

Assessment of microbiological quality of water in the Nowohucki Reservoir with particular regard to microorganisms potentially dangerous to humans

Ocena stanu mikrobiologicznego wód Zalewu w Nowej Hucie ze szczególnym uwzględnieniem drobnoustrojów potencjalnie niebezpiecznych dla człowieka

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- (c) conducting research
- (d) analysis of results and drawing conclusions
- (e) preparation of the manuscript

ABSTRACT

Introduction. This study was aimed to assess the microbiological quality of water in the Nowohucki Reservoir (Kraków, Poland) as well as to determine whether its waters contain microorganisms potentially dangerous from an epidemiological point of view.

Material and methods. Microbiological analyses included the determination of the number of mesophilic and psychrophilic bacteria, coliforms, fecal *E. coli*, as well as *E. faecalis*, *C. perfringens*, *Staphylococcus* spp. and *Salmonella* spp.. Water samples were collected 4 times per year on April 27th 2015 (spring), July 10th 2015 (summer), October 12th 2015 (autumn) and December 29th 2015 (winter) at 5 points within the area of the reservoir. Water and air temperature was measured onsite.

Results. It was found that the prevalence of the analyzed microorganisms was affected by changing water and air temperature as well as by using this reservoir during holiday season for swimming purposes by local residents. All analyzed microbiological indicators of poor water quality were found in the analyzed water samples, which may pose a potential health risk to people swimming in the considered reservoir.

Conclusions. From an epidemiological point of view, it is reasonable to include the Nowohucki Reservoir into a constant sanitary monitoring programme.

Key words: microbiological contamination of water, mesophilic bacteria, *E. coli*, *E. faecalis*, surface water, Nowohucki Reservoir.

STRESZCZENIE

Wstęp. Badania miały na celu ocenę stanu mikrobiologicznego wód Zalewu w Nowej Hucie (Kraków, Polska) a także określenie, czy w zbiorniku występują drobnoustroje potencjalnie niebezpieczne z epidemiologicznego punktu widzenia.

Materiał i metody. Analizy mikrobiologiczne obejmowały określenie liczebności bakterii mezofilnych, psychrofilnych, bakterii grupy coli, form kałowych *E. coli*, a także *E. faecalis*, *C. perfringens*, *Staphylococcus* spp. i *Salmonella* spp.. Próbkę wód pobierane były 4 razy w roku w następujących terminach: 27.04.2015 r. (wiosna), 10.07.2015 r. (lato), 12.10.2015 r. (jesień) i 29.12.2015 r. (zima) w 5 punktach na terenie zalewu. Ponadto, w każdym punkcie pomiarowym mierzono temperaturę wody oraz powietrza.

Wyniki. Stwierdzono, że na liczebność badanych drobnoustrojów wpływała zmieniająca się temperatura wody i powietrza oraz użytkowanie w sezonie wakacyjnym zalewu w charakterze kąpieliska przez okolicznych mieszkańców. W pobranych próbkach zidentyfikowano wszystkie badane wskaźniki czystości mikrobiologicznej wód, które stanowią potencjalne zagrożenie dla kąpiących się w zbiorniku ludzi.

Wnioski. Z epidemiologicznego punktu widzenia zasadnym jest objęcie wód Zalewu w Nowej Hucie stałym monitoringiem sanitarnym.

Słowa kluczowe: mikrobiologiczne zanieczyszczenie wody, bakterie mezofilne, *E. coli*, *E. faecalis*, wody powierzchniowe, Zalew w Nowej Hucie.

INTRODUCTION

The assessment of water contamination in Poland is currently conducted based on microbiological, physico-chemical and hydrobiological analyses according to the guidelines presented in both national and EU standards and regulations [1–7]. Depending on its usage profile (drinking water, bathing, water intended for domestic purposes, etc.), the quality of surface water is evaluated based on the indicators included in the Regulations of the Minister of Environment.

