# Original Research

# Sanitary and Ecological Characteristics of Water in the Municipal Lake of Rusałka in Szczecin

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### Abstract

Our studies were aimed at bacteriological analysis of water in the small municipal lake of Rusałka, in Szczecin. The studies were conducted over four years, in monthly intervals, assuming that the results will enable detailed analysis of bacteriological characteristics of the water in the tested reservoir and will yield sanitary and ecological characteristics of the reservoir. The quantitative estimation included index bacteria for the extent of pollution, index bacteria of sanitary condition, bacteria of selected physiological groups (ammonification bacteria, denitrification bacteria) as well as the presence of thermophile bacteria, sporulating bacteria, bacteria capable of sulphate reduction (*Desulfotomaculum nigrificans*). Also, the ratio was calculated for foecal-type coli group bacteria to foecal streptococci, treated as an index of origin of foecal-type pollution. The obtained results were subjected to statistical analysis.

In the studied lake high values of all the examined parameters were disclosed. The high numerical force of index bacteria for the extent of pollution (TVC 20°C, TVC 37°C) and of index bacteria for sanitary condition (coli group bacteria, foecal type coli group bacteria, foecal streptococci) pointed to contamination of the lake by household sewage. The observed high numbers of bacteria participating in turnover of nitrogen compounds (ammonification and denitrification bacteria) indicated a continuous inflow of nitrogen-rich compounds and development of water self-purification. The values of most studied parameters obtained by us were in general higher than the values noted in the same lake in previous years and higher than the values noted in other municipal lakes in Poland.

Keywords: water, municipal lake, microbiological analysis

#### Introduction

Poland contains approximately 9,300 lakes [1] of various size. However, the National Monitoring of Environment includes mainly lakes of over 100 ha area [1], which are innumerous both in the country and in the voivodship. In West Pomerania voivodship the most numerous group of water reservoirs includes lakes of below 10 ha area. Some of them are situated within administrative limits of the city of Szczecin, frequently in recreational areas like Rusałka lake, Syrenie Stawy or Szmaragdowe lake or close to settlements, like Słoneczne lake. Despite such a position, the containers are subjected to no testing by state institutions, which would evaluate the purity of their waters. The situation may rise to the rank of a problem, in particular because the inlowing waters bring in contamination [1]. Considering easy and frequent contact of town inhabitants with water of the containers and absence of any evaluation of classification of such lakes, we thought it proper to cover them with studies that would permit us to appraise their sanitary and ecological condition and to point out the direction of

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activities which are indispensable for their protection. The first studies of the type were started in mid 1993 by the Inter-chair Team for Evaluation of Water Environment, Faculty of Natural Sciences, University of Szczecin [2, 3]. They were implemented based first of all on microbiological [3] and chemical [2] indices of the water. The studies were continued in subsequent years [4-12]. The results pointed to the significant extent of microbiological contamination of studied lakes and their high propensity for degradation. They also documented purposefulness of systematic studies, including a wide range of bacteriological parameters [3-8]. It is worth stressing that principal part of the till now conducted microbiological studies pertaining to water reservoirs of 10 to 100 ha area and larger ones [quoted after 1]. Much less frequently microbiological studies were undertaken on small or very small lakes, of less than 10 ha in area [quoted after 1]. The low number of studies involved microbiological tests on municipal lakes [3-8, 13-29]. In West Pomerania voivodship alone the problem involves 1,031 lakes of up to 10 ha area and 448 lakes of 10 to 50 ha area or, in other words, a total of at least 10,000 hectares of water area. Thus, considering the number and situation of the lakes on the voivodship map, any initiative seems justifiable which aims at quality evaluation and protection of water reservoirs of small area. This seems particularly important due to both the economic and recreational significance of the lakes.

Present studies are aimed at bacteriological analysis of water in the small municipal lake of Rusałka in Szczecin. The studies were conducted over 4 years, in monthly intervals, assuming that they will permit us to characterize in detail bacteriological patterns of water in the container and to provide sanitary and ecological characteristics of the lake.

## **Experimental Procedure**

Rusałka lake is situated in the centre of Szczecin. Its area amounts to 3.7 ha, its maximum width is 40 m and its maximum depth is 2 m. The lake has an elongated shape and its long axis covers the East-West direction. It manifests neither thermal stratification nor intense waves. The bottom of the lake is flat, with a thick layer of mud and is contaminated. The lake is a flow-through reservoir, supplied from the western side by Osówka stream. The stream provides input of water from the drainage area including areas of the Lasek Arkoński forest and from residential quarters of Szczecin. The excess of water is drained on the eastern side of the lake by a subterranean pipeline to the Western Odra river.

