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Original Research

Survival of *Escherichia Coli* Serotype O157:H7 in Water and in Bottom-Shore Sediments

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Abstract

Survival of a single strain *E. coli* serotype O157:H7, isolated from milk in Poland, was examined in water environment (water, bottom-shore sediments and in muddy water over sediments) at 6°C and 24°C. Pathogenic bacteria were not detected (direct plating method) in water within 32 and 51 days of incubation at 6°C and within 21 and 32 days of incubation at 24°C (except water from one of the rivers, where the disappearance of serotype O157:H7 was noticed only after 54 incubation days). The target bacteria survived in muddy water 49-65 days at 6°C and 26-60 days at 24°C. Bacteria died at the slowest rate in bottom-shore sediments. The disappearance of the enterohemorrhagic serotype in these environmental materials was noticed only after 73-100 (6°C) and 30-60 (24°C) incubation days. The obtained results evidently indicate the existence of possible hazards connected with relatively long survival periods of pathogenic bacteria in water environment. The bottom-shore sediments in particular can be a reservoir of this bacteria.

Keywords: *Escherichia coli* O157:H7, survival, water, bottom-shore sediments

Introduction

The first reports on *Eschericha coli* serotype O157:H7 date back to 1982 when this bacteria caused food poisoning of 47 people in Oregon and Michigan after eating undercooked hamburgers originating from the same fast food restaurant chain [1]. Since that time, outbreaks of the illness have been noted after ingestion of contaminated water, foods of bovine origin (ground beef, fermented sausages, raw milk and milk products) as well as fruit and vegetable products and plant sprouts [1-4]. This organism causes a spectrum of disease symptoms of varying severity, from haemorrhagic colitis manifested by bloody diarrhea to haemolytic uremic syndrome/thrombotic thrombocytopenic pupura that can lead to death [1].

The principal reservoir of *E. coli* O157:H7 serotype is the intestinal tract of ruminants, especially cattle [1]. These animals are only carriers of pathogenic bacteria, which does not cause any disease symptoms [1, 5, 6]. According to Zhao et al. [7], Faith et al. [8], Kudva et al. [9], Orr [10] and Uradziński [11] the prevalence of carrier status of *E. coli* O157/ O157:H7 in cattle ranges from 0.3% to 10.8%.

Bacteria *E. coli* serotype O157:H7 can get to rivers and water reservoirs along with partially purified waste water from animal farms and cattle-houses as well as with communal waste. They also can be washed out from cultivable soils (fertilized naturally or by waste) and from

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pastures during rainstorms, floods or snow melting. Water supplies for animals and bathing areas also are possible sources of water contamination. If contaminated surface water is used for drinking without any physical or chemical processing before consumption, this can be the source of the epidemic food poisonings. In Cabool, Missouri 243 persons became ill in late 1989 and early 1990; the cause of the epidemic was water from the town water supply contaminated with the enterohemorrhagic serotype [12]. In 1999 in Washington County, New York, 921 persons were poisoned after consumption of non-chlorinated *E. coli* O157:H7 bacteria-contaminated water [13]. In Alpine, Wyoming, numerous illnesses were noticed in a group of persons drinking mineral water from a spring [14]. It was found that previously the spring was accessible to cattle. In Swaziland, South Africa [15], in the period from October to November 1992, 40,912 persons suffered from hemorrhagic diarrhea caused by serotypes *E. coli* O157; the occurrence of pathogenic bacteria was noticed in water from drilled wells, water supply lines and from house water collectors.

Attacks of illness can occur also after bathing in surface water contaminated with pathogenic bacteria. Thus, in Atlanta, 26 children bathing in a swimming pool located in a recreation park suffered from a dangerous episode of poisoning [14]. Illnesses after bathing in water reservoirs were noticed also in Portland, Oregon in 1994 [1] and in Clark County, Washington, in 1999 [12].

Investigations oriented towards bacteria *E. coli* serotype O157:H7 monitoring in water environment are at the initial stage. Johnson et al. [16] carried out two-year research on the frequency of these bacteria on the enormous area of the Old Man River watershed in Southern Alberta, Canada (agriculturally developed land). The enterohemorrhagic serotype was detected in 0.9% of samples from the total sample number of 1483.

Taking into account that the pathogenic bacteria, occurring in water and entering the human body directly or indirectly, can cause serious illness, it would be important to determine the survival of the microorganisms in this natural environment.

