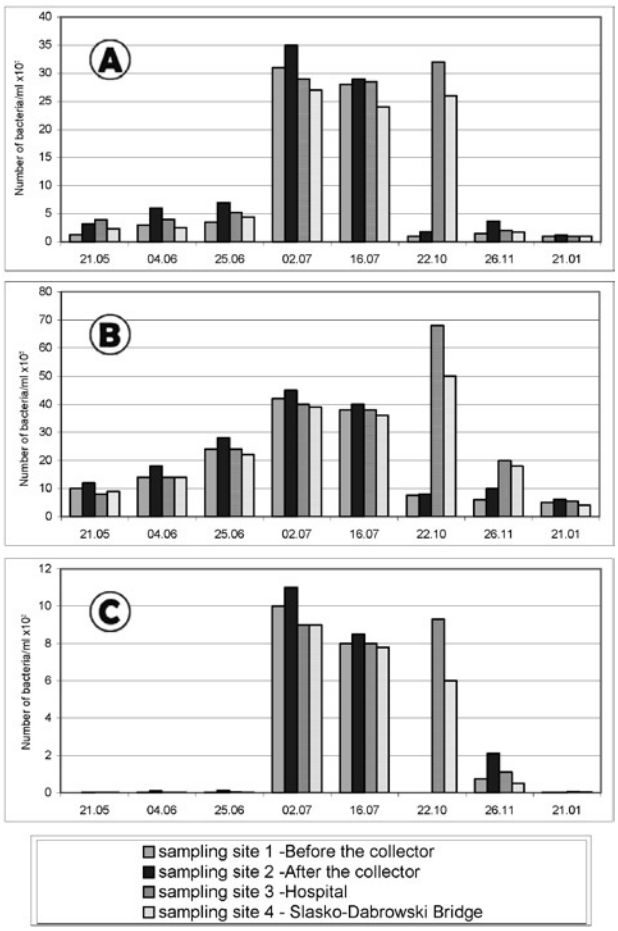

Fig. 1. Changes of bacteria number in Vistula River.
A - Differences in number of coli-type bacteria at 1, 2, 3, 4 sampling sites; B - Differences in number of bacteria at 1, 2, 3, 4 sampling sites (bacteria incubated at 37°C); C - Seasonal changes of bacteria numbers at 1, 2, 3, 4 sampling sites (bacteria incubated at 20°C).

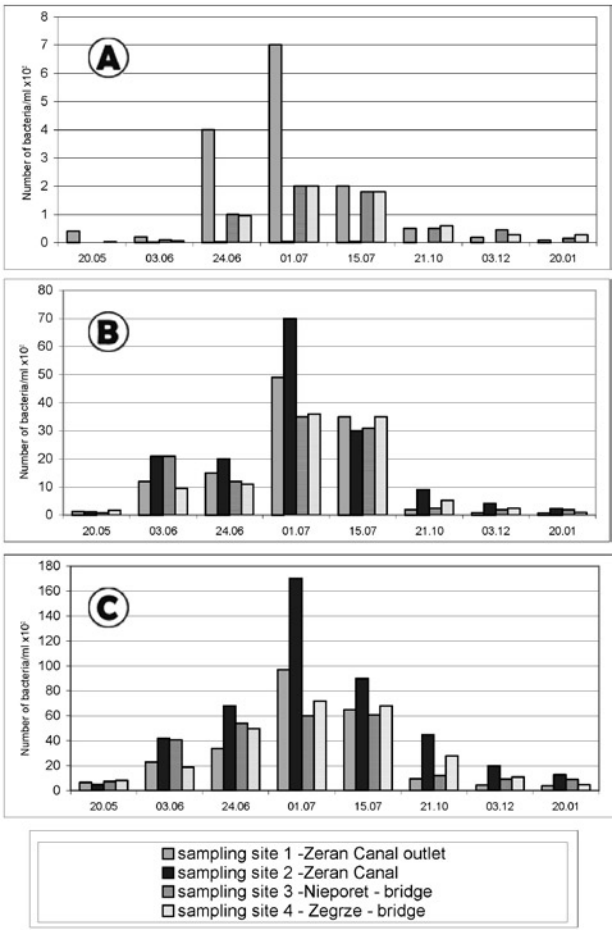

Fig. 2. Changes of bacteria number in Zegrze Reservoir.
A - Differences in number of coli-type bacteria at 1, 2, 3, 4 sampling sites; B - Differences in number of bacteria at 1, 2, 3, 4 sampling sites (bacteria incubated at 37°C); C - Seasonal changes of bacteria numbers at 1, 2, 3, 4 sampling sites (bacteria incubated at 20°C).

