IMPACT OF WATER QUALITY ON SUMMER BACTERIOPLANKTON IN SPRINGS OF GRYŻYŃSKI LANDSCAPE PARK

P. Zieliński[1*], A. Szczucińska[2], A. Stopińska[1], W. Pol[1], E. Jekatierynczuk-Rudczyk[1]

[1]Department of Water Ecology, Faculty of Biology, University of Bialystok, Ciolkowskiego 1J, 15-245 Białystok, Poland
[2]Hydrometry Research Unit, Faculty of Geographic and Geological Sciences, Adam Mickiewicz University, Krygowskiego 10, 61-680 Poznań, Poland
*p.zielinski@uwb.edu.pl

Abstract

The study aimed to analyze hydrochemical and bacteriological features in selected springs of the Gryżyński Landscape Park (GLP) in western Poland. The basic hydrochemical parameters and the abundance and biomass of bacteria were analyzed in water samples collected from 22 springs in GLP in July 2018. The hydrochemical composition of the GLP spring waters depends mainly on the specific geological structure and character of catchment land use. The research showed significant differences in the water quality of GLP springs in the case of many hydrochemical parameters, e.g., dissolved organic carbon concentration, biogenic substances which influence the bacterial abundance and biomass. The average bacterial number (BN) in the waters of the Gryżyński Landscape Park springs was 0.656 10^6 cells/ml, with a range from 0.181 10^6 cells/ml to 1.656 10^6 cells/ml. The coefficient of variation of this parameter was 75.67%. In 12 investigated springs of GLP, BN was below 0.5 10^6 cells/ml, in 6 springs, the average BN was between 0.5 and 1 10^6 cells/ml, and in 4 springs BN, it was in the range from 1 to 2 10^6 cells/ml. The mean bacterioplankton biomass was 22.98 µgC/l and from 6.35 to 61.54 µgC/l. Based on the collected material, many correlations between BN and selected water quality parameters were found (temperature, phenolics, phosphates, nitrites, and dissolved organic carbon). The microbiological condition of investigated springs depends mainly on the geological structure of the spring niches and water quality. Moreover, the research shows that GLP springs are susceptible to human activity in the direct catchment area. Therefore, this research indicates the potential possibility of using hydrochemical and microbiological parameters together to assess the ecological condition of lowland springs or in planning protective measures.

Key words: bacterioplankton, hydrochemistry, spring

Introduction

Microbiological tests of waters focus mainly on their sanitary and epidemiological condition. Less attention is paid to the role of aquatic microorganisms in shaping the ecological state and the condition of the ecosystem. Among inland waters, most studies concern bacterioplankton in lakes, rivers, and dam reservoirs. Scientists consider bacteria in natural groundwater outflows much less frequently. They can be divided, among others, by the way water flows out (springs, effluents), the type of aquifer (fissures, karst, pore springs – fed with water from loose formations), or the direction of the hydrodynamic force (ascension, descent) (Moniewski 2007). All these features determine the quality of water, and thus also the groups of organisms inhabiting them. It is worth noting, especially, that the springs are characterized by very high natural value and usually occur in areas with a low degree of anthropogenic transformation. Although springs have been recognized as necessary, rare, and globally threatened ecosystems, there is no consistent and comprehensive classification or common lexicon for springs, especially in lowland areas (Springer et al. 2008). Springs play a critical role in periods without rainfall, because by supplying rivers they determine, among others, the amount of water flowing in the river beds. Hence, it is crucial to protect the springs to maintain the proper level of surface waters. In Poland, especially in lowland areas, the degradation and disappearance of wetlands and springs are observed (Siwek 2004); therefore, research on the springs is becoming more and more critical (Michalczyk 2001, Michalczyk et al. 2004, Michalczyk et al. 2015, Puczko, Jekatierynczuk-Rudczyk 2020). The waters of Quaternary springs are exposed to area pollution of agricultural origin or resulting from urbanization in the direct catchment area (Jekatierynczuk-Rudczyk 2008). This affects the changes in the chemical composition of groundwater manifested by a disturbance in the proportion of micronutrients (Górniak, Jekatierynczuk-Rudczyk 1997, Małecki 1998, Jekatierynczuk-Rudczyk 1999, Szczucińska 2017) and dissolved organic matter (Zieliński et al. 2020). The organisms that respond to the changes in water quality in the first place are water microorganisms. The number of bacterioplankton is a good illustration of the intensity of biogeochemical changes in these naturally poor habitats. Moreover, the abundance of bacterioplankton in the water of the springs is determined by different geological, hydrological, and catchment conditions. To better understand their ecology in the area with little anthropogenic transformation, hydrochemical and microbiological studies of selected springs of the Gryżyński Landscape Park were carried out. The study considers the nature of the outflows and the type of land development as additional factors determining bacterioplankton in springs.