Materials and Methods

Target Bacteria

In model experiments on the survival of *Escherichia coli* O157:H7 serotype in water environment, the strain isolated from milk by the employees of the Veterinary Institute in Puławy was used. Its pathogenicity was confirmed by the studies of Łękowska-Kochaniak et al. [17].

Inoculum Preparation for Model Experiments

Suspension of *E. coli* serotype O157:H7 (250 mL), necessary for inoculation of materials from water environment, was prepared after a two-stage culture process. In the first stage, 1 loop of the biological material was introduced into 10 mL of bioMerieux Trypcase Soy Broth and incubated for 6 h at 37°C. The entire content of the test tube was then transferred to 250 mL of the above-mentioned broth and incubated at the same temperature for 24 h. Subsequently, bacterial suspension was centrifuged (6,350 x g, 5 min), washed two times in saline (0.85% NaCl) solution and resuspended in 25 mL of the same solution. The count of bacteria was estimated on Trypticase Soy Agar after incubation at 37°C for 24 h. An inoculum contained 10^9-10^{10} bacterial cells per 1 mL.

Model Experiments

Samples from Polish rivers and lakes (water, bottom-shore sediments and muddy water over sediment) were collected at various sites in Mazowieckie Province. At the beginning of experiments the environmental materials were characterized with respect to their physicochemical properties and to the level of natural microflora. Each sample was tested for the presence of O157 according to the procedure described in ENISO 16654 [18]. Samples of 150 mL volume (water, muddy water) or 150 g mass (bottom-shore sediment) were added to sterile 300 mL Erlenmayer flasks. Each sample was inoculated with 3.0 mL of bacterial suspension. The samples were incubated at 6°C and 24°C. Flasks with water and muddy water samples were incubated in the shaker (80 rpm), whereas flasks with sediment samples were maintained in stationary conditions. Samples for analyses were collected within the indicated time. The frequency of analyses of *E. coli* survival depended on the death rate in specific types of experiments. Environmental samples (1 mL of water or 1 g of sediment) were serially diluted (1/10) in saline solution and assayed for *E. coli* O157:H7 counts by direct plating (0.1 or 0.5 mL of initial sample and each dilution) in duplicate on bioMerieux Coli O157:H7 ID Agar with additional selective substances (0.5 g of sodium tellurite and 0.01 g of cefixim per 1 L of a medium). Petri dishes were incubated at 37°C for 24 hours. The study was terminated when target bacteria were not detected in three consecutive daily analyses.

When the incubation of specific material was at an end, physicochemical and microbiological characteristics were repeated in the same range as in the time-zero incubation.

Microbiological Analyses of Environmental Materials

The total bacteria count was determined on Oxoid Plate Count Agar; Petri dishes were incubated at 20°C for 72-96 hours.

Coliform count was determined on Oxoid *E. coli* /Coliform Agar and β-glucuronidase-positive *E. coli* count on Oxoid TBX medium; Petri dishes were incubated for 24 hours at 37°C or 44°C, respectively.

Confirmation Procedure for Determination of the *E. coli* O157:H7 Presence in the Environmental Materials

Long-term incubation of water and water sediments affected the morphology of *E. coli* O157:H7 colonies. In doubtful cases, material from suspected colonies was transferred to Petri dishes with bioMerieux Endo Agar. After 24-h incubation at 37°C, the presence of metallic colonies was checked. These colonies were subject to testing with the use of a Merck Singlepath *E. coli* O157 test (immunochromatographic rapid test based on gold-labelled antibodies specific to *E. coli* O157).

Physicochemical Analyses

Dry mass content in muddy water and sediments was determined by drying 10 g and 20 g material samples to a constant weight at 105°C. The presented results are arithmetic means of results for both weighed portions.

Inorganic substances content in muddy water and sediments was determined by burning 10 g and 20 g material samples in a muffle furnace at 500°C during 1 hour. The presented results are arithmetic means of results for both weighed portions.

Organic substances content in muddy water and sediments was mathematically determined by the difference between dry mass content and inorganic substances content.

The chemical oxygen demand (COD) of water was determined using the dichromate method [19].

Results and Discussion

The survival of *E. coli* serotype O157:H7 in water environments (water, muddy water and bottom-shore sediments) was evaluated in samples collected from five rivers and a lake located in the Warsaw area. Samples from the Wisła River were collected at two sites several tens of kilometers apart. The materials were taken from places located near large pastures (rivers) or near a large bathing area (the lake). Flooded areas were preferred due to the fact that pathogenic bacteria most often transmit to water environments from such areas. All samples were collected in autumn, after the end of the bathing season and cattle feeding on meadows. No environmental sample was *E. coli* O157 contaminated.

