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Microbiological Contamination of Water in Fountains Located in the Ciechocinek Health Resort

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1. Introduction

Municipal fountains, normally situated in parks and city centres, play mainly a recreational role for people, but they also serve as a habitat and watering place for birds and other animals. Shared use of fountains by animals and people may be conducive to the spread of pathogens and hazardous to the health and life of human beings (Biedunkiewicz 2009). Unlimited access to fountains often encourages people to use them for bathing as well, which leads to an enormous sanitary hazard (Burkowska-But et al. 2013, Eisenstein et al. 2008). Fountain water is characterised by a very good oxygenation and is not subject to disinfection, which is conducive to the growth of pathogens. Microorganisms detected in fountain water include coliform bacteria, staphylococci, streptococci, bacteria of genus *Salmonella* and microscopic fungi (Kirian et al. 2008). Their presence causes mainly gastrointestinal and skin infections (Minshew et al. 2000). Water particles carried by wind form the so-called water-air aerosol, which has the long-awaited soothing effect in summer. However, the aerosol may spread microbiological contamination, including the hazardous *Legionella* spp. bacteria. Inhalation of contaminated aerosol may cause the bacteria penetration into the lungs and may cause the respiratory disease called the Legionnaires' disease (Haupt et al. 2012). The lifespan of pathogens in the environment is generally sufficiently long to

pose a potential threat of spreading diseases by water. The viability of microorganisms in water depends on the type of microorganism and physical and chemical conditions and it may range from several days to several months (Szejniuk et al. 2013). Due to the fact that there are no legal regulations in Poland concerning the sanitary requirements for water from the fountains, it is justified to quote data concerning water used for recreational purposes. Therefore, the quality of water and air around such facilities is crucial for patients who use the fountains. Furthermore, the water is not legally subject of sanitary and epidemiological inspections. The facilities are not examined regularly, while sporadic water analyses are based on the requirements specified in the Regulation of the Minister of Health of 8 April 2011 on the water quality supervision in bathing facilities (Dz.U. 2011 no 86 item 478). The report entitled "Surveillance for Waterborne Disease Outbreaks and Other Health Events Associated with Recreational Water" indicates that between the years 2007 and 2008 in 38 countries a total of 134 recreational water epidemics were recorded that led to at least 13.966 disease cases. More than 60% of them involved gastrointestinal tract disorders, 18% – skin infections, and ca. 13% – respiratory system diseases. Their major epidemiological factors were parasites (64.5%), bacteria (21.0%), viruses (4.8%), chemical substances used (8.6%) and synergic impact of several factors (1.0%) (Office of Surveillance, Epidemiology, and Laboratory Services, Centers for disease control and prevention (CDC), U.S. Department of health and human services, 2011). Fountains located in health resorts frequently serve as therapeutic waters intended for recreational and balneological purposes (Walczak & Lalke-Porczyk 2011).

Conducted research aimed at the assessment of the degree of bacterial and fungal contamination of water samples taken from fountains located in the Ciechocinek health resort from the perspective of biosafety of tourists and patients.

2. Material and methods

2.1. Water sampling

Water samples for testing were taken from four fountains located in the Ciechocinek health resort in the Kujawsko-Pomorskie Province. Fountain A "Jaś i Małgosia" is the most famous fountain in Ciechocinek

situated in the central part of the "Park Zdrojowy". Fountain B "Żaba" is located at the medicinal water pump room in the "Park Zdrojowy". Fountain C "Grzybek" in the city centre serves as a natural inhalatorium due to the properties of brine water. Fountain D "Windsor 600" is the newest fountain located at the famous Hellwig Promenade in Ciechocinek. Closed circulation of water and mechanical filtration was applied in all fountains. Surface of water for individual fountains is respectively: A – 29.21 m^2; B – 66.15m^2; C – 66.60m^2; D – 23.32m^2. It attracts particular attention among tourists, because of its water jet that changes every several minutes. The research was conducted during the period from June to October 2014 in 7 research series. Water samples from individual fountains were taken to sterile glass 1000 ml bottles according to standard PN-EN ISO 19458:2007. The bottles were filled to the ¾ of their volume. Samples were transferred to the laboratory of the Department of Animal Hygiene and Microbiology of the Environment at University of Science and Technology in Bydgoszcz, where, on the same day. A total count of bacteria and fungi, a count of coliform bacteria, *Escherichia coli*, and a count of faecal staphylococci and streptococci were determined in the samples tested.