The primary objective of this study was to assess the quality of water in the Nowohucki Reservoir, which was put into operation in 1957 as an artificial reservoir and became an object of recreation for the residents of the old part of the Nowa Huta (the largest district of Kraków, Poland). This reservoir was built in a two-kilometer green protection zone, separating the steelworks (formerly Tadeusz Sendzimir Steelworks, now ArcelorMittal Poland JSC) from the housing estates of the Nowa Huta. In total, this area covers approx. 17 ha, whereas the area of the reservoir itself is more than 7 ha [8]. In the 50s and 60s of the 20th century, the Nowohucki Reservoir was a place of rest and recreation for the residents of the Nowa Huta district. In subsequent years, this area was neglected, but now the reservoir has four basic functions: it acts as a fishery managed by the Fishing Circle, a place for breeding and growth of water birds, protected amphibians and reptiles, as well as it is an unregistered bathing area and a reservoir which supplies the nearby allotment gardens. Waters of the reservoir are not subject to hygienic and sanitary tests performed by the SANEPID (sanitary and epidemiological station). It should be noted that despite clear bathing prohibition, hundreds of people swim and rest by this reservoir during the holiday season each year. Its water may contain pathogenic microorganisms, such as *Escherichia coli*, *Clostridium perfringens*, or *Enterococcus faecalis* excreted by people, dogs or wild animals swimming in the reservoir. Thus, it is very important to determine whether microorganisms inhabiting the Nowohucki Reservoir may pose threat from an epidemiological point of view and may become the cause of infections and health issues. Therefore, two types of criteria for the assessment of microbiological quality of water were applied in this study, i.e. those contained in the Regulation of the Minister of Health on the requirements for surface water [5] as well as for water in swimming reservoirs [4, 7].

This paper was aimed to determine the changes in the quantity of the selected microorganisms which occur in the water of the Nowohucki Reservoir and which are important from the sanitary and/or epidemiological point of view. Additionally, species identification of the isolated microbial strains was conducted with particular emphasis on bacteria potentially pathogenic to people bathing in the reservoir.

MATERIAL AND METHODS

Water samples in the amount of 1000 ml were collected into sterile containers from five sites located within the reservoir in Nowa Huta (Nowohucki Reservoir), which is the largest district of Kraków (Poland). With regard to the seasonality, the samples of water were collected four times per year on: April 27th 2015 (spring), July 10th 2015 (summer), October 12th 2015 (autumn) and December 29th 2015 (winter) [6]. Water was collected at 5 sites: 1 – inflow; 5 – outflow; 2, 3, 4 – sites within the reservoir, distant from one another (Tab. I; Fig. 1). During collection, water and air temperature were measured onsite at each of the sampling sites using an electronic thermometer (Biowin).

Table I. Coordinates of the sampling sites

Tabela I. Współrzędne punktów pomiarowych

No.	GPS position	Description
1	N 50°4'52'73" E 20°3'3'91"	Inflow
2	N 50° 4'49'11" E 20°3'7'77"	Artificial beach
3	N 50° 4'43'66" E 20°3'5'07"	Angler's House
4	N 50° 4'40'88" E 20°3'16'04"	Pumping station
5	N 50° 4'37'36" E 20°3'17'73"	Outflow

Membrane filtration method was used to determine the number of: coliforms (purple colonies with metallic sheen, cultured on ENDO agar at 37°C, 48 h); thermotolerant (fecal) forms of *E. coli* (purple colonies with metallic sheen, cultured on ENDO agar at 44°C, 48 h); *Enterococcus faecalis* (small, dark-purple colonies, cultured on Slanetz-Bartley agar at 37°C, 72 h); and *Clostridium perfringens* (black colonies cultured in anaerobic conditions on Wilson-Blair agar at 37°C, 48 h). Plate dilution method was used to determine the number of: mesophilic bacteria (cultured on MPA medium at 37°C, 48 h); psychrophilic bacteria (cultured on MPA medium at 22°C, 72 h); *Staphylococcus* spp. (Chapman medium at 37°C, 48 h) and *Salmonella*

spp. (SS agar, at 37°C, 48 h) [1–5, 7]. After incubation the colonies were counted and the results of analysis were expressed, depending on the analyzed microorganisms, as the number of colony forming units per 100 ml of water – membrane filtration method (CFU/100 ml – coliforms, *E. coli*, *E. faecalis*, *C. perfringens*) or per 1 ml of water – plate dilution method (CFU/ml – mesophilic and psychrophilic bacteria, *Staphylococcus* spp., *Salmonella* spp.).