On the basis of microbiological analyses of the material samples used in model experiments, it was found that the total count of mesophilic bacteria changed in the range between $7.9 \cdot 10^2$ and $4.3 \cdot 10^4$ CFU/mL in water, in the range between $4.7 \cdot 10^5$ and $2.2 \cdot 10^6$ CFU/mL in muddy water and in the range between $2.4 \cdot 10^6$ and $6.2 \cdot 10^6$ CFU/g in bottom-shore sediments (Table 1). The majority of coli group bacteria occurred in sediments, slightly less in muddy water and the least — in water.

β-glucuronidase-positive *Escherichia coli* bacteria occured in all muddy water and bottom-shore sediment samples. The highest number was noticed in muddy water (1.1×10^2 CFU/mL) and bottom-shore sediment (1.4×10^2 CFU/g) of the Wkra River. Moreover, *E. coli* bacteria were discovered in water from the Wkra River and Czerniakowskie Lake and their count amounted to $2.2 \cdot 10^1$ and $8.7 \cdot 10^1$ CFU/mL, respectively.

It has been found after physicochemical analyses that environmental samples were close to neutral or slightly alcalic (pH in the range 7.14 to 8.06) and in the majority of cases pH of muddy water and bottom-shore sediments was slightly lower than pH of water (Table 2 and 3). COD of water varied between 24 and 139 mgO_2/L, whereas the values above 100 mg O_2/L were noticed in water collected from one site at the Wisła River and from the Bug River and the smallest values in water collected from the Sona River (Table 2). COD of muddy water varied in a wide range between 33 and 504 mgO_2/L; the lowest and the highest values were characteristic for the Sona and Bug Rivers, respectively (Table 2). Dry mass from bottom-shore sediments varied in principle between 71.4% and 83.1% and from muddy water between 0.6% and 1.5% (Table 2 and 3). Only the sediment from the Wisła River at collection site 1 was characterized by the very low dry mass content (46.8%), while it was connected with very high dry mass content of muddy water (10.4%) in the layer just above the sediment. Bottom-shore sediments in the majority of rivers and water reservoirs contained small amounts of organic substances between 0.2% and 0.6% of dry mass (Table 3). An exception was the sediments from the Wisła River, where the organic substances content amounted to 4.4% and 1.5% of dry mass. Attention should be paid to the high content of organic substances in muddy water, in the order of 8-9%; only in one case — Czerniakowskie Lake — was content 1.6% (Table 2).

It was found in model experiments on survival of pathogenic bacteria in water at 6°C that the population of *E. coli* O157:H7 bacteria decreased to an undetectable level (<1 CFU/ ml) as determined by agar plate within 32 and 51 incubation days (Fig. 1, top curves). The longest survival (ca. 50 days) was observed for target bacteria in the Bug and Sona Rivers as well as Czerniakowskie Lake. At 24°C the survival period was shorter and varied between 21 and 32 days (with the exception of the Sona River, where the absence of pathogenic bacteria was first noticed only after 54 incubation days) (Fig. 1, bottom curves).

Bacteria serotype O157:H7 survived slightly longer in muddy water than in water. At 6°C the pathogenic bacteria were not detected after 49-65 days, whereas at 24°C after 26-60 days (Fig. 2). At 24°C bacteria in muddy water of the Sona River died last; and in water likewise.

It seems that COD level as well as pH of water and muddy water did not play a significant role in the survival of *E. coli* O157:H7. There were observed only slight changes in COD level of water and muddy water after