2.2. Microbiological contamination determination procedure

The sanitary analysis of fountain waters included:

- determination of total mesophilic bacteria count incubated at temperature 37°C during 24 hours on nutrient agar, according to the Polish standard PN-EN-ISO 8199:2010;
- determination of coliform bacteria count on Endo agar and *Escherichia coli* on Tergitol®7 agar with TTC, incubated at temperature 37°C during 24 hours, according to the standard PN-EN-ISO 9308-1:2004;
- determination of enterococci on agar with kanamycin, aesculin and azide, incubated at temperature 37°C during 24-48 hours, according to the Polish standard PN-ISO 7899-2: 2004;
- determination of staphylococci on Chapman agar, incubated at temperature 37°C during 24-48 hours, according to the standard PN-Z-11001-3:2000;
- determination of total fungi count on Sabouraud dextrose agar with chloramphenicol, incubated at temperature 26°C during 5-7 days, according to the Polish standard PN-EN-ISO 8199:2010.

The determination of a total count of mesophilic bacteria and a total count of fungi in the water samples was conducted by using plate method and surface culture technique. In order to detemine total bacterial and fungi count by usage of plate method, a series of decimal dilutions were performed in the range from 10^{-1} to 10^{-4} with condiseration of estimated degree of microbiological contamination in taken samples. The plate method results were read from plates taken from two consecutive dilutions, which demonstrated growth of 15-300 colonies of bacteria and 10-150 colonies of fungi.

The final result expressed in colony forming units (cfu) in a 1 ml of sample was calculated using the following formula:

$$L = \left[\frac{C}{(N_1+0{,}1N_2)\cdot d}\right] \cdot a \quad (1)$$

where:
L – a total count of bacteria or fungi (cfu in 1 ml), C – a total colony count on plates selected for counting, N_1 – number of plates of the first counted dilution, N_2 – number of plates of the second counted dilution, d – dilution factor corresponding to the first counted dilution, a – volume factor of culture material.

For the quantitative determination of coliform bacteria, *Escherichia coli*, staphylococci and streptococci, the membrane filter technique was used. Sterile cellulose ester filters with 0.45 μm pores were used in the tests. The first stage involved dilutions 10^{-1} of water samples. Then occurred filtration of 100 ml undiluted and diluted 10^{-1} water samples, filtered in three repetitions. Following the filtration of a water sample, the filter with retained microorganisms was transferred to a Petri dish with appropriate solid medium. Final result of determination was obtained through counting characteristic colonies that grew on the surface of the filter and taking into account the dilution factor; the result was expressed in cfu/100 ml.

The results of microbiological tests in a logarithmic form were calculated using the Microsoft Excel 2007 program. For calculating the basic statistical data, the Statistica 2010 program was used.

3. Results and discussion

Tests results regarding microbiological contamination of fountain water in the Ciechocinek health resort are presented in Tables 1-6 and Figures 1-6. Throughout the period of testing, the total bacteria count in research series from individual fountains ranged on average from $2.86 \cdot 10^2$ to $5.17 \cdot 10^2$ cfu/ml (Table1). Microorganisms were the most abundant in water taken from fountain B, while their lowest amount was isolated from water samples taken from fountain D, with the difference between the two results amounted to 0.24 log cfu/ml (Fig. 1). Research by Burkowska-But et al. (2013) indicates that the population size of mesophilic bacteria in water samples taken from fountains ranged from $5.0 \cdot 10^0$ to $1.52 \cdot 10^3$ cfu/ml. Furthermore, Burkowska & Donderski (2007) have found that the bacteria may enter air in the form of water aerosol, with their count ranging from 0 to $1.3 \cdot 10^3$ cfu/m^3 depending on the month. However, the mesophilic bacteria content in air ranged from 23 to 566 cfu/m^3, with their highest concentration occurring in August (Burkowska et al. 2012). Szczygłowska et al. (2012) have found that the bacteria content in water-air aerosol reached $1.4 \cdot 10^3$ cfu/m^3, while in contaminated water the number was higher and amounted to $1.9 \cdot 10^4$ cfu/ml.

Table 1. Total number of bacteria (cfu/ml) in the water samples of investigated fountains
Tabela 1. Ogólna liczba bakterii (jtk/ml) w próbkach wody z badanych fontann

Research series	Object			
	A	B	C	D
1	$2.64 \cdot 10^2$	$5{,}91 \cdot 10^2$	$2{,}64 \cdot 10^2$	$2.82 \cdot 10^2$
2	$4.73 \cdot 10^2$	$7.18 \cdot 10^2$	$1{,}81 \cdot 10^2$	$2.27 \cdot 10^2$
3	$8.55 \cdot 10^2$	$6.55 \cdot 10^2$	$5{,}73 \cdot 10^2$	$2.09 \cdot 10^2$
4	$3.27 \cdot 10^2$	$5.64 \cdot 10^2$	$3{,}55 \cdot 10^2$	$3.27 \cdot 10^2$
5	$4.91 \cdot 10^2$	$2.73 \cdot 10^2$	$4{,}09 \cdot 10^2$	$3.18 \cdot 10^2$
6	$4.36 \cdot 10^2$	$4.90 \cdot 10^2$	$3{,}36 \cdot 10^2$	$2.73 \cdot 10^2$
7	$6.45 \cdot 10^2$	$3.27 \cdot 10^2$	$2, 82 \cdot 10^2$	$3.64 \cdot 10^2$
Mean	$4.99 \cdot 10^2$	$5.17 \cdot 10^2$	$3{,}43 \cdot 10^2$	$2.86 \cdot 10^2$

Fungi were also identified in the water samples taken from the relevant municipal fountains (Table 2). Their highest mean count of $1.8 \cdot 10^3$ cfu/ml was recorded for fountain D, while the lowest for fountain B ($2.75 \cdot 10^2$ cfu/ml).