Materials and methods

Study area

Gryżyński Landscape Park (GLP) is located in the west of Poland, in Lubuska Upland. It was established on April 15, 1996. The area of the park is 3064.80 ha, and the buffer zone is 7911.20 ha. The park was established to protect postglacial relief, hydrography, various habitats, and rare flora and fauna. The landscape is dominated by a post-glacial gutter drained by the river Gryżynka, 17 km long. The gutter is cut into the sander surface to a depth of 30 m. A deep erosive incision is conducive to the functioning of groundwater outflows, of which more than 350 have been inventoried in the gutter. These outflows are located mainly under the slopes and drain Quaternary waters from alternating sand and gravel pores. They are characterized by high-performance stability throughout the year and a stable temperature. The spring zone with the highest efficiency of approximately 50 dm^3/s is located in Gryżyna. The average temperature of spring waters is around 9°C. More than 70% of these waters belong to the hydrochemical type HCO_3-SO_4-Ca. This chemical composition is characteristic of shallow groundwater in the hypergeneic zone.

However, for some indicators, e.g., nitrates, sulfates, iron, and manganese, different concentrations were found even in the adjacent outflows. This diversity may be influenced by how the catchment area and the immediate vicinity of the outflow are managed. Forests dominate the direct drainage catchment. They cover 76% of the GLP area and are dominated by pine forests. Less numerous are deciduous forests, preserved mainly in the Gryżyński Potok valley. Some spring niches are located near farmland and built-up areas. Agricultural land and built-up land, which can be a source of anthropogenic pollution, account for 16% and 4%, respectively. Spring zones are a place where groundwater flows to the surface of the area, and hence they are a convenient place for sampling and obtaining information about the quality of the water drained by them. The GLP area is slightly anthropogenically transformed, and therefore it was selected to recognize both the hydrochemical and microbiological status of groundwater occurring in the semi-natural area. The study covered 22 springs located in the GLP area (Figure 1).

Research methods

Twenty-two springs in the Gryżyński Landscape Park were selected for detailed hydrochemical and microbiological analyses (Table 1, Figure 1). The field studies were conducted in the summer (July 9, 2018). Temperature, pH, electrolytic conductivity (EC), and oxygen concentration in water were measured directly in the field using the Hach-Lange HQ-40d multi-parameter probe. Water samples for hydrochemical analyses were collected following the recommendations for the Geochemical Map of Europe (Salminen et al. 1998) and samples for microbiological determinations were collected in sterile 50 ml containers. Hydrochemical analyzes were performed at the Department of Water Ecology at the University of Białystok. Concentrations of anions: NO_3, NO_2, PO_4 and cations: NH_4 were determined by high-performance liquid chromatography (HPLC). The content of total phosphorus (TP) was determined by the molybdate method with ascorbic acid and the total iron content (TFe) was determined using the phenanthroline method. SpecraMaxM2 spectrofluorimeter was used in spectrophotometric determinations. The analyses of dissolved organic carbon (DOC) and total nitrogen (TN) concentrations were carried out using the high-temperature catalytic combustion method with Shimadzu carbon and nitrogen analyzer (TOC 5050A, Japan). Water samples for DOC determinations were filtered through a 0.45 µm filter, acidified with 2 M HCl to a pH of about 2, and rinsed with pure nitrogen to remove traces of inorganic carbon.

The total number of bacteria (BN) was determined in formalin preserved samples (2% final conc.) using epifluorescence microscopy. DAPI-stained bacterial cells were counted on 0.2 µm pore-size black polycarbonate membrane filters (Nuclepore, Whatman) with Olympus epifluorescence microscopy (BX61, Japan) using the Porter and Feig (1980) method. BN was recalculated for bacterial biomass (BB) using the Theil-Nielsen, Sondergaard (1998) method.