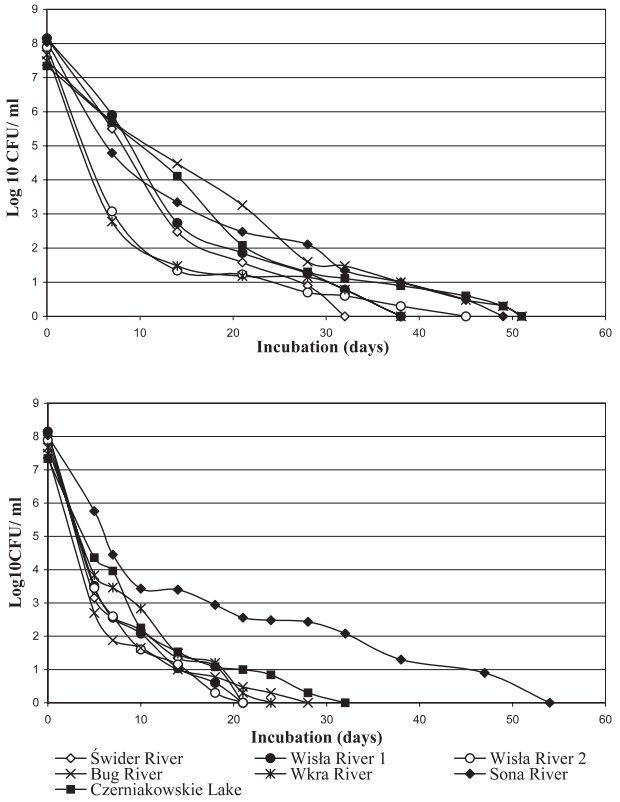

Fig. 1. Survival of *E. coli* O157:H7 serotype in water at the temperature of 6°C (top curves) and at 24°C (bottom curves). Each point is the mean of values from duplicate experiments

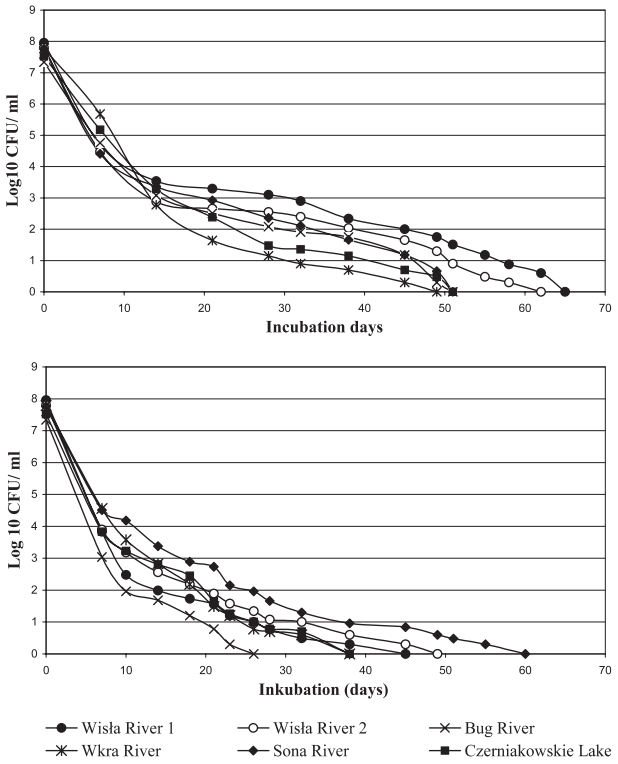

Fig. 2. Survival of *E. coli* O157:H7 serotype in muddy water at the temperature of 6°C (top curves) and at 24°C (bottom curves). Each point is the mean of values from duplicate experiments

sample incubation at both temperatures (data not presented). On the other hand, pH of water increased from 7.57-8.06 to 8.03-8.32 (6°C) and 7.78-8.22 (24°C) as well as muddy water from 7.57-7.92 to 7.83-8.06 (6°C) and 7.76-8.15 (24°C) (data not presented), but these values were outside the optimal pH range 6-7 for *E. coli* [20].

Comparing the results obtained in the frames of present work and the results of Wang and Doyle [21], it can be stated that at low temperature *E. coli* O157:H7 strain isolated in Poland survived shorter in water than a mixture of O157:H7 strains examined by the above-mentioned scientists. The strains introduced by these authors were still present in water from town water supplies and in water from two lakes after 91 days of incubation at 8°C. Bacteria survival at 24-25°C in present work and in Wang and Doyle study were much the same. In 4/5 water samples from Poland and in two water samples from USA lakes O157:H7 bacteria were undetectable (<1 CFU/ ml) by agar plating method after 21-35 days of incubation.

The investigations oriented on the determination of serotype O157:H7 survival in natural mineral water [22] indicated that these bacteria died in this medium only after 70 incubation days. Simultaneously, the authors concluded that autochtonic microflora not only do not exert any antagonistic action on serotype O157:H7 but even slowed down its death rate in this medium.