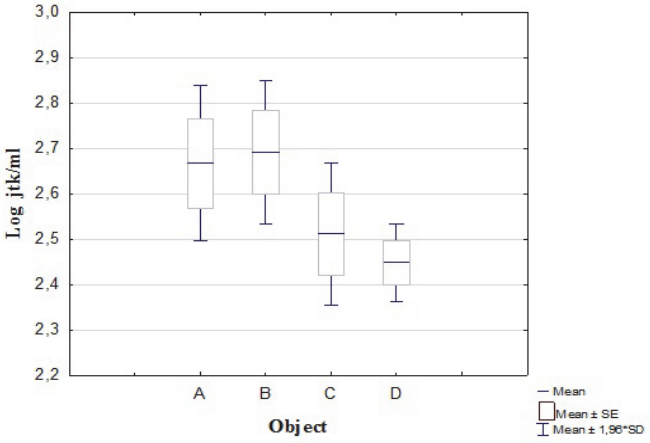

Fig. 1. Average number of bacteria in the water from individual fountains
Rys. 1. Średnia liczba bakterii w wodzie z poszczególnych fontann

In the water samples taken from fountain A, the mean fungi count amounted to $1.3 \cdot 10^3$ cfu/ml, while the water samples from fountain C contained $1.6 \cdot 10^3$ cfu/ml of these microorganisms. It must be noted that for all the fountains analysed, there were samples that did not contain any fungi at all. As the data presented in Figure 2 demonstrate, the largest difference in the total fungi count was recorded between fountain A and fountain B, while a similar count was found in the water samples taken from fountain C and fountain D. It should be noted that the water originating from all fountains was not deprived of spores of fungi, even the water taken from fountain "Grzybek" (C), which had highest degree of salinity, which exceeded 4,51%. Similar results were conducted by Biedunkiewicz (2009), who found a significant fungal contamination of fountain water, demonstrating that 14 out of 60 water samples taken from five municipal fountains contained microfungi. Among them, there were four species of mould fungi (*Aspergillus fumigatus*, *Aspergillus niger*, *Syncephalastrum racemosum* and *Trichoderma viridae*) and 23 species of yeast-like fungi, where *Candida* was the dominating genus (34.78%). During the research, none of the fountains was found to be completely free from fungal contamination (Biedunkiewicz 2009).

Fungal spores may also be present in water-air aerosol. Szczygłowska et al. (2012) argues that their population in air is higher

than that of bacteria and amounts to $5.3 \cdot 10^2$ cfu/m^3. On the other hand, research conducted by Burkowska & Donderski (2006) demonstrates that the highest amount of mould fungi in bioaerosol is reported in June (2600 cfu/m^3). Research by Burkowska-But et al. (2013) shows that bio-indicators of faecal contamination were found in the water of 74% of fountains examined.

Table 2. Total number of fungi (cfu/ml) in the water samples from individual fountains
Tabela 2. Ogólna liczba grzybów (jtk/ml) w próbkach wody z poszczególnych fontann

Research series	Object			
	A	B	C	D
1	$3.36 \cdot 10^2$	$1.09 \cdot 10^2$	$4.55 \cdot 10^2$	$3.00 \cdot 10^2$
2	0	0	$0.45 \cdot 10^0$	0
3	$9.09 \cdot 10^2$	$9.09 \cdot 10^2$	$9.09 \cdot 10^2$	$2.73 \cdot 10^3$
4	0	0	0	0
5	$4.55 \cdot 10^3$	0	9.09×10^3	$9.09 \cdot 10^3$
6	$5.73 \cdot 10^2$	0	7.27×10^2	$4.73 \cdot 10^2$
7	$2.73 \cdot 10^3$	$9.09 \cdot 10^2$	0	0
Mean	$1.30 \cdot 10^3$	$2.75 \cdot 10^2$	$1.60 \cdot 10^3$	$1.80 \cdot 10^3$