The higher number of bacteria incubated at 20°C was determined at higher water temperature. With a decrease of water temperature the number of bacteria also decreased. In the case of mesophilic bacteria we ought to underline that their number also depends upon the character and inflow of wastewater.

Similar to the Vistula results, changes in E.coli numbers correlated with bacteriophages.

There were two bacterial hosts for bacteriophages. The better one appeared to be E.coli strain ATCC 35218, rather than the wild E.coli strain isolated from the natural environment.

Discussion

Everyday occurrence of bacteria in inland waters has been well known for years [3]. The quantity and distribution of microorganisms in waters and their mutual correlation depends on many factors: natural conditions, climate, season, etc. Their life and development are submitted to certain laws, the final results of which are the result of numerous opposed processes [24].

To estimate the sanitary state of the Vistula River and Zegrze Reservoir, some standard parameters, including

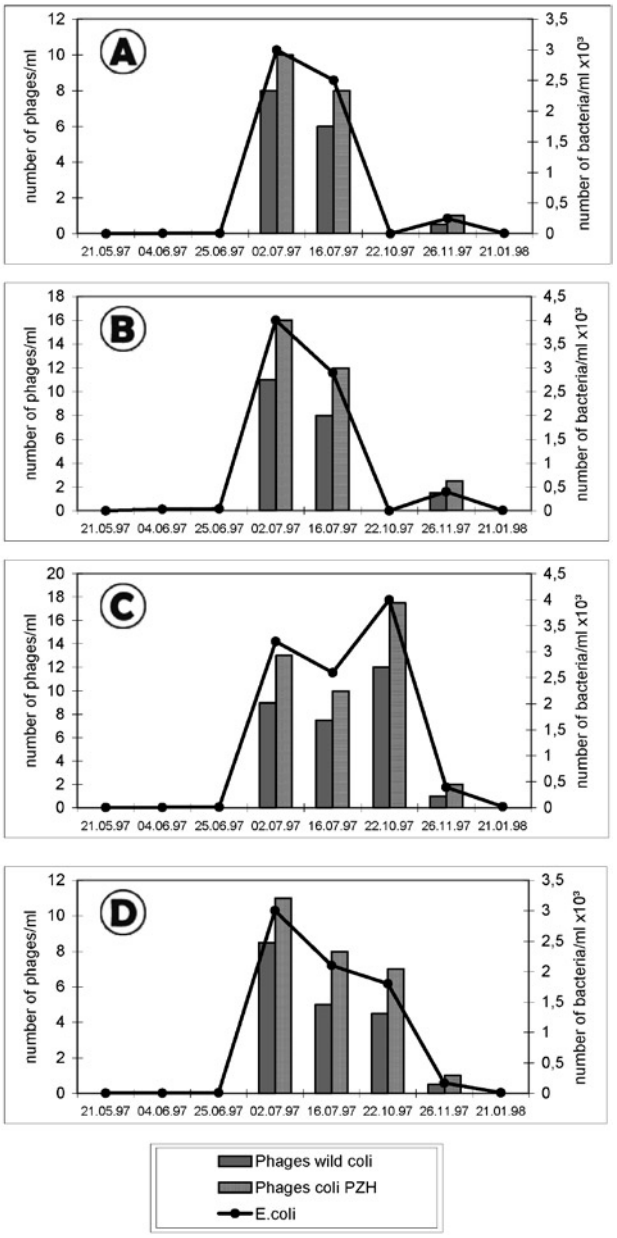

Fig. 3. Correlation between the changes in bacteria number with phages number at all sampling sites.
A - Before the collector; B - After collector; C - Hospital; D - Śląsko-Dąbrowski Bridge.