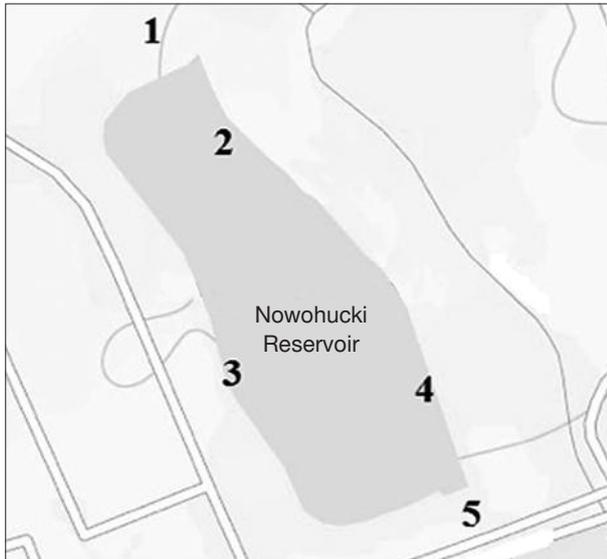


Fig.1. Location of the sampling sites [9]
Ryc.1. Lokalizacja punktów poboru [9]

Statistical analysis aimed to calculate the mean number of microorganisms in the analyzed water samples was conducted using Statistica v. 10 (StatSoft). Analysis of variance (ANOVA) was conducted to assess the significance of both temporal and spatial diversity in the number of the tested microorganisms. The correlation between the mean number of analyzed microbial groups and water and air temperature observed in different seasons of the year was also determined.

RESULTS

The conducted study of the microbiological quality of water in the Nowohucki Reservoir in five sampling sites in 2015 showed large variations in the number and species composition of the analyzed microorganisms (Tab. II). The number of mesophilic bacteria ranged from 31 CFU/ml in spring at the site No. 4 (Pumping station) to 30770 CFU/ml at the site No. 1 (Inflow) in summer. On the other hand, the largest number of psychrophilic bacteria

(45250 CFU/ml) was observed at the site No. 1 (Inflow) in spring, then it decreased to 10 CFU/ml at the site No. 5 (Outflow) in summer. What is more, this annual study indicated that the ratio of mesophilic and psychrophilic bacteria was changing significantly. In spring and autumn, the number of psychrophilic bacteria was much larger than mesophilic bacteria, while in summer this ratio was reversed (Fig. 2).

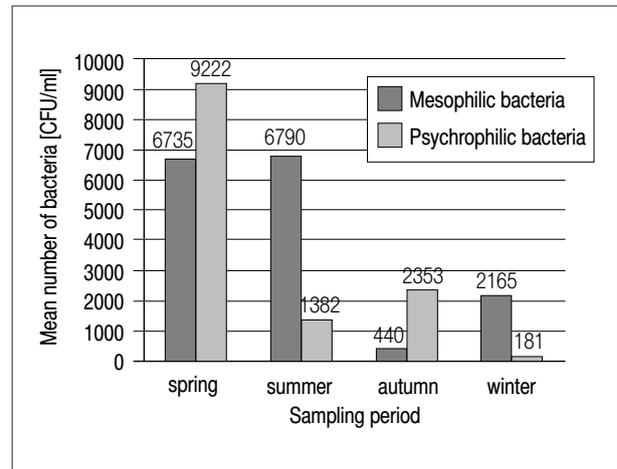


Fig.2. Changes in the mean number of mesophilic and psychrophilic bacteria in the analyzed research period

Ryc. 2. Zmiany średniej liczebności bakterii mezofilnych i psychrofilnych w analizowanym okresie badawczym