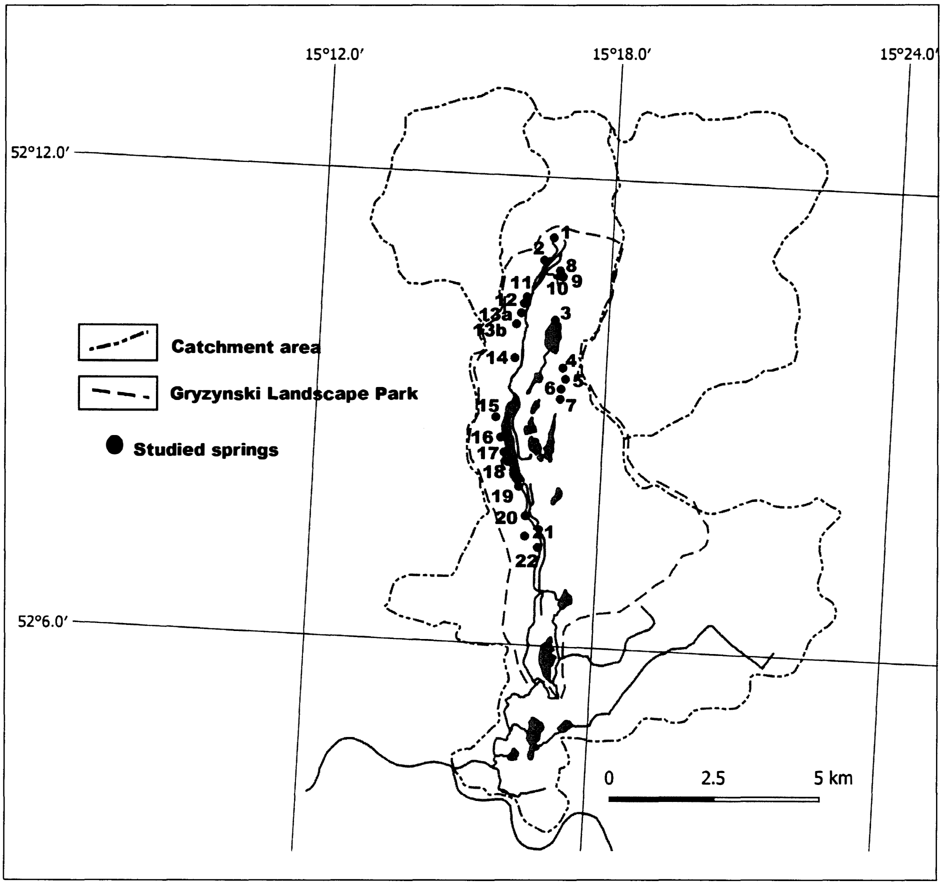

Figure 1. Distribution of the studied springs in the Gryżyński Landscape Park, considering the Gryżynka River catchment area.

Table 1. Hydrological characteristics of the studied springs in GLP.

Spring No.	Morphological type	Q (dm^3/s)	Outflow type	Development
1	bottom-slope	0.5	descending	rural buildings
2	bottom-slope	0.5	descending	forest
3	bottom-slope	2.5	ascending	forest
4	bottom-slope	0.1	descending	forest
5	bottom-slope	0.1	descending	forest
6	bottom-slope	0.1	descending	forest
7	bottom-slope	0.1	descending	forest
8	bottom-slope	0.2	descending	forest
9	bottom-slope	0.3	descending	forest
10	bottom-slope	0.4	descending	forest
11	bottom-slope	0.1	descending	field, forest
12	bottom-slope	0.15	descending	field, forest
13	bottom-slope	2.5	descending	field, forest
14	bottom-slope	0.8	descending	forest
15	bottom-slope	0.8	descending	forest
16	bottom-slope	6	descending	forest
17	bottom-slope	0.3	descending	forest
18	bottom-slope	0.15	descending	forest
19	bottom-slope	0.15	descending	forest
20	slope	0.1	descending	forest
21	bottom-slope	0.6	descending	forest
22	valley	0.1	ascending	forest

Results

Hydrochemical water quality

The average air temperature during the sampling period (July 2018) in GLP was 20.8°C, while the average water temperature of the studied springs was 12.2°C. The water temperature in the samples from GLP ranged from 10.1°C in spring 13 to 16.1°C in spring No. 1. A low coefficient of temperature variation was noted for the waters of GLP springs (16.5%). The average water pH in the GLP springs was 7.45. The average electrolytic conductivity in the GLP springs was 410.78 µS/cm. The EC values ranged from 159.9 to 538 µS/cm. The dissolved oxygen content in the waters of GLP springs was on average 4.29 mg/l.

The amount of oxygen in the water ranged from 0.11 in spring No. 22 to 9.04 mg/l in spring No. 11 (Table 2).

Table 2. Statistical characteristics of physical and chemical parameters in the springs of the Gryżyński Landscape Park, average results for the summer period of 2018.

Parameter	Number of samples	Mean	Standard deviation	Min-max value	Variation coefficient
Temperature (°C)	22	12.24	2.02	10.1 – 16.1	16.48
pH	22	7.45	0.098	7.28 – 7.61	13.4
Specific electrolytic conductivity (μS/cm)	22	410.27	92.27	159.9 – 538	23.01
Oxidation-reduction potential (mV)	22	175.04	6.580	155.9 – 185.6	3.84
Oxygen (mg/l)	22	4.35	2.917	0.11 – 9.04	68.32
Oxygen saturation (%)	22	42.38	29.69	1.1 – 92.3	71.21
Ammonium ions (μgN/l)	22	62.81	35.52	1.0 – 138	56.55
Nitrites (μgN/l)	22	2.65	2.27	0.4 – 13.2	100.94
Nitrates (μgN/l)	22	411.95	849.98	2.4 – 3360	206.33
Total nitrogen (μgN/l)	22	876.81	1239	197 – 5012	141.32
Phosphates (μgP/l)	22	10.97	5.56	2.4 – 22.7	50.75
Total phosphorus (μg/l)	22	64.8	27.1	13.5 – 145.3	41.72
Total iron (mg/l)	22	0.329	0.138	0.028 – 0.560	87.93
DOC (mg/l)	22	2.75	1.20	1.58 – 6.48	43.46
Phenolics (mg/l)	22	0.39	0.12	0.19 – 0.61	31.08