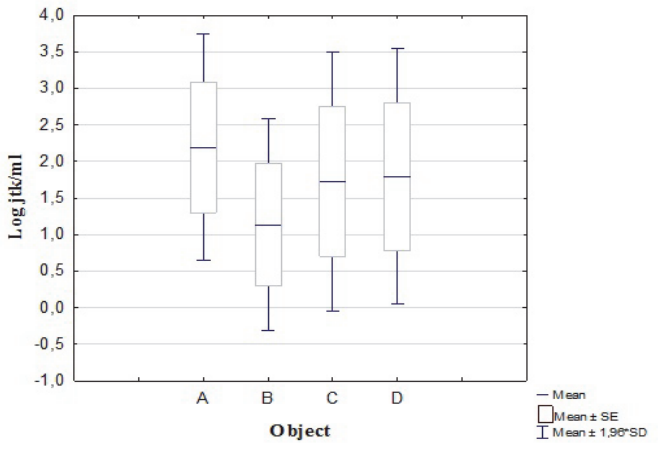

Fig. 2. Mean number of fungi in the water from individual fountains
Rys. 2. Średnia liczba grzybów w wodzie z poszczególnych fontann

Table 3. Number of faecal streptococci (cfu/100 ml) in the water from individual fountains
Tabela 3. Liczebność paciorkowców kałowych (jtk/100 ml) w wodzie z poszczególnych fontann

Research series	Object			
	A	B	C	D
1	0	$2.00 \cdot 10^0$	0	$6.00 \cdot 10^0$
2	$1.20 \cdot 10^1$	$2.50 \cdot 10^1$	0	$1.00 \cdot 10^0$
3	$1.40 \cdot 10^1$	$2.97 \cdot 10^1$	$7.00 \cdot 10^0$	$1.07 \cdot 10^1$
4	$8.67 \cdot 10^0$	0	0	0
5	$1.00 \cdot 10^0$	$1.33 \cdot 10^0$	0	$3.17 \cdot 10^1$
6	0	$2.50 \cdot 10^0$	$6.00 \cdot$	$1.03 \cdot 10^1$
7	0	$5.00 \cdot 10^0$	0	$4.33 \cdot 10^0$
Mean	$5.10 \cdot 10^0$	$9.36 \cdot 10^0$	$1.86 \cdot 10^0$	$9.14 \cdot 10^0$

Own studies indicate that the number of faecal streptococci in the water samples was relatively low (Table 3). Mean amount of these bacteria in the water samples from fountain B was $9.36 \cdot 10^0$ cfu/100 ml and was higher by only 0.22 cfu than the amount isolated from the samples taken from fountain D. The water samples from fountain A were characterised by the presence of faecal streptococci amounting to $5.10 \cdot 10^0$ cfu/100 ml. The water samples from fountain C contained the least amount of these bacteria ($1.86 \cdot 10^0$ cfu/100 ml). A maximum number of faecal streptococci was identified in research series 5 from fountain D ($3.17 \cdot 10^1$ cfu/100 ml). In samples number 4 from fountain B and D and in most of the water samples from fountain C, those bacteria were not found (Table 3). Figure 3 demonstrates the occurrence of faecal streptococci in the water samples taken from individual fountains. As the data indicate, the largest differences regarding the population of those microorganisms were between water samples taken from fountain B and C. Burkowska-But et al. (2013) demonstrate that the content of faecal streptococci in water samples taken from fountains ranged from $3.6 \cdot 10^1$ cfu/ml to $1.99 \cdot 10^3$ cfu/ml. The ratio of the amount of *E. coli* to the number of faecal streptococci FC/FS indicates contamination of animal origin in 3/4 of the fountains examined. According to Hoebe et al. (2004), the number of faecal streptococci in similar facilities amounts to an average of $3.5 \cdot 10^3$ cfu/100 ml. However, Flores et. al. (2013) argue

that the amount of enterococci in fountain water reaches an average of $4.10 \cdot 10^0$ cfu/100 ml. There are two species dominating in this genus: *Enterococcus faecium* and *E. faecalis*. Own studies indicate that the number of faecal streptococci in the water samples ranged from $1.86 \cdot 10^0$ to $9.14 \cdot 10^0$ cfu/100 ml.

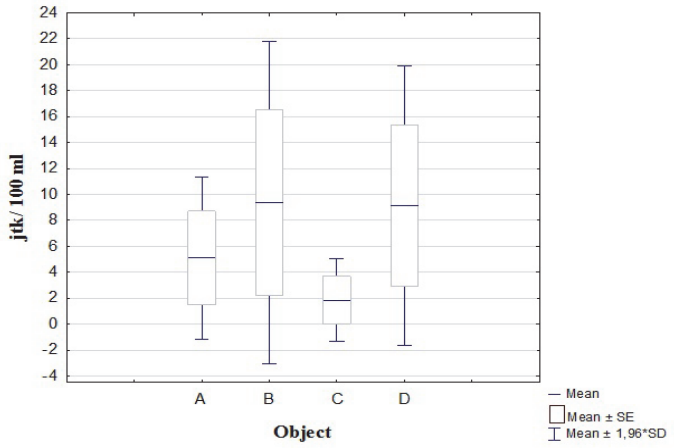

Fig. 3. Occurrence of faecal streptococci in the water from individual fountains
Rys. 3. Występowanie paciorkowców kałowych w wodzie pochodzącej z poszczególnych fontann