Taking into account ecological interview and shape of the terrain, two water sampling points were selected, marked A and B. Point A was situated close to the inflow of the Osówka stream (on the western bank) and point B on the opposite, eastern bank of the lake, close to its outflow.

The studies were performed between January 2000 and December 2003. The water was sampled every 4 weeks, at a depth of 15-20 cm below water surface. Sampling of the water for the studies [30-34] and bacteriological procedures [35-42] followed rules of the Polish Standards.

The determined variables included:

- index bacteria for the extent of pollution: the total number of bacteria in one ml of water grown on a nutrient agar following 24 h of culture at a temperature of 37°C (TVC37°C), and following 72 h of culture at 20°C (TVC20°C) [35],
- index bacteria for the sanitary condition: NPL (the most probable number) of coli group bacteria in 100 ml water (TC), estimated by the tube fermentation technique [36]; NPL of foecal type coli group bacteria in 100 ml water (FC), estimated by the tube fermentation technique [39]; NPL of foecal streptococci in 100 ml water (FS), estimated using membrane filters [41]; NPL of anaerobic bacteria, sporulating bacteria, sulphite-reducing bacteria (*Clostridium*) in 100 ml water, estimated using a culture in a liquid medium [40],
- bacteria of selected physiological groups: NPL of ammonification bacteria in 100 ml water, estimated by the tube technique [37]; NPL of denitrification bacteria in 100 ml water, estimated by the tube technique [38], NPL of sporulating bacteria and sulphate-reducing bacteria (*Desulfotomaculum nigrificans*), estimated using culture in a liquid medium [42].

Results of the tests permitted us to calculate the ratio of foecal-type coli group bacteria (FC) to foecal streptococci (FS), treated as an index of a foecal source of the contamination.

Results of the studies were subjected to statistical analysis using the STATISTICA 6.0 software. The analysis included the W test of Shapiro-Wilk, which permitted us to demonstrate abnormal distribution of studied parameters. The latter required that non-parametric statistics were used to calculate correlation coefficients and correlation coefficients of R Spearman were calculated. All the calculations were conducted using p < 0.05 level of significance.

#### Results

Results of the above listed studies were presented in the form of ranges of values and mean values obtained in individual years (2000–03) and for individual sampling points (A and B). Moreover, in Table 1 percentages are listed of water sample numbers in which the presence of sulphite-reducing bacteria was disclosed. Values of FC: FS ratio are shown in Table 2. Results of statistical analyses of the studies and significance of the differences are provided in Tables 3 and 4.

Index bacteria for the extent of pollution. In the period of 2000-03 numbers of TVC 20°C bacteria ranged in sampling point A from 26 bacteria per 1 ml (year 2000) to 440,000 bacteria per 1 ml (2003), and in the sampling point B from 60 (2002) to 1,080,000 bacteria per 1 ml (2003). The annual mean values of the parameter were as follows: in point A the lowest mean value was noted in 2000 and in point B in 2002, the highest numbers in both

Table 1. Frequency of water samples in which the presence of sulphite-reducing bacteria was disclosed.

Year	Sampling point	Presence of sulphite-reducing bacteria	
2000	А	50%	
	В	68%	
2001	А	92%	
	В	100%	
2002	А	92%	
	В	92%	
2003	А	100%	
	В	92%	

Table 2. Frequencies of various ranges of FC: FS values.

Value of EC:ES ratio	Sampling point		
value of FC.FS fatio	А	В	
<0.7	37.5%	29.2%	
0.7 - 4.0	12.5%	16.6%	
>4.0	50%	54.2%	

sampling points were detected in the year 2003. Between the 2000 and 2003 the number of TVC 37°C bacteria in point A was 3 cells per ml (year 2000) to 300,000 cells per ml (year 2001), and in point B it was 20 bacteria per ml (year 2000) to 91,000 bacteria per ml (year 2001). The lowest annual mean values were obtained in 2003 in point A and in 2002 in point B. The highest annual means in both points were noted in 2001.