Nitrogen compounds

The average concentration of ammonium ions in the GLP springs was 62.8 μgN/l (Table 1). The minimum values of NH_4 were recorded in springs No. 13 and 18 (26 and 32 μgN/l, respectively). A significantly higher NH_4 concentration, exceeding 120 μgN/l, was found in springs No. 1 and 2, located close to human settlements. The average concentration of nitrates in the GLP springs was 0.412 mg/l. The values of this parameter ranged from 24 (spring No. 6) to 3368 μgN/l (spring No. 9). Due to the high variability of this parameter, a high coefficient of variation was noted (206.33%). The less variable parameter in terms of concentration in water were nitrites (CV = 100.94%). The average concentration of nitrites in the GLP springs was 2.65 μgN/l. The concentration of nitrites in the GLP springs ranged from 0.4 μgN/l to 13.2 μgN/l (in springs No. 13 and 3, respectively). The mean value of total nitrogen in the GLP springs was small and amounted to 0.88 mg/l. In GLP springs, the value of this parameter ranged from 0.197 mg/l to 5.12 mg/l. In GLP, a high coefficient of variation was noted: 149.49% (Table 2).

Phosphorus compounds

The average concentration of phosphates (SRP) in the waters of GLP springs was 10.97 μgP/l. The concentration of SRP ranged from 2 μgP/l to 23 μgP/l. The average concentration of total phosphorus in the GLP springs was 65 μgP/l. This concentration in the GLP springs ranged from 14 μgP/l to 145 μgP/l (Table 2).

Organic compounds are essential for the functioning of microorganisms in spring niches. The average content of dissolved organic carbon in GLP was 2.754 mg/l. In the waters of the GLP springs DOC range was between 1.577 and 6.478 mg/l DOC. The mean concentration of phenolics in the GLP springs was 0.391 mg/l. On the other hand, the content of phenolics ranged from 0.197 mg/l to 0.612 mg/l (Table 2).

Total number and biomass of bacteria

The average number of bacteria in the waters of the GLP springs was 0.611 10^6 cells/ml, and the coefficient of variation was 75.67%. The number of bacteria in the samples from GLP ranged from 0.182 10^6 cells/ml (spring No. 10) to 1.656 10^6 cells/ml (spring No. 13). In 12 of the examined GLP springs, the number of bacteria did not exceed 0.5 10^6 cells/ml, in 6 springs, the average BN ranged from 0.5 to 1 10^6 cells/ml, and in 4 springs, BN ranged from 1 to 2 10^6 cells/ml (Figure 2). Thus, bacterial biomass in water samples from GLP springs was small and amounted to 22.975 μgC/l. Bacterial biomass ranged from 6.352 (spring No. 10) to 61.537 μgC/l (spring No. 13).

Based on the performed statistical analyses, numerous dependencies were found between water quality parameters and the BN. For example, positive correlations were found between BN and water temperature (Figure 3), the content of dissolved organic matter (Figure 4), and the concentration of phenolics (Figure 5). On the other hand, negative correlation coefficients were noted between BN and the concentration of phosphate ions (Figure 6) or the concentration of NO_2 ions (-0.377; $p<0.005$).