The occurrence of coliform bacteria in the water samples taken from the relevant fountains shows Table 4. During the entire period of research, the mean number of coliform bacteria in the water samples ranged from $0.29 \cdot 10^0$ cfu/100 ml to $7.10 \cdot 10^0$ cfu/100 ml. The highest amount of those bacteria was determined in research series 6 from fountain D ($2.27 \cdot 10^1$ cfu/100 ml). The highest frequency of occurrence of coliform bacteria was found for the water samples taken from fountain A, while within the entire period of research regarding the water samples taken from fountain C, only research series 5 contained those bacteria, the amount of which was 2 cfu in 100 ml of water. Figure 4 shows the distribution of coliform bacteria population in the water samples, with their sampling point indicated. The results obtained indicate that the highest differences in the number of coliform bacteria occurred between the water samples taken from fountain C and D.

Table 4. Occurrence of coliform bacteria (cfu/100 ml) in water from individual fountains
Tabela 4. Występowanie bakterii z grupy coli (jtk/100 ml) w wodzie z poszczególnych fontann

Research series	Object			
	A	B	C	D
1	$3.00 \cdot 10^0$	$1.10 \cdot 10^1$	0	$1.00 \cdot 10^1$
2	0	$4.00 \cdot 10^0$	0	0
3	$6.00 \cdot 10^0$	$2.00 \cdot 10^0$	0	0
4	$2.00 \cdot 10^0$	0	0	0
5	$1.47 \cdot 10^1$	$1.10 \cdot 10^1$	$2.00 \cdot 10^0$	$1.70 \cdot 10^1$
6	$1.00 \cdot 10^1$	$8.00 \cdot 10^0$	0	$2.27 \cdot 10^1$
7	$3.67 \cdot 10^0$	0	0	0
Mean	$5.62 \cdot 10^0$	$5.15 \cdot 10^0$	$0.29 \cdot 10^0$	$7.10 \cdot 10^0$

The water samples taken from the fountains contained an average of $9.0 \cdot 10^1$ cfu/100 ml to $2.5 \cdot 10^4$ cfu/100 ml of coliform bacteria, with the population of *E. coli* ranging from 0 to $1.96 \cdot 10^3$ cfu/100 ml (Burkowska-But et al. 2013). However, according to Hoebe et al. (2004), the population of coliform bacteria in fountain water amounts to $1.0 \cdot 10^3$ cfu/ml, while the number of *E. coli* reaches $7.7 \cdot 10^3$ cfu/100 ml. According to Fleming et al. (2000) the amount of *E. coli* in water was $5.0 \cdot 10^0$ cfu/100 ml. However, Flores et. al. (2013) detected *E. coli* in 13 fountains located in Porto (Portugal) a mean population these bacteria amounted to $3.52 \cdot 10^2$ cfu/100 ml. There were $2.1 \cdot 10^3$ cfu/ml of coliform bacteria in the water taken from an interactive fountain, with a mean population of *E. coli* amounting to 40 cfu/ml (Jones et al., 2006). Research by Roscoe et al. (2000) indicates that the water samples taken from fountains contained more than $4.8 \cdot 10^1$ cfu/100 ml of coliform bacteria. The highest number of these bacteria was more than $8.0 \cdot 10^1$ cfu/100 ml. Research by Sezen et al. (2012) indicates that the water samples contained from $3.8 \cdot 10^1$ to $3.0 \cdot 10^2$ cfu/100 ml of coliform bacteria. The number of *E. coli* ranged from 2.2 x 10^1 to $1.0 \cdot 10^2$ cfu/100 ml. Fernández et al. (2002) detected coliform bacteria and *E. coli* respectively in 75% and 49% of samples taken from five fountains located in Guadalajara (Mexico). Research by Hlavsa et al. (2014) confirms that *E. coli* occur also in recreational water in the USA. The use of treated water led to the infections with the strain *E. coli* O157:H7 – 14 cases were

reported. The contact with untreated water, on the other hand, caused infections in 17 people.

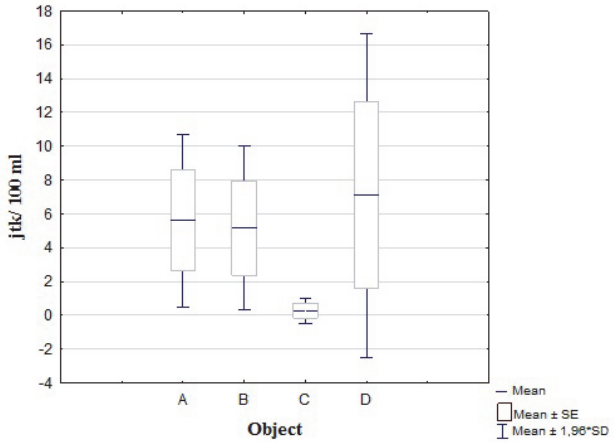

Fig. 4. Mean population of coliform bacteria isolated from water from individual fountains
Rys. 4. Średnia liczebność bakterii z grupy coli wyizolowana z wody z poszczególnych fontann

Table 5 demonstrates research findings regarding the occurrence of *Escherichia coli* isolated from the water samples taken from individual fountains.