The pathogenic strain, isolated in Poland, survived in water longer than another strain *E. coli* W3110 [23]. Absence of this strain at 4°C was noticed after 12 days while at 20°C after 8 incubation days. The investigations from Rhodes and Kator, Virginia Institute of Marine Science, USA [24], once again confirmed the dependence between the survival of *E. coli* and *Salmonella* species and water temperature. Both types of bacteria died at the fastest rate at Ware River mouth in February at the highest temperature and at the slowest rate in May. In frames of around a 12-day-long experiment, the number of bacteria *E. coli* and Salmonella decreased in the first month from 6 log CFU/mL respectively to <1 log CFU/mL and around 2 log CFU/mL, whereas in the second month to the level of around 3.5 and 4.5 log CFU/mL.

In the present investigations on survival of serotype O157:H7 in bottom-shore sediments, the dependence between the survival of pathogenic bacteria and temperature of sample incubation was evidently observed. At 6°C indetectable level (< 1 CFU/ g) of pathogenic bacteria after agar plating method was noticed after 73-100 incubation days, whereas at 24°C after 30-60 incubation days (Fig. 3). Similar results concerning the survival of fecal bacteria in river sediments were obtained by Davies et al. [25]. The authors found out that after a 60-day incubation period of samples at 20°C bacteria *E. coli* either died completely or their number decreased to the level close to 7 log CFU/mL down to around 3 log CFU/mL, depending on sediment source.

Table 1. Changes in the bacterial population of environmental samples after incubation at temperatures at 6°C and 24°C.

River/ Lake	Type of Sample	Time-zero of incubation			After incubation at 6°C				After incubation at 24°C			
		Total count of bacteria[/1]	Coliforms[/1]	*E. coli*[/1]	Days	Total count of bacteria[/1]	Coliforms[/1]	*E. coli*[/1]	Days	Total count of bacteria[/1]	Coliforms[/1]	*E.coli*[/1]
Świder River [/2]	Water Bottom-shore sediment	8.3×10^3 2.4×10^6	4.9×10^1 1.5×10^3	ND[/3] 2.8×10^1	32 90	4.7×10^4 1.4×10^6	3.2×10^1 1.4×10^3	ND <$10^{/4}$	21 30	1.8×10^4 3.5×10^5	2.2×10^1 1.4×10^2	ND < 10
Wisła River - collection site 1	Water Muddy water Bottom-shore sediment	2.3×10^4 1.2×10^6 3.0×10^6	1.0×10^2 3.0×10^3 5.4×10^3	ND 7.0×10^1 4.0×10^1	38 65 96	4.6×10^4 1.5×10^6 1.9×10^6	5.2×10^1 3.7×10^2 8.7×10^3	ND ND <10	21 45 60	4.8×10^3 1.8×10^6 1.7×10^6	3.8×10^1 4.2×10^3 2.0×10^4	ND ND <10
Wisła River – collection site 2	Water Muddy water Bottom-shore sediment	7.9×10^2 1.8×10^6 4.8×10^6	9.0×10^1 9.5×10^1 5.8×10^4	ND 4 3.6×10^1	45 62 90	2.7×10^3 3.0×10^6 1.1×10^6	2.2×10^1 2.1×10^2 5.5×10^3	ND ND <10	21 49 30	4.0×10^3 5.2×10^5 3.8×10^6	2.0×10^1 2.3×10^2 2.4×10^3	ND ND <10
Bug River	Water Muddy water Bottom-shore sediment	7.9×10^3 1.7×10^6 2.5×10^6	2.3×10^2 9.8×10^2 2.7×10^4	ND 6.0×10^1 8.8×10^1	51 51 73	3.0×10^3 2.3×10^5 3.3×10^6	8.9×10^1 1.2×10^2 1.2×10^2	ND 1.0×10^1 2	28 26 36	7.1×10^3 1.1×10^5 1.6×10^6	4 6.6×10^1 1.3×10^2	ND ND <10
Wkra River	Water Muddy water Bottom-shore sediment	3.6×10^4 6.8×10^5 5.9×10^6	2.0×10^2 1.1×10^4 2.0×10^3	2.2×10^1 1.1×10^2 1.4×10^2	38 49 80	4.3×10^4 3.1×10^5 1.1×10^6	2.4×10^1 1.6×10^2 1.0×10^3	ND ND <10	24 38 30	1.8×10^4 4.0×10^4 4.4×10^5	4.2×10^1 6.3×10^3 3.5×10^3	ND ND <10
Sona River	Water Muddy water Bottom-shore sediment	6.3×10^3 4.7×10^5 6.2×10^6	3.8×10^1 6.7×10^2 7.4×10^3	ND 3.2×10^1 3.7×10^1	49 51 80	1.4×10^4 2.4×10^5 1.1×10^6	1.2×10^1 2.3×10^2 8.5×10^1	ND ND 1.7×10^1	54 60 40	2.3×10^3 3.3×10^5 2.6×10^6	2.1×10^1 5.6×10^1 3.8×10^3	ND ND <10
Czerniakowskie Lake, Warsaw	Water Muddy water Bottom-shore sediment	4.3×10^4 2.2×10^6 3.3×10^6	1.4×10^2 5.0×10^4 6.9×10^4	8.7×10^1 7.5×10^1 9.0×10^1	51 51 100	1.6×10^2 2.4×10^5 1.4×10^6	8.7×10^1 3.0×10^2 2.2×10^4	ND 2.3×10^1 <10	32 38 56	1.3×10^4 9.3×10^4 7.9×10^5	2.8×10^1 1.6×10^2 1.5×10^4	ND ND <10