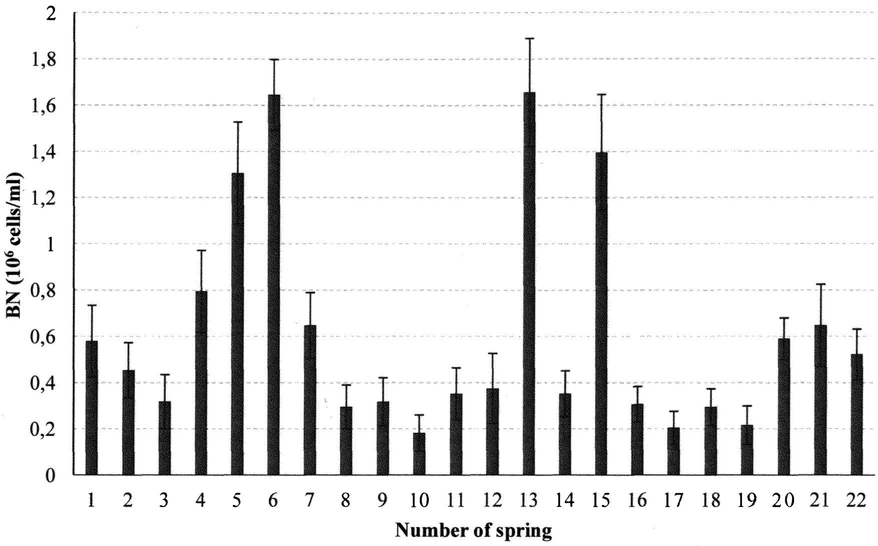

Figure 2. The average number of bacteria (BN) in the studied springs of the Gryżyński Landscape Park, average results from the summer period of 2018.

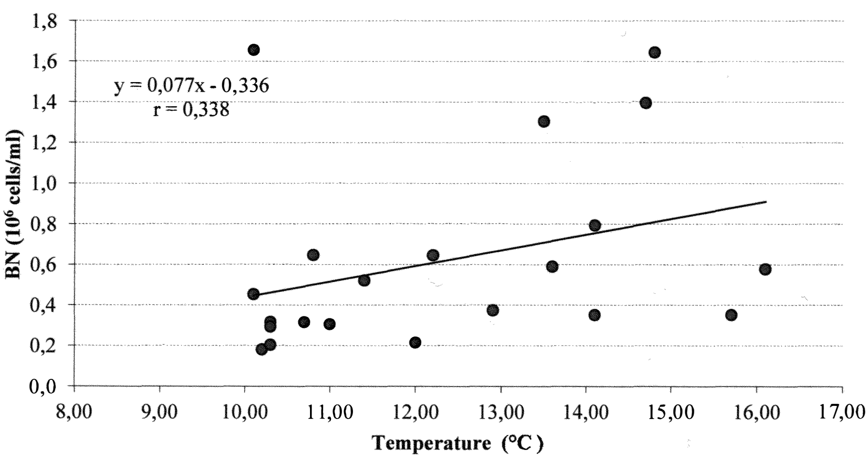

Figure 3. Correlation between the number of bacteria (BN) and the temperature in the waters of the Gryżyński Landscape Park springs.

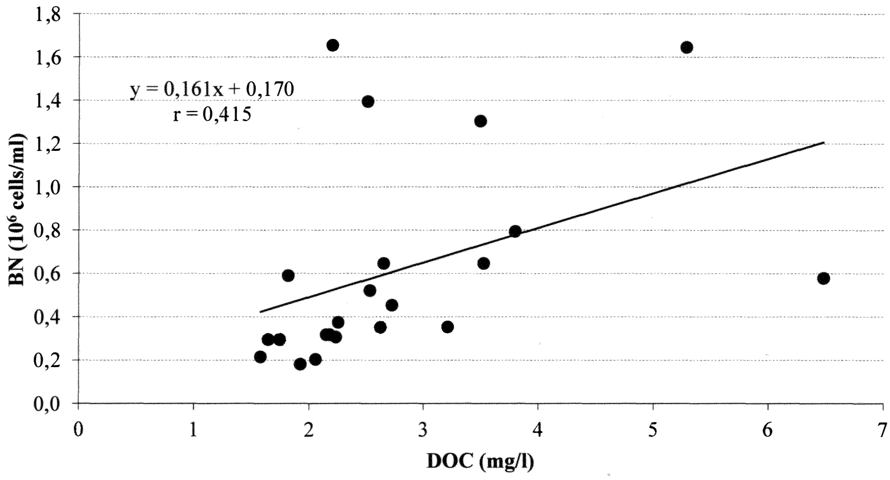

Figure 4. Correlation between the number of bacteria (BN) and the concentration of dissolved organic carbon (DOC) in the waters of the Gryżyński Landscape Park springs.

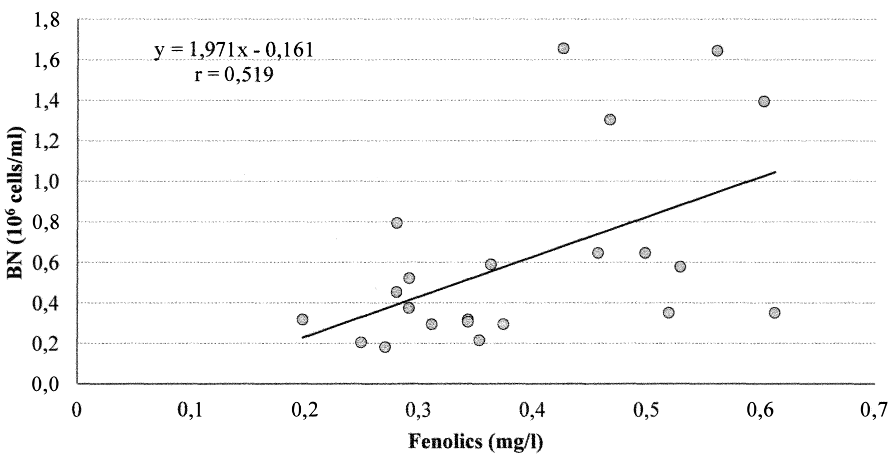

Figure 5. Correlation between the number of bacteria (BN) and the concentration of phenols in the waters of the Gryżyński Landscape Park springs.