Table 5. Number of *Escherichia coli* (cfu/100 ml) in the water from individual fountains
Tabela 5. Liczebność bakterii *Escherichia coli* (jtk/100 ml) w wodzie z poszczególnych fontann

Research series	Object			
	A	B	C	D
1	$3.00 \cdot 10^0$	$6.00 \cdot 10^0$	0	$8.00 \cdot 10^0$
2	0	$3.90 \cdot 10^0$	0	0
3	$3.00 \cdot 10^0$	$1.00 \cdot 10^0$	0	0
4	$2.00 \cdot 10^0$	0	0	0
5	$1.17 \cdot 10^1$	$1.10 \cdot 10^1$	$1.00 \cdot 10^0$	$1.20 \cdot 10^0$
6	$9.00 \cdot 10^0$	$7.67 \cdot 10^0$	0	$2.03 \cdot 10^1$
7	$1.50 \cdot 10^0$	0	0	0
Mean	$4.31 \cdot 10^0$	$4.22 \cdot 10^0$	$0.14 \cdot 10^0$	$5.76 \cdot 10^0$

The mean amount of these bacteria in the water samples taken from fountain D was $5.76 \cdot 10^0$ cfu/100 ml, which was more than 40 times higher than the mean population of these microorganisms isolated from the water taken from fountain C ($0.14 \cdot 10^0$ cfu/100 ml). Water samples taken from fountains A and B had similar content of *E. coli,* which amounted to respectively $4.31 \cdot 10^0$ cfu/100 ml and $4.22 \cdot 10^0$ cfu/100 ml. The highest amount of *Escherichia coli* was determined in research series 6 taken from fountain D ($2.03 \cdot 10^1$ cfu/100 ml). The highest frequency of the occurrence of the *E. coli* bacilli was recorded in fountain A, while they occurred once in research series 5 taken from fountain C Figure 5 demonstrates the occurrence of *E. coli* in water samples taken from individual fountains. Similar to other microorganisms, the highest discrepancies in their amount occurred in fountains C and D. Own studies demonstrate that the least number of coliform bacteria and *E. coli* was found in fountain C, where 4.51% brine water was used. Considering the fact that brine is used for medicinal purposes in Poland, there is insufficient data on the sanitary and hygiene condition of these waters. It is presumed that salt has negative effect on the growth and viability of microorganisms. Previous research indicated that *E. coli* occurred in brine water within the Ciechocinek spa resort and their content ranged from 10 to 140 cfu/ml (Walczak & Lalke-Porczyk 2011).

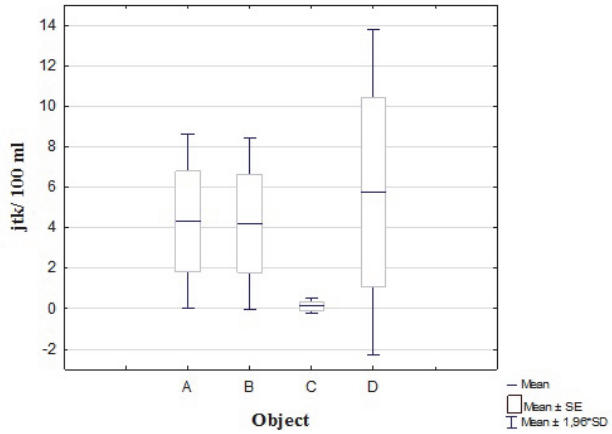

Fig. 5. Mean number of *E. coli* isolated from water from individual fountains
Rys. 5. Średnia liczebność bakterii *E. coli* wyizolowana z wody z poszczególnych fontann

Table 6 demonstrates research findings regarding the occurrence of staphylococci isolated from the water samples taken from individual fountains. The mean population of staphylococci in the water samples taken from fountains ranged from $1.88 \cdot 10^1$ to $3.85 \cdot 10^1$ cfu/100 ml. The water samples taken from fountain A contained an average of $3.25 \cdot 10^1$ cfu/100 ml, while from fountain B – $3.85 \cdot 10^1$ cfu/100 ml. The highest number of staphylococci during the entire research period was determined in water research series 3 taken from fountain D ($7.9 \cdot 10^1$ cfu/100 ml), while in water in research series 1 taken from fountains A and C and in research series 6 from fountain B, no such bacteria were found (Table 6).