[/1] CFU/ mL or g; [/2] No muddy water layer over sediment in this river [/3] Not detected in 1 mL of liquid samples [/4] Not detected in 1 mL of first dilution of sediment samples

Table 2. Physicochemical characteristics of water and muddy water used in the experiments on the *E. coli* serotype O157:H7 survival.

River/ Lake	Type of sample	pH	COD (mg O_2/L)	Dry mass d.m. (%)	Inorganic substances (% d.m.)	Organic substances (% d.m.)
Świder River	Water	7.63	84	-	-	-
Wisła River 1	Water	7.83	68	-	-	
	Muddy water	7.77	136	10.4	91.3	8.7
Wisła River 2	Water	8.06	139	-	-	-
	Muddy water	7.86	171	1.5	92.6	7.4
Bug River	Water	7.95	115	-	-	-
	Muddy water	7.57	504	0.6	91.3	8.7
Wkra River	Water	7.68	48	-	-	-
	Muddy water	7.38	80	1.0	92.2	7.8
Sona River	Water	7.92	24	-	-	-
	Muddy water	7.65	33	1.2	92.2	7.8
Czerniakowskie Lake	Water	7.57	69	-	-	-
	Muddy water	7.72	178	0.8	98.4	1.6

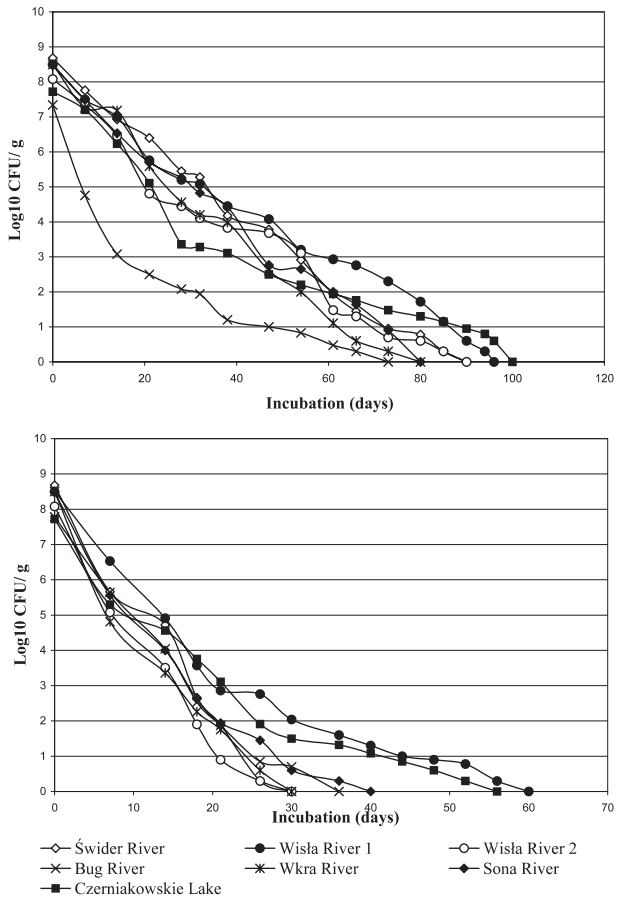

Fig. 3. Survival of *E. coli* O157:H7 serotype in bottom-shore sediments at 6°C (top curves) and 24°C (bottom curves). Each point is the mean of values from duplicate experiments