Index bacteria of the sanitary status. In analysis of TC bacteria numbers in the studied period values of the parameter in point A ranged between 23 cells per 100 ml (year 2003) and 240,000 cells per 100 ml (years 2001 and 2002) while in point B they ranged between 23 cells per 100 ml (year 2002) and 23,000 cells per 100 ml (year 2000). The lowest mean annual values for the coli group bacteria in point A were noted in 2000, in point B in 2001 while the highest values were noted in point A in 2002 and in point B in 2000. In turn, the level of FC bacteria in 2000-03 ranged in point A between 23 cells in 100 ml (2003) and 230,000 cells per 100 ml (2002), and in point B between 13 cells per 100 ml (2002) and 23,000 cells per 100 ml (2000). In the range of foecal type coli group bacteria the lowest mean annual values manifested in point A in 2000, in point B in 2001, while the highest respective values in point A were observed in 2002, and in point B in 2000. In the studied period the content of FS bacteria ranged in point A between 0 (in all the years) to 11,000 cells per 100 ml (year 2002), and in point B between 0 (in all the years) to 8200 cells per 100 ml (year 2002). The lowest average content of foecal streptococci in both samAnalysis of sulphite-reducing bacteria in 2000-03 disclosed that in point A the values ranged from <3 bacteria per 100 ml (years 2000 and 02) to 2,400 bacteria per 100 ml (years 2001 and 03), while in point B they ranged from <3 cells per 100 ml (years 2000-02) to 1500 cells per 100 ml (year 2002). The lowest average annual values were noted in both sampling points in 2000, the highest ones in 2003.

#### Bacteria of Selected Physiological Groups

In the studied period the level of ammonification bacteria ranged in point A from 9300 bacteria per 100 ml (2001) to 240,000,000 bacteria per 100 ml (2001); in point B - from 1500 bacteria per 100 ml (2000) to 240,000,000 cells per 100 ml (2001-03). In this scope, the lowest mean annual values in point A were noted in 2003 and in point B in 2000, while the highest values in both sampling points were detected in 2001. The numbers of denitrification bacteria ranged in point A from 400 cells per 100 ml (2000) to 240,000,000 cells per 100 ml (2003); in point B from 230 cells per 100 ml (2000) to 240,000,000 cells per 100 ml (2003). The lowest mean annual values of NPL of denitrification bacteria in point A manifested in 2001 and in point B in 2000, while the highest ones were detected in both points in 2003.

Thermophile bacteria, sporulating bacteria, sulphitereducing bacteria in the studied period were detected in most of samples, both in A and in B sampling points (Table 1).

Distribution of percentage values in the FC: FS ratio demonstrated that in both water sampling points most samples showed the value of the coefficient >4.0, proving that foecal contamination was of human origin. Also, a relatively high proportion of the samples demonstrated contamination with animal foeces (values of FC: FS < 0.7) (Table 2).

#### Statistical Analysis

In the scopes of index bacteria for the extent of pollution and those for sanitary condition in both places of water sampling a correlation was disclosed between TVC 20°C and TVC 37°C as well as between TVC 20°C / TVC 37°C and numbers of foecal streptococci and also between NPL of coli group bacteria and NPL of foecal type coli group bacteria (Table 3). Moreover, in sampling point A a correlation was noted between TVC 20°C and NPL of coli group bacteria and NPL of foecal type coli group bacteria and between the numbers of foecal streptococci on one hand and NPL of coli group bacteria and NPL of foecal type coli group bacteria on the other (Table 3).

In the scope of index bacteria for pollution and bacteria of physiological groups both in A and B sampling points a correlation was noted between TVC 20°C and TVC 37°C, NPL of denitrification bacteria and NPL of ammonification bacteria. Also in both sampling points a

Parameters	Point	TVC 20°C	TVC 37°C	TC	FC	FS
TVC 20°C	A	-	0.58	0.39	0.33	0.36
TVC 37°C		0.58	-	-	-	0.41
TC		0.39	-	-	0.77	0.41
FC		0.33	-	0.77	-	0.33
FS		0.36	0.41	0.41	0.33	-
TVC 20°C	В	-	0.42	-	-	0.43
TVC 37°C		0.42	-	-	-	0.46
TC		-	-	-	0.65	-
FC		-	-	0.65	-	-
FS		0.43	0.46	-	-	-

Table 3. Spearman's rank correlation between content of index bacteria for the extent of contamination and those for sanitary condition p < 0.05.