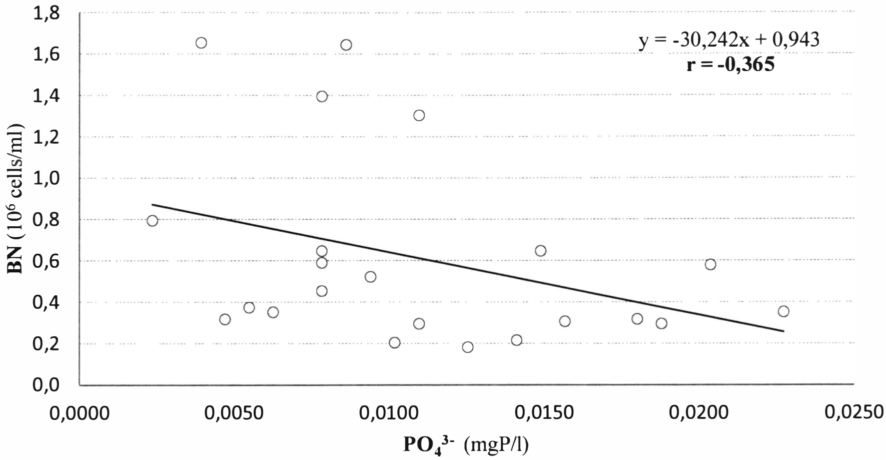

Figure 6. Correlation between the number of bacteria (BN) and the concentration of soluble reactive phosphates (PO_4^{3-}) in the waters of the Gryżyński Landscape Park springs.

Discussion

Microorganisms, including bacteria, constitute the most numerous group of organisms living in all aquatic ecosystems. The specific aquatic environments include springs, where the degree of recognition of the diversity of organisms is still insufficient (Puczko et al. 2018, Zieliński et al. 2020). The high hydrochemical stability of natural groundwater outflows allows for identifying those factors that determine the development of bacterioplankton in lowland spring niches. The number of bacteria depends on many hydrological, physical, and chemical parameters of water. For spring waters, BN is usually from several dozen to several hundred thousand cells per milliliter. The total number of bacterioplankton in the GLP springs exceeded several hundred thousand cells per milliliter (Figure 2). It may be related to the research period — the high growing season. According to the literature, usually, the highest BN values are recorded in warmer periods (Sinreich et al. 2014). Probably the increased BN value in GLP springs is primarily the result of favorable thermal and oxygen conditions. Psychrophilic bacteria predominate in the examined GLP springs because the water temperature of these springs did not exceed 20 °C. The groundwater of lowland areas is characterized by relatively stable temperature (Jekatierynczuk-Rudczyk 2007, Szczucińska, Wasilewski 2013). It depends mainly on the climatic conditions in a given area and the geological structure and management of the catchment area (Michalik, Szczucińska 2011). The slight variation in water temperature in the springs is one of the main factors affecting the occurrence and diversity of aquatic microorganisms (Rychła et al. 2015, Sinreich et al. 2014), which is confirmed by this study (Figure 3). The development of aquatic bacteria is favored by a pH that is close to neutral. The optimal pH for bacterial growth ranges from 5.5 to 8.5 (Chomutowska 2009). This feature of water mainly depends on the electrolytic dissociation of water and the dissociation and hydrolysis of the compounds dissolved in it. Most shallow groundwater in post-glacial areas is naturally neutral (Bodora 2016, Jekatierynczuk-Rudczyk et al. 2017). The pH range noted in GLP springs favors the development of bacterioplankton (Table 2). The periodic changes in the water reaction in the springs are also influenced by the quantity and quality of precipitation, the content of carbonates, the nature of the substrate, and the degree of anthropogenic pollution (Jekatierynczuk-Rudczyk 1999, Zieliński et al. 2020). In the case of GLP springs, there were no differences in water pH resulting from changes in the catchment area management. Another factor influencing the growth of bacteria is the availability of oxygen. The oxygen in the water of the springs comes from the atmosphere and, to a lesser extent, from photosynthesis. The content of dissolved oxygen in groundwater is usually lower than in surface waters, hence more challenging conditions for the development of bacteria. The average oxygen concentration in the waters of GLP springs was similar to that in the lowland NE springs of Poland, among others, Knyszyn Forest Landscape Park and Suwałki Landscape Park (Jekatierynczuk-Rudczyk 2008, Jekatierynczuk-Rudczyk et al. 2017, Zieliński et al. 2020). As the springs show a relatively constant water temperature daily and annually, they rarely experience extreme oxygen conditions. No oxygen deficits were found in the examined GLP springs, which can also be considered a factor favoring the growth of bacteria.