Table 6. Number of staphylococci (cfu//100 ml) in water from individual fountains
Tabela 6. Liczebność gronkowców (jtk/100 ml) w wodzie z poszczególnych fontann

Research series	Object			
	A	B	C	D
1	0	$1.40 \cdot 10^1$	0	$2.90 \cdot 10^1$
2	$6.65 \cdot 10^1$	$5.00 \cdot 10^1$	$1.03 \cdot 10^1$	$1.30 \cdot 10^1$
3	$4.50 \cdot 10^1$	$6.12 \cdot 10^1$	$9.82 \cdot 10^0$	$7.90 \cdot 10^1$
4	$4.67 \cdot 10^0$	$5.04 \cdot 10^1$	$1.65 \cdot 10^1$	$2.47 \cdot 10^1$
5	$3.13 \cdot 10^1$	$6.33 \cdot 10^1$	$1.70 \cdot 10^1$	$4.07 \cdot 10^1$
6	$5.37 \cdot 10^1$	0	$7.50 \cdot 10^1$	$3.67 \cdot 10^1$
7	$2.63 \cdot 10^1$	$2.97 \cdot 10^1$	$3.00 \cdot 10^0$	$2.43 \cdot 10^1$
Mean	$3.25 \cdot 10^1$	$3.85 \cdot 10^1$	$1.88 \cdot 10^1$	$3.53 \cdot 10^1$

Figure 6 demonstrates the distribution of staphylococci population in the water samples, with their sampling point indicated. The data indicate that the highest differences were observed staphylococci identified in the water samples taken from fountain B and C. Burkowska & Donderski (2006) confirm that they are also present in water-air aerosol generated by fountains, with their mean amount reaching 140 cfu/m^3. At the same time, the researchers pointed out that the highest amount of those microorganisms occurred during the holiday season (Burkowska & Donderski, 2007).

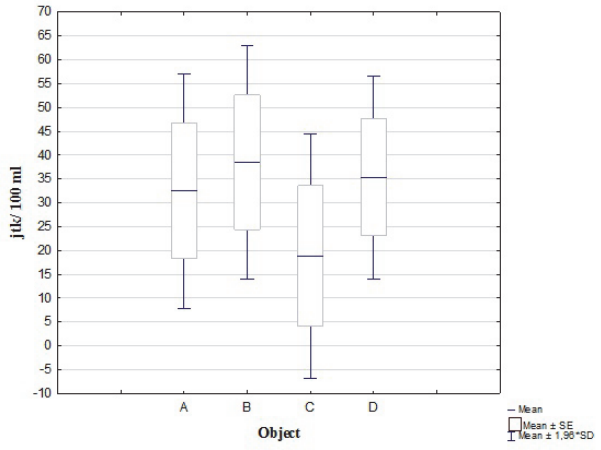

Fig. 6. Mean number of staphylococci in water from individual fountains
Rys. 6. Średnia liczebność gronkowców w wodzie z poszczególnych fontann

Own studies demonstrate that also in this case, the least number of staphylococci was found in fountain C (Table 6), which used brine water ($1.88 \cdot 10^1$ cfu/100 ml). Research conducted by Walczak & Lalke-Porczyk (2011) confirms that brine water from Ciechocinek does not contain *Staphylococcus aureus.*

4. Conclusions

1. The water from fountains located within the Ciechocinek health resort contained bacterial and fungal contamination, with microscopic fungi being the most abundant group, while the least contaminated was the water with highest degree of salinity.
2. Bioindicators, such as coliform bacteria, *Escherichia coli* and faecal streptococci, were found in the water samples taken from the fountains. The analyses also revealed the presence of mannitol positive staphylococci.
3. The microbiological contamination of water may pose a risk of diseases for people who use the fountains, that's why it is important to monitor the sanitary state of water originating from such sources.
4. The research indicated that it is necessary to monitor fountain water and introduce legal regulations regarding its sanitary and hygiene condition.

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Zanieczyszczenie mikrobiologiczne wody pochodzącej z fontann zlokalizowanych w uzdrowisku Ciechocinek

Abstract

Fountains located in health resorts frequently serve as therapeutic waters intended for recreational and balneological purposes. Therefore, the quality of water and air around such objects is crucial for patients using the fountains. Fountain water conducive to the occurrence of pathogen microorganisms, such as: coliform bacteria, staphylococci, streptococci, *Salmonella* spp. and microscopic fungi. They mostly cause a gastrointestinal and skin infections. The research here presented aimed at the assessment of the degree of bacterial and fungal contamination of water samples taken from fountains located in the Ciechocinek health resort from the perspective of biosafety of tourists and patients. Microbiological analysis of the water sample was taken from four fountains (object A-D), one of which (object C) was supplied with brine water.The plate method was used to determination of a total count of bacteria and a total count of fungi in the water samples. For the quantitative determination of coliform bacteria, *Escherichia coli*, staphylococci and streptococci, the membrane filter technique was employed. The studies showed that the samples of water from fountains contained bacteriological and mycological contamination in which microfungi being the most abundant group. The water samples contained a few cells of *E. coli*, fecal streptococci and staphylococci. The most frequently indentified indicator bacteria in sample water from fountain D were faecal streptococci ($3.17 \cdot 10^1$ cfu/100 ml). The water samples coming from the object B and D and in most water samples from the object C, there was no presence of these bacteria. As the data indicate, the largest differences regarding the population of those microorganisms were between water samples taken from Fountain B and C. The highest frequency of the occurrence of the *E. coli* bacilli was recorded in fountain A, while they occurred once in research series 5 taken from fountain C. The mean amount of these bacteria in the water samples taken from Fountain D was $5.76 \cdot 10^0$ cfu/100 ml, which was more than 40 times higher than the mean population of these microorganisms isolated from the water taken from Fountain C ($0.14 \cdot 10^0$ cfu/100 ml). The studies demonstrate that the least number of coliform bacteria and *E. coli* was found in fountain C, where 4.51% brine water was used. The highest number of staphylococci during the entire research period was determined in water sample taken from fountain D ($7.9 \cdot 10^1$ cfu/100 ml), while in water sample taken from fountains A and C and in sample from fountain B, no such bacteria were found. Occurrence of faecal bacteria, staphylococci and microscopic fungi in water may pose a risk of diseases for people who use the fountains. The research indicated that it is neces-