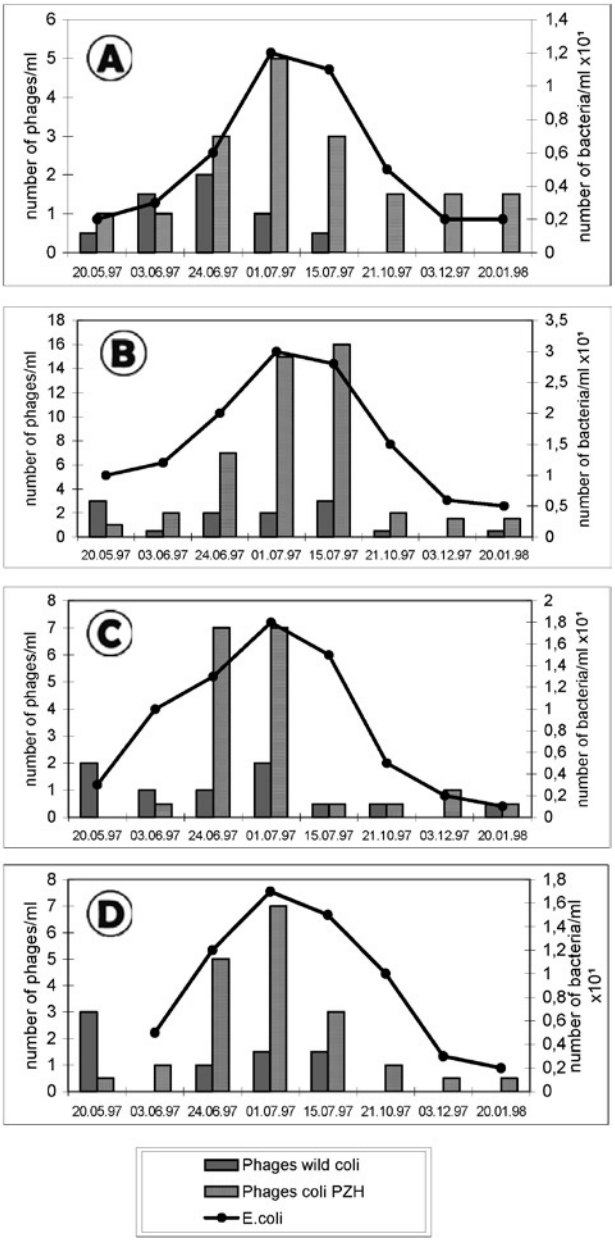

Fig. 4. Correlation between the changes in bacteria number with phages number at all sampling sites.
A - Żerań Canal outlet; B - Żerań Canal; A - Nieporęt - bridge; B- Zegrze- bridge.

the presence of E.coli bacteriophages, in chosen sites, were determined.

The number of bacteria in water undergoes significant fluctuations for all determined parameters. Due to the fact that the experiments were conducted in all seasons of the year we can state that the bacteria incubated at 20°C are present at seasonal periods related to temperature. The optimal growth temperature for this kind of bacteria is 12-32°C [21]. For all sampling sites the number of these bacteria increased with temperature, the maximal peak in the summer when the temperature was 20-22°C. Comparison between spring, autumn and winter months suggest a connection with temperature, because in the spring (14-19°C) the number of bacteria was higher than in the autumn-winter months (water temperature 8-4°C).

Besides seasonal number changes of saprophyte bacteria connected with water temperature variations, the inflow of wastewaters has influenced the number of bacterial population.

The high number of these microorganisms may suggest considerable organic matter inflow to investigated water [3]. The experiments conducted confirmed that each waste inflow was connected with an increase in the number of bacteria. This effect was most visible (Fig.1) at sampling site numbers 3 (hospital) and 4 (Śląsko-Dąbrowski Bridge) in October and at sampling site number 2 (after the collector) in November, where besides low temperature significant peaks in quantity of microorganisms were observed.