In the investigations presented here, the longest survival at both temperatures was found for bacteria from Wisła River sediments (collection site 1) and from Czerniakowskie Lake. This phenomenon could not be explained based on analysis of physicochemical and microbiological indexes, determined in the frames of present investigations. For example, this effect could not be related to the high organic substances content of these materials (it was observed during experiments on the O157:H7 serotype survival in soils — Czajkowska et al. [26]). In sediment from the Wisła River this content was in fact high (4.4% dry mass) and amounted to 4.4-4.5% after incubation period, whereas in sediment from Czerniakowskie Lake it was low (0.5% dry mass) and increased after the end of incubation only to the level of 0.6-1.0% dry mass (Table 3). Initial pH of sediments from Wisła River (collection site 1-) and Czerniakowskie Lake (No 7), being outside the optimal pH range *E. coli*, did not differ significantly from pH of sediments from the majority of other rivers (Table 3). Also, the final pH of sediments from the Bug (No 4) and Wkra Rivers — No 5 (for samples incubated at temperature 6°C, 7.38 and 7.13, respectively) was closer to optimal pH than to pH of sediments from Wisła River and Czerniakowskie Lake (7.51 and 7.45, respectively) and nevertheless, the survival of pathogenic bacteria in these sediments was 10-20 days shorter (Fig. 3, top curves, and Table 3).

After incubation sediments at both temperature, dry mass content was decreased (Table 3). We also observed negligible changes in organic and inorganic substance content (Table 3). It could be assumed that there were more due to the heterogeneity of the samples itself than to the activity of microorganisms present in the samples.

Table 3. Changes in pH, dry mass, inorganic substance and organic substance contents in bottom-shore sediments after the end of incubation at 6°C and 24°C.

River/ Lake	Time-zero of incubation				After incubation at 6°C					After incubation at 24°C				
	pH	Dry mass [%]	Inorganic substances [% d.m.]	Organic substances [% d.m.]	Days	pH	Dry mass [%]	Inorganic substances [% d.m.]	Organic substances [% d.m.]	Days	PH	Dry mass [%]	Inorganic substances [% d.m.]	Organic substances [% d.m.]
Świder River	7.14	74.8	99.4	0.6	90	8.03	74.4	99.3	0.7	30	7.85	69.7	99.5	0.5
Wisła River 1	7.67	46.8	95.6	4.4	96	7.51	44.7	95.5	4.5	60	7.82	43.6	96.6	4.4
Wisła River 2	7.75	74.7	98.5	1.5	90	7.54	49.2	98.6	1.4	30	7.65	55.0	98.6	1.4
Bug River	7.72	71.4	99.4	0.6	73	7.38	69.6	99.1	0.9	36	7.52	67.0	99.6	0.4
Wkra River	7.84	78.9	99.8	0.2	80	7.13	75.8	99.5	0.5	30	7.93	74.2	99.6	0.4
Sona River	7.58	82.8	99.7	0.3	80	7.64	76.3	99.3	0.7	40	7.73	69.9	99.4	0.6
Czerniakowskie Lake	7.64	83.1	99.5	0.5	100	7.45	77.7	99.0	1.0	56	7.62	80.0	99.4	0.6

No regularities were observed in the changes of quantity of mesophilic bacteria population in all materials from water environment after the end of incubation at both temperatures (Table 1). Nevertheless, it was concluded that there was a tendency to a decline in the number of bacteria from the coli group in the majority of samples. Environmental bacteria *Escherichia coli* were noted in the materials where bacteria serotype O157:H7 were no longer detectable. This fact confirmed the previously observed phenomenon of the faster death of pathogenic bacteria from *Enterobacteriaceae* family than of other non-pathogenic strains from the same family.

Conclusion

The obtained results of investigations indicate evidently an existence of a possible hazard connected with the relatively long survival of serotype O157:H7 in materials from a water environment. The bottom-shore sediments in particular can be a reservoir of pathogenic bacteria. In the investigations presented here the bacteria of pathogenic serotype died faster than environmental bacteria *Escherichia coli*. Nevertheless, taking into account cited results of investigations from other authors, it can be supposed that the lack of indicative bacteria *E. coli* will not always be the confirmation of absence of dangerous pathogens. Investigations on other strains of *E. coli* O157:H7, isolated in Poland, should be continued to establish the final conclusions.

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