sary to perform monitoring of fountain water and introduce legal regulations regarding its sanitary and hygiene condition.

Streszczenie

Fontanny zlokalizowane na terenach uzdrowiskowych często są wykorzystywane jako wody lecznicze przeznaczone do celów rekreacyjnych oraz balneologicznych. W związku z tym bardzo ważna dla kuracjuszy korzystających z fontann jest jakość wody i powietrza wokół tych obiektów. Woda pochodząca z fontann sprzyja występowaniu mikroorganizmów chorobotwórczych takich jak: bakterie grupy coli, gronkowce, paciorkowce, pałeczki z rodzaju *Salmonella* oraz grzyby mikroskopowe. Powodują one przede wszystkim zakażenia o charakterze żołądkowo-jelitowym oraz infekcje skórne. Celem pracy była ocena stopnia zanieczyszczenia bakteriologicznego i mikologicznego próbek wody pobieranych z fontann, zlokalizowanych na terenie uzdrowiska Ciechocinek w aspekcie bezpieczeństwa sanitarnego turystów i kuracjuszy. Analizie mikrobiologicznej poddano próbki wody pobrane z czterech fontann (obiekty A-D), z których jedna (obiekt C) była zasilana wodą solankową. Metodą płytkową oznaczono ogólną liczbę bakterii i grzybów mikroskopowych. Do oznaczenia ilościowego bakterii grupy coli, *Escherichia coli*, paciorkowców kałowych oraz gronkowców wykorzystano metodę filtrów membranowych. W wyniku przeprowadzonych badań stwierdzono, że próbki wody pochodzące z fontann zawierały zanieczyszczenia bakteriologiczne i mikologiczne, przy czym najliczniej reprezentowane były grzyby mikroskopowe. W próbkach wody występowały pojedyncze komórki *E. coli* a także paciorkowce kałowe oraz gronkowce. Najliczniej paciorkowce kałowe identyfikowano w próbce wody pobranej z fontanny D ($3{,}17 \cdot 10^1$ jtk/100 ml). W próbkach wody pochodzących z obiektu B i D oraz w większości próbek wody z obiektu C nie stwierdzono obecności tych bakterii. Największe różnice dotyczące liczebności tych mikroorganizmów wystąpiły w przypadku próbek wody pochodzących z fontanny B i C. W obiekcie A notowano najwyższą częstotliwość występowania pałeczek *E. coli*, natomiast w przypadku fontanny C występowały one w jednym przypadku w wody pochodzącej z 5 serii badawczej. Średnia liczba bakterii *Escherichia coli* w próbkach wody pobranej z obiektu D wynosiła $5{,}76 \cdot 10^0$ jtk/100 ml i była ponad 40-krotnie wyższa od średniej liczebności tych drobnoustrojów izolowanych z wody pochodzącej z obiektu C ($0{,}14 \cdot 10^0$ jtk/100 ml). Badania wykazały, że najmniej bakterii grupy coli i *E. coli* stwierdzono w obiekcie C, w którym wykorzystywano wodę solankową o zasoleniu 4,51%. Największą liczbę gronkowców w ciągu całego okresu badań oznaczono w próbce wody pobranej z obiektu D ($7{,}9 \cdot 10^1$ jtk/100 ml), natomiast w niektórych próbkach wody ujętej z obiektu A i C oraz B nie stwierdzono obecności tych bakterii.

Występowanie w wodzie bakterii fekalnych, gronkowców, jak również grzybów mikroskopowych może stwarzać ryzyko zachorowań u ludzi korzystających z fontann. Przeprowadzone badania wskazują na konieczność prowadzenia monitoringu wody pochodzącej z fontann, a także na wprowadzenie regulacji prawnych dotyczących ich stanu sanitarno-higienicznego.

Słowa kluczowe:
woda, fontanny, zanieczyszczenie, bakterie, grzyby mikroskopowe

Keywords:
water, fountains, contamination, bacteria, microscopic fungi