Phenolics are organic compounds that occur naturally in water (leached from the soil) or constitute industrial, less so communal, pollution. The amount of phenolics in groundwater depends mainly on the immediate catchment area and soil erosion. Favorable conditions for accumulating phenols in the soil are created by low soil pH, increased humidity, and easy oxygen access. In the waters of GLP springs, relatively low concentrations of phenols were found (Table 2). Still, it turns out that due to the limited access to organic matter (Table 2), phenolics can be an attractive source of substrates for bacteria (Figure 5), similarly to other organic compounds dissolved in water (Figure 4). Other studies confirm a substantial contribution of dissolved organic carbon to groundwater microbial biomass build (Schw b et al. 2017).

Nitrogen compounds of both inorganic and organic origin play an essential role in the proper functioning of aquatic ecosystems. These compounds can get into the water from both natural and anthropogenic sources. Some bacteria can fix nitrogen from various compounds (Adamczy , Jachimowski 2013), while others release this element during the reduction of nitrates. The average concentration of total nitrogen and nitrates in the GLP springs was lo and did not differ from other glacial areas (Table 2). However, in 2006-2007 Michalik and Szczucińska (2011) recorded much higher values of nitrate concentrations in GLP waters. Thus, the condition of the springs' waters in terms of these parameters has improved within 14 years.

On the other hand, increased mean concentrations of nitrites and ammonium ions in GLP springs were noted (Table 2). In the summer period, intensive denitrification processes probably occur in the studied niches, which are much more efficient at higher BN values. Recent studies demonstrated a high potential for denitrification linked to the capacity for anaerobic ammonium oxidation in springs (Savio et al. 2019).

The source of various phosphorus compounds (including orthophosphates) is the natural process of weathering of phosphate minerals and human economic activity (Igras, Jadczyszyn 2008). However, the average concentration of orthophosphates is so high that it is not a factor limiting the growth of bacteria in the springs of GLP. Therefore, it can be assumed that phosphorus from anthropogenic sources is a good indicator of the microbiological condition of the springs. Presumably, bacterioplankton of the springs develop better under conditions of lower SRP values, hence the observed negative relationship between the concentration of SRP and BN (Figure 6).

The increased value of the summer BN in the waters of GLP springs is probably the result of the specific geological structure, water quality of the springs, and the fact that they are not mountain springs (springs efficiency). The GLP area is characterized by high forest cover, and forest areas constantly supply organic matter to the water and thus increase the number of bacteria. The relatively small range of the total number of bacteria in GLP results from the fact that the springs are located close to each other, are supplied from practically the same aquifer, and lie within a protected area. In GLP, the highest abundance of bacterioplankton was recorded at spring No. 13 (Figure 2), where a field and a forest surround a descending spring. The impact of land use on shallow groundwater quality is often observed in shallow groundwater environment (Eftimi, Zojer 2015, Ramos et al. 2018). Low concentrations of most chemical parameters of water were found in forest springs. More considerable changes in the chemical composition of water occurred in the outflows located in arable lands, rural areas, and the greatest in the rural development zone; similar observations were recorded by Michalczyk et al. (2015), Jekatierynczuk-Rudczyk et al. (2017). However, this did not lead to significant differences in BN between springs with different catchment area management. It is also worth noting that the assessment of the microbiological condition of springs may play an essential role in determining their ecological status. In the future, using this parameter should be considered an obligatory factor in assessing the condition of natural groundwater outflows.

References

1. Adamczyk W., Jachimowski A. (2013) Wpływ składników biogennych na jakość i eutrofizację powierzchniowych wód płynących, stanowiących źródło wody pitnej Krakowa. (Influence of nutrients on the quality and eutrophication of flowing surface waters, constituting the source of Krakow's drinking water). Żywność Nauka Technologia Jakość, 20(6). (in Polish)