Coliforms were detected in all samples tested outside the period of winter. A similar trend was observed in the case of fecal forms of *E. coli*, which even though present in winter, at sites No. 2 (Artificial beach) and No. 5 (Outflow), their number in both cases was at the level of 10 CFU/100 ml of water. In addition, both coliforms and fecal *E. coli* were more numerous in the analyzed reservoir in spring and summer (Tab. II; Fig. 3). *E. faecalis* occurred in the samples from the Nowohucki Reservoir in spring and summer, with numbers ranging from 10 to 342 CFU/100 ml, while at the site No. 4 (Pumping station) *E. faecalis* was not detected in any of the analyses. Also *C. perfringens* were more frequently isolated in summer and their mean number in this period at all sampling sites was 363 CFU/100 ml (Fig. 3). On the other hand, no *Staphylococcus* spp. was found in spring in the analyzed water samples. The highest mean number of staphylococci was observed in summer (58 CFU/ml) and winter (52 CFU/ml) (Fig. 3). *Salmonella* spp. rods were detected only at the site No. 1 (Inflow) in spring (8 CFU/ml) and summer (7 CFU/ml) (Tab. II).

Table II. The prevalence of the selected microbial groups in waters of the Nowohucki Reservoir (CFU/100 ml: coliforms, fecal *E. coli*, *E. faecalis*, *C. perfringens*; CFU/1 ml: mesophilic and psychrophilic bacteria, *Staphylococcus* spp., *Salmonella* spp.) as well as water and air temperature at the sampling sites (°C)

Tabela II. Częstość występowania wybranych grup drobnoustrojów w wodach Zalewu w Nowej Hucie (jtk/100 ml: bakterie z grupy coli, formy katowe *E. coli*, *E. faecalis*, *C. perfringens*; jtk/1 ml: bakterie mezofile i psychrofilne, *Staphylococcus* spp., *Salmonella* spp.) oraz temperatura wody i powietrza w punktach pomiarowych (°C)

Sam-pling date	Sam-pling site	Coliforms	Fecal coliforms	<i>E. fae-calis</i>	<i>C. perfrin-gens</i>	<i>Staphy-lococ-cus</i> spp.	Meso-philic bacteria	Psycho-philic bacteria	<i>Salmo-nella</i> spp.	Water temp.	Air temp.
spring	1 Inflow	10800	2440	342	50	0	27550	45250	8	9.2	8.1
summer		9200	9100	100	710	192	30770	5394	7	14.3	17.9
autumn		370	190	0	200	32	227	3375	0	7.3	9.1
winter		0	0	0	55	136	8226	500	0	3.8	4.1
Mean		6790	3910	221	254	120	16693	13630	8	–	–
spring	2 Artificial beach	130	90	10	150	0	2376	165	0	8.5	8.3
summer		1350	437	0	125	24	324	41	0	22.8	20.8
autumn		747	445	0	150	18	580	7750	0	8.1	9.8
winter		0	10	0	50	31	1063	139	0	3.4	3.1
Mean		742	247	10	119	24	1086	2024	0	–	–
spring	3 Angler's house	170	100	10	345	0	2350	343	0	8.0	7.9
summer		210	495	0	430	28	254	69	0	20.1	2.4
autumn		175	40	0	240	18	151	54	0	7.9	9.4
winter		0	0	0	105	37	34	33	0	3.3	3.4
Mean		185	212	10	280	28	697	125	0	–	–
spring	4 Pumping station	50	30	0	330	0	31	130	0	8.0	8.0
summer		255	0	0	325	22	2243	25	0	19.6	18.4
autumn		130	100	0	260	16	139	385	0	7.8	8.9
winter		0	0	0	50	26	1033	122	0	3.7	2.9
Mean		145	65	0	241	21	862	166	0	–	–
spring	5 Outflow	155	20	100	305	0	1367	221	0	8.7	8.1
summer		500	65	0	225	23	359	10	0	19.9	19.1
autumn		50	20	0	95	6	1104	200	0	7.4	9.3
winter		0	10	0	50	32	469	113	0	3.3	3.6
Mean		235	29	100	169	20	825	136	0	–	–