The number of both psychrophilic and mesophilic bacteria was always higher at both sampling site 2, because the inflow at this site was much more polluted and therefore the bacteria number was higher than at sampling site 1.

Mesophilic bacteria incubated at 37° C was the second systematically examined parameter. These microorganisms are classified as allochtonic bacteria because water is not their natural environment. A great number of these microorganisms testify to the pollution of water by wastewaters [3], a rich source of organic and mineral compounds.

The highest inflow of wastewater was observed Oct. 22 at sampling site 3 (hospital), when the highest amount of allochthonic bacteria was noticed (Fig.1). The above-mentioned inflow caused a significant presence of allochthonic bacteria at sampling site 4 (much further after Śląsko-Dąbrowski Bridge). Comparing numbers of bacteria, one can state lower bacteria number at sampling site 4. This fact can testify to water self-purification. Determining this process only by participation of microflora is not precise and ought to be completed by specifying the proportions among physiological types of bacteria.

In the summer, when the water temperature reached the highest values (20-22°C), it was found that the highest number of allochthonic (mesophilic) bacteria overlaps with the highest number of bacteria at 20°C. It must be remembered that downpours and flood preceded the drawing of the samples. So, the inflow of floodwater and downpours probably caused an increase in the number of bacteria. The same effect was observed by other authors [7].

It is also noticeable that the number of bacteria at 20°C as at 37°C was smaller July 16 than in July 2 at all sampling sites. It was allowed to expect contrary results with regard to the arrival of a flood wave on July 16 to all the tested sampling sites. The explanation of this fact may be connected with harmful compounds present in water as the result of flood and antagonistic reactions among microorganisms, which regulated their number. The data presented in Fig.1 clearly show that the number of autochthonic (psychrophilic) bacteria was always higher than that of the allochthonic (mesophilic) ones. This is compatible with literature data [25].

To estimate the sanitary state of the examined waters it is necessary to determine coli type bacteria and among them E.coli [16]. The highest number of coli type bacteria and E.coli was detected in the summer, when the number of mesophilic bacteria is significant (Figs. 1, 2). From the bacteriological point of view the examined sector of the Vistula belongs to III class of water purity. Zegrze Reservoir may be classified at (in summer) water purity class III or IV.

Using the E.coli bacteriophages as indicators of fecal-polluted waters were undertaken. The phages are not a constant part of water microflora but their presence is usually connected with wastewater inflow rich in animal and human excrement. Their survivability depends upon physical-chemical environmental conditions. Phages are very resistant to environmental factors; hence water samples containing viruses may be appreciated as free from microbial disease factors when pathogen bacteria are not detected [5].

The changes in E.coli numbers were correlated with changes of bacteriophage numbers (Figs. 3, 4). There were two bacteria hosts for bacteriophages. E.coli strain ATCC 35218 appeared to be better than wild E. coli strain isolated from the natural environment. Probably, this result was related with some protective system existing in wild E. coli as the defensive mechanism against phage attack. Coli type bacteria were always detected in much higher number 4 than phages (Figs. 3, 4). This is in agreement with the results of other workers like Bell [2] and Zaiss [35]. According to their results, the relation between the number of E.coli and coli phages is 100-1000: 1 (wastewater inflow) and decreases in the course of distance from wastewater source to 1-10:1. It may suggest bacteriophages' better environmental survivability.

Conclusions

1. Zegrze Reservoir as well as the examined Vistula sector belong to microbiologically contaminated waters rich in the summer period in coli type and E.coli bacteria.
2. The changes in E.coli numbers are correlated with phage number in two examined waters.
3. E.coli strain ATCC 35218 appeared to be a better host

for bacteriophages (present in Vistula river and Zegrze Reservoir) than wild E.coli strain isolated from the natural environment.
4. Bacteriophages can be an additional test for determing fecal-polluted waters.
5. Coli bacteriophages used as potential indicators of fecal pollution is promising, but it is doubtful that they could substitute for such traditional indicator bacteria as E.coli.

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