Legend: - No significant correlation

Table 4. Spearman's rank correlation between content of index bacteria for the extent of contamination and content of selected physiological groups of bacteria, p < 0.05.

Parameters	Point	TVC 20°C	TVC 37°C	NPL A	NPL D
TVC 20°C	A	-	0.58	0.50	0.46
TVC 37°C		0.58	-	0.36	-
NPL A		0.50	0.36	-	0.69
NPL D		0.46	-	0.69	-
TVC 20°C	В	-	0.42	0.31	0.59
TVC 37°C		0.42	-	-	-
NPL A		0.31	-	-	0.48
NPL D		0.59	-	0.48	-

Legend: NPL D – most probable number of denitrification bacteria; NPL A – most probable number of ammonification bacteria; – no significant correlation

correlation was detected between NPL of denitrification bacteria and NPL of ammonification bacteria. Moreover, in point A a correlation was noted between TVC 37°C and NPL of ammonification bacteria.

# **Discussion of Results**

Analysis of the results allows us to note that the detected numbers of TVC 20°C bacteria were higher than most of the levels presented in literature, both for municipal water reservoirs resembling Lake Rusałka in size [3-6, 25] and for lakes of greater area [13-16, 20, 22, 27-29], and higher than the values noted in earlier studies on Lake Rusałka [3, 4, 6, 8]. Values much higher than the ones noted at present in Rusałka were detected only in Kortowskie Lake [23] and Ukiel Lake [24], used as recreation sites. Similarly to TVC 20°C, the numbers of TVC 37°C bacteria noted in our studies proved to be much higher than the values obtained by other authors in municipal lakes [5, 18, 20, 25, 27, 29] and in the earlier studies on Rusałka [3, 4]. Much higher numbers of such bacteria were demonstrated only in large municipal lakes [23, 24, 26] and in Syrenie Stawy Lake [4].

In general, results of our own studies on NPL of coli group bacteria were higher than those observed in other water reservoirs, both in small municipal lakes subjected to anthropopression [3, 5, 25] and in larger reservoirs [23, 24, 26, 27], as well as the data obtained in earlier studies on Rusałka [3, 4, 8]. In a similar way, the values of NPL for foecal-type coli group bacteria obtained in our studies proved to be much higher than those noted in other reservoirs [3, 5, 23-25, 27] and in earlier studies on Rusałka [3, 4]. Following analysis of foecal streptococci in Rusałka it could be concluded that the values were lower or slightly higher than those detected in our studies were disclosed in large municipal lakes [23, 24, 26, 27], while similar values were detected in small municipal [4, 5-7, 25] and in earlier studies on Rusałka [4, 6]. In turn, the levels of sulphite-reducing bacteria (*Clostridium*) noted in our studies were lower compared to those observed in lakes Syrenie Stawy [5] and Kortowskie [23].

In analysis of mean annual levels of index bacteria for the pollution level and for sanitary conditions obtained in Rusałka it should be noted that they were higher in the A sampling point, positioned in a direct vicinity of Osówka stream inflow to the lake. Close to the water outflow from the lake, in sampling point B, numbers of the bacteria were much lower. The observed bacteriological pattern proved that the Osówka stream imported a significant load of pollution to Rusałka. In sampling point B the numbers of TVC 37°C, TC, FC, FS and *Clostridium* bacteria decreased, most probably due to their decay and consumption by zooplankton.

Analysis of our own results obtained in studies on Rusałka and the earlier obtained results [3, 4, 6, 8] documented a lack of changes in the bacteriological status of the lake which would be related to elapsing time. The observed bacteriological condition of the lake proves that the process of polluting the lake continues or even becomes increasingly intense.

Determination of index bacteria for sanitary condition of the lake, which point to the potential for foecal contamination of the lake, indirectly allows us to draw conclusions as to probable presence in the water of pathogenic microbes in the studied reservoir [43]. However, it should be noted that such a relationship cannot be taken as absolute, as indicated by studies of Hörman et al. [44]. Nevertheless, in this context it is worth noting that in 31 per 96 tested samples of water of Rusałka >2000 cells have been noted of foecal-type coli group bacteria per 100 ml water.