The statistical analysis of the results indicated that the changes in the number of mesophilic bacteria between the analyzed water sampling sites are statistically significant. On the other hand, the seasonal differences in the number of the analyzed microorganisms throughout the year are statistically significant only in the case of *C. perfringens* (Tab. III). In addition, the statistical analysis of the rela-

tionship between the mean numbers of microorganisms in the water samples and the mean water and air temperature confirmed in most cases that there was almost full, or very high positive correlation between these values ($p < 0.05$). Only in the case of psychrophilic bacteria this correlation was negative or very low (Tab. III).

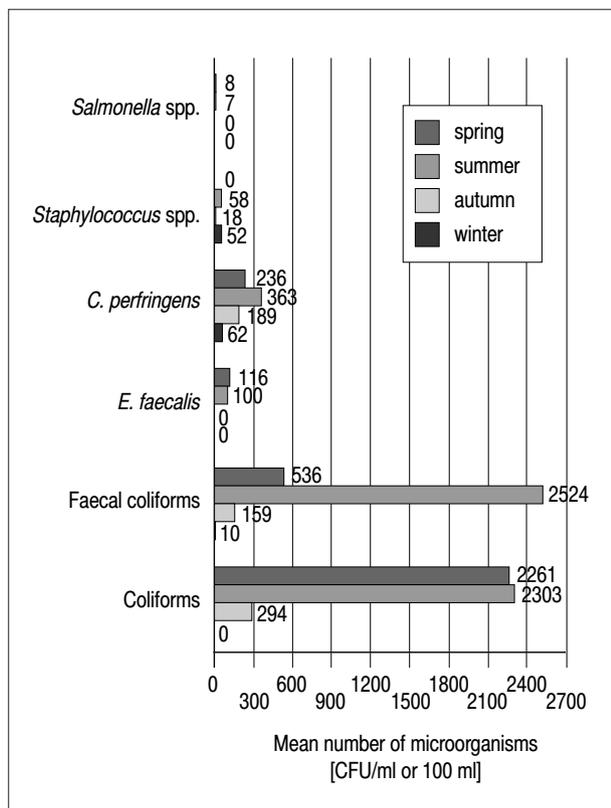


Fig. 3. Changes in the mean prevalence of indicator bacteria in the study period

Ryc. 3. Zmiany średniej liczebności bakterii wskaźnikowych w analizowanym okresie badawczym

Table III. Results of analysis of variance of the spatial and temporal variability in the number of microorganisms and the value of Pearson's correlation coefficient *r*

Tabela III. Wyniki analizy wariancji dotyczącej czasowego i przestrzennego zróżnicowania liczebności mikroorganizmów oraz wartość współczynnika korelacji *r* Pearsona

Microorganism	Coefficient F (*values are significant at p<0.05)		Correlation coefficient (p<0.05)	
	Sampling site	Season	Water temp. (°C)	Air temp. (°C)
Coliforms	2.87	0.81	0.74	0.67
Faecal coliforms	1.75	1.00	0.98	0.95
<i>E. faecalis</i>	1.56	1.67	0.64	0.56
<i>C. perfringens</i>	0.59	4.27*	0.95	0.94
<i>Staphylococcus</i> spp.	2.40	1.94	0.34	0.34
Mesophilic bacteria	4.49*	0.64	0.64	0.55
Psychrophilic bacteria	1.49	0.80	-0.05	-0.11
<i>Salmonella</i> spp.	2.97	0.67	0.65	0.57

DISCUSSION

The quality of surface water depends on numerous factors, both of natural and anthropogenic origin [10, 11]. Microorganisms in aquatic environment comprise the so-called autochthonous microflora (indigenous microorganisms, for which the considered reservoir is the primary ecological niche) and allochthonous (extraneous) microflora [12, 13]. The distribution and abundance of both indigenous and extraneous microorganisms in water is affected among others by the availability the availability of nutrients typical for aquatic ecosystems, water and air temperature, amount of dissolved oxygen as well as pH of water [14, 15]. This study was aimed to determine the occurrence of autochthonous and allochthonous microorganisms based on the prevalence of psychrophilic and mesophilic bacteria, respectively. Psychrophilic bacteria do not pose threat to the human health and they play a very important role in the mineralization of organic substances and the circulation of elements in the environment, thus contributing to the process of self-purification of water [15]. They are more frequently isolated from water in periods characterized by low temperatures, which to some extent was confirmed in this study. The highest mean number of psychrophilic bacteria was found in spring, those numbers were average in summer and autumn and then dropped to be the lowest in winter, which could have been caused by gradual depletion of organic compounds in the reservoir and therefore by its purification [16]. On the other hand, changes in numbers of mesophilic bacteria are often very dynamic and occurrence of these bacteria in the reservoir may be periodic. Mesophilic bacteria are classified as allochthonous microorganisms, whose presence in water is unwanted and may be the effect of its contamination by e.g. sewage [17]. The distribution and abundance of mesophilic bacteria is subject to significant changes because they are microorganisms that in most cases are not capable of reproduction in aquatic environment; nevertheless, they can occur temporarily, especially in the forms of spores [12, 14, 18].

While assessing the microbiological quality of surface water, most attention is paid to microorganisms important from sanitary or epidemiological point of view. Such microorganisms primarily include pathogenic bacteria that can migrate to surface water together with municipal sewage, effluent originating from animal breeding farms and rainwater, or they can originate from feces of wild animals [12, 14, 15, 18, 19]. Contact with water contaminated with pathogenic microorganisms – by its con-

sumption, bathing or usage for production of e.g. food – may pose threat from an epidemiological perspective. Therefore, monitoring the presence of fecal microorganisms in waters is critical, as they can be the cause of serious diseases both in humans and animals. The presence of coliforms (mainly *Escherichia coli* and genera such as *Enterobacter*, *Citrobacter* and *Klebsiella*) as well as fecal types of *E. coli* (thermotolerant strains, able to grow at 44°C), that were also determined in this research, are basic microbiological indicators of the sanitary quality of water [20, 21]. In this study, the highest numbers of coliforms and fecal *E. coli* were observed in summer, when there are many people bathing in the Nowohucki Reservoir. Although swimming in this reservoir is prohibited, still during each summer holidays it is the place for bathing and/or swimming for the residents of Nowa Huta. Fecal enterococci, which – like coliforms – are constantly present in feces of people and animals, are another well recognized indicators of sanitary contamination of water. Fecal enterococci which include *E. faecalis*, also determined in this study, when they significantly outnumber coliforms, may indicate the contamination of water with animal feces or effluent from animal breeding farms [15]. Such relationship was not observed in this study, i.e. small numbers of *E. faecalis* were detected in spring and summer only in some sampling sites. *E. faecalis* cannot reproduce in water, hence its presence evidences fresh fecal contamination of water [18, 22]. On the other hand, the presence of anaerobic rods of the genus *Clostridium* may evidence – due to its ability to produce spores – that the fecal contamination of water is more distant in time [18, 23, 24]. Bacteria *C. perfringens* were isolated throughout the year from water collected at all sampling sites, but their highest abundance was observed in summer. Microbiological analysis of water was supplemented by the determination of *Staphylococcus* spp. Water-borne staphylococci may enter human organism through damaged skin, mucous membranes as well as through the urogenital system [25]. Coagulase-positive species of *Staphylococcus* are particularly dangerous, because they may become the cause of purulent infections of skin, subcutaneous tissues and soft tissues, systemic infections and intoxications [26]. Moreover, staphylococci – contrary to most of indicator bacteria – are characterized by longer survivability in water, therefore they pose greater threat to people swimming in the reservoir [25]. The presence of rods of the genus *Salmonella* was also assessed in this research, as they are frequently isolated from water environment. Nevertheless, their pres-

ence and number can significantly vary in time, which is affected by – among others – lack of ability to form spores by these bacteria [27]. All species of the genus *Salmonella* are considered obligatory pathogenic. They can be the cause of food poisonings with fever, typhoid fever, sepsis, skin infections, as well as respiratory and urinary tract infections [27, 28]. Infection is most often a result of contact with sick people or those who are vectors, through contaminated water and food [29]. As demonstrated by the results of this study, *Salmonella* spp. were extremely rarely isolated from the analyzed water samples. Nevertheless, considering the possibility of their occurrence in surface waters used for recreation, it is legitimate to routinely determine the occurrence of *Salmonella* spp., since during holiday season a great number of people access the contaminated water, which causes serious health risks for them [29].