The conducted tests permitted us also to classify in microbiological respect water in the studied reservoir, in line with principles of the basic monitoring of lakes [In Polish] [45]. In sampling point A most tested water samples fitted the norms of class II (35%) or III (27%) of cleanness while in sampling point B the respective proportions amounted to 44% and 33%. At the same time 4% to 21% of samples manifested pollution exceeding any norms. In the entire testing period and in each sampling point the test values ranged from those typical for any class of water purity to values characterizing pollution exceeding any norms. Results of the presented studies have confirmed the extensive effect of anthropogenic pollution on water quality in the examined lake (Table 2) and they have pointed to the risk that biological potential of the water reservoir has been exhausted. The problem seems a significant one since Rusałka has been found to be a reservoir extremely prone to degradation, in line with the criteria of the Water Quality Evaluation System [11].

Numerical force of bacteria which participate in nitrogen turnover, including ammonification and denitrification bacteria, in municipal lakes till has now been determined in relatively few research projects [5, 16, 28, 29]. The published data indicate that the obtained levels of ammonification bacteria were much lower than those obtained by us [5, 16, 28, 29]. On the other hand, levels of denitrification bacteria resembling the Rusałka levels were noted in Syrenie Stawy Lake [5]. The presence of denitrification bacteria was disclosed also in Długie and Miejskie lakes in Szczytno [16], but in these cases no quantitative tests were conducted. The documented high levels of ammonification and denitrification bacteria prove that the organic substances inflowing to a lake are actively used by the studied physiological groups of microbes. However, the continuing import of high amounts of pollution may exhaust the potential for self-purification of the water and lead to degradation of the water container.

In evaluation of water quality, the sulphate-reducing bacteria are one of the least studied groups of microbes. In this study we have undertaken such tests but they have been restricted to detection of the presence of such bacteria in tested water samples and have involved no quantitative assays. The results manifested the presence of such microbes in most tested water samples. Similar results were obtained in the earlier studies on Syrenie Stawy Lake [5] as well as on Długie and Miejskie lakes in Szczytno [16], in which sulphate-reducing bacteria were present in the superficial and bottom layers as well as in bottom sediments.

In analysis of the mean annual levels of physiological group bacteria in Rusałka higher values could have been noted in the B sampling point than in A. The results might have reflected the fact that numbers of allochthonous bacteria which are introduced to the container with Osówka stream water decreases and numbers of autochthonous bacteria increases with growing distance from the source of pollution. The latter bacteria actively take advantage of the available organic substances and in this way participate in self-cleaning of the container.

In the studied lake a correlation has been disclosed between TVC 20°C and TVC 37°C bacteria. It is worth noting that TVC 20°C bacteria represent the autochthonous flora of water containers while the TVC 37°C bacteria are thought to represent an allochthonous flora. The correlation between the two groups of bacteria may be explained by the fact that TVC 37°C bacteria have been introduced to the water with the sewage. On the other hand, the observed increase in levels of TVC 20°C bacteria has reflected continuous inflow to the lake of bacterial nutrients. The other observed relationship has involved the relationship between TVC 37°C bacteria and numbers of foecal streptococci, present in the aqueous environment as an allochthonous flora. In turn, the correlations between contents of index bacteria for the sanitary condition of i.e. TC, FC and FS bacteria may be explained by the presence of all the groups of bacteria in foeces of humans and homoiothermic animals, which contaminate the water directly or which inflow to the container with water of tributaries.

The correlation between the level of NPL of ammonification bacteria and TVC 37°C bacteria represents a natural phenomenon in as much as most of the ammonification bacteria belong to mesophilic bacteria. In turn, the correlation between ammonification and denitrification bacteria reflects their common involvement in turnover of nitrogen compounds. The parallel manifestation of high numbers of the two metabolic groups of microbes was disclosed also by Niewolak et al. [43].

# Conclusions

Water of the studied container was found to exhibit high values of all tested variables. The high numerical force of index bacteria for the extent of pollution (TVC 20°C, TVC 37°C) and of index bacteria for sanitary condition (TC, FC, FS) pointed to contamination of the lake with household sewage. The observed high levels of bacteria which participate in turnover of nitrogen compounds (ammonification and denitrification bacteria) pointed to a continuous inflow to the container of nitrogen-rich substances and to manifestation of self-cleaning processes in the water container.

The obtained levels of most studied variables in general proved to be higher than those observed in the same container in the preceding years and higher than the levels noted in other municipal lakes in Poland and published in the literature of the subject.

In view of the documented heretofore high and persisting extent of pollution in Rusałka lake it is indispensable to immediately restrict inflow of pollution to the lake and to undertake regular monitoring of its water quality.

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