The most severe contamination of water in the Nowohucki Reservoir, including potentially pathogenic microorganisms, was reported during holidays, when the reservoir, despite the prohibition, acts as a public bathing area. This is probably affected by large number of people bathing in the reservoir, who can be the source of the analyzed microorganisms. The presence of microorganisms was also significantly affected by the temperature of water and air, because with its decrease, the number of waterborne microorganisms declined proportionally. In addition to temperature and intensive use of the reservoir, it was noted that the number of indicator microorganisms in water varies depending on the sampling site. The greatest contamination, including pathogenic bacteria, was observed in points No. 1 (Inflow) and No. 2 (Artificial beach), regardless of the season. Then the number of microorganisms decreased in the following sampling sites: No. 3 (Angler's House), 4 (Pumping station) and 5 (Outflow). The obtained results were interpreted based on the limits provided by the Regulations of the Minister of Health [4, 5, 7], determining the parameters of sanitary quality of surface water and bathing water. Water samples collected from the Nowohucki Reservoir were qualified as class II and III of purity (in the five-point scale). Class II comprises waters of good quality, which meet most of the requirements and are subject to only slight anthropogenic impact. On the other hand, class III includes waters of satisfactory quality, which require purification before their use for drinking purposes and are subject to moderate anthropogenic influence [5]. When interpreting the results obtained in this study in relation to the standards to be met by bathing water, it can be stated

that the numbers of fecal *E. coli* and coliforms recorded at the site No. 1 (Inflow) exceed the limits and then drop in the other sampling sites to fall within the limit values [4]. The presence of other indicators of microbial contamination of bathing waters corresponds to the respected values [4, 7]. Based on the conducted study it can be concluded that the quality of water in the Nowohucki Reservoir is satisfactory, despite the presence of microorganisms potentially dangerous to humans. On the other hand, due to high interest of the Nowa Huta residents in the considered reservoir, it is legitimate to consider including this reservoir in the permanent monitoring programme of sanitary water quality.

CONCLUSIONS

This study assessed the microbiological quality of water in the Nowohucki Reservoir and determined whether waters of this reservoir contain microorganisms that can be epidemiologically dangerous to humans. Regular analyses conducted in 2015 allowed to determine the number of mesophilic and psychrophilic bacteria as well as microorganisms that are potential pathogens of humans, i.e. coliforms, fecal *E. coli* and *E. faecalis*, *C. perfringens*, *Staphylococcus* spp. and *Salmonella* spp. The collected results indicated that numbers of microorganisms considered in this study changed dynamically and were shaped by both climatic conditions and the use of the Nowohucki Reservoir during the holiday season as a bathing area by the local residents.

Poor microbiological quality of water makes it difficult, and sometimes even prevents the use of water resources for the needs of people. Moreover, it impairs the quality of water environment, causing changes in water and water-dependent ecosystems. The human impact on the quality of water resources is extremely complex and multilateral. We can distinguish various sources of water contaminated with pathogenic bacteria, such as: municipal sewage, hospital wastewater (particularly from departments of infectious diseases), effluent from animal farms and leachate from landfills. The consequences of microbiological contamination of water may be very severe. Water-transmitted pathogenic bacteria may cause infectious diseases, quickly spreading within the population. The most susceptible group to such infections includes children, elderly or immunocompromised people. Taking into account the results of this study, indicating the possibility of bacteriological contamination of people bathing in the

Nowohucki Reservoir, undertaking permanent control of sanitary and hygienic condition of water in this reservoir should be seriously considered.

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