2. Chomutowska H. (2009) Wpływ wybranych czynników fizycznych na bakterioplankton rzeki Rozpuda (Influence of selected physical factors on bacterioplankton of the Rozpuda river). Ochrona Środowiska i Zasobów Naturalnych, 40: 513–523 (in Polish).
3. Eftimi R., Zojer H. (2015) Human impacts on karst aquifers of Albania. Environmental Earth Sciences, 74(1): 57-70.
4. Górniak A., Jekatierynczuk-Rudczyk E. (1997) Anthropogenic changes in spring water quality in the area of the town of Bialystok (Northeastern Poland). Acta Hydrobiologica, 39(1-2): 19-28.
5. Igras J., Jadczyszyn T. (2008) Zawartość azotanów i fosforanów w płytkich wodach gruntowych w Polsce (Content of nitrates and phosphates in shallow groundwater in Poland). Problemy Inżynierii Rolniczej, 16: 91-102 (in Polish).
6. Jekatierynczuk-Rudczyk E. (1999) Effects of drainage basin management on the chemical composition of waters in lowland springs. Acta Hydrobiologica, 41(6): 97-105.
7. Jekatierynczyk-Rudczyk E. (2007) Charakterystyka jakości wody naturalnych wypływów wód podziemnych Białegostoku (Water quality characteristics of natural water outflows underground in Białystok). w: Jokiel P., Moniewski P., Ziułkiewicz M. (red.). Źródła Polski. Wybrane problemy krenologiczne. Wydział Nauk Geograficznych Uniwersytetu Łódzkiego, Łódź, 234-241. (in Polish)
8. Jekatierynczuk-Rudczyk E. (2008) Threats to a small river and its urban catchment: Hydrological and hydrochemical assessment of Jaroszówka River in Białystok, Poland. Ecohydrology & Hydrobiology, 8(1): 77-87.
9. Jekatierynczuk-Rudczyk E., Zieliński P., Puczko K. (2017) Czy ochrona źródlisk w Puszczy Knyszyńskiej jest skuteczna i zadowalająca? (Is the protection of the springs in the Knyszyn Forest effective and satisfactory?) Chrońmy Przyrodę Ojczystą, 73(2): 135-147. (in Polish)
10. Michalczyk Z. (2001) Źródła Wyżyny Lubelskiej i Roztocza (Springs of the Lublin Upland and Roztocze). Wyd. UMCS, Lublin, 298 pp. (in Polish)
11. Michalczyk Z., Chmiel S., Głowacki S., Zielińska B. (2004) Zmiany wydajności i chemizmu wód źródlanych Wyżyny Lubelskiej i Roztocza (Changes in the yield and chemistry of the spring waters of the Lublin Upland and Roztocze). Ann. UMCS, seria B, 54: 107–122. (in Polish)
12. Michalczyk Z., Chmiel S., Głowacki S., Zielińska B. (2015) Monitoringowe badania źródeł Wyżyny Lubelskiej i Roztocza (Monitoring studies of the springs of the Lublin Upland and Roztocze). Przegląd Geologiczny, 63(10/2): 935-939. (in Polish)
13. Michalik A., Szczucińska A. (2011) Wykorzystanie analizy wiązkowej do interpretacji składu chemicznego wód ze źródeł na terenie Świętokrzyskiego Parku Narodowego i Ggryżyńskiego Parku Krajobrazowego (The application of cluster analysis for studying spring water chemistry and quality bin Świętokrzyski National Park and Gryżyński Landscape Park), Biuletyn Państwowego Instytutu Geologicznego 445: 405–412. (in Polish)
14. Moniewski P. (2007) Zagadnienia terminologiczne i współczesne kierunki badań krenologicznych. In: Jokiel P., Moniewski P., Ziułkiewicz M. (eds.). Źródła Polski. Wybrane problemy krenologiczne. (Springs of Poland. Selected crenological problems). Regina Poloniae, Częstochowa, Łódź: 15-29.
15. Porter K.G., Feig Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora 1. Limnology and oceanography, 25(5): 943-948.

16. Puczko K., Zieliński P., Jusik S., Kołakowska A., Jekatierynczuk-Rudczyk E. (2018) Vascular plant and bryophyte species richness in response to water quality in lowland spring niches with different anthropogenic impacts. Environmental monitoring and assessment, 190(6): 1-16.
17. Puczko K., Jekatierynczuk-Rudczyk E. (2020) Analysis of urban land cover influence to organic carbon and nutrients in surface water via impacted groundwater. Environmental monitoring and assessment, 192(2): 1-16.
18. Ramos S.T.B., Mafra M.S.H., Rech T.D., Siegloch A.E., Rech Â.F. (2018) Water quality of springs in areas under different land uses in the southern highlands of Santa Catarina. Revista Ambiente & Água, 13(4).
19. Rychła A., Buczyńska E., Szczucińska A.M. (2015) The environmental requirements of Crunoecia irrorata (Curtis, 1834) (Trichoptera: Lepidostomatidae) and the potential of the species for use as an indicator: an example from the Vistulian glaciation area, J. Limnol., 74(3): 421-432.
20. Salminen R., Tarvainen T., Demetriades A., Duris M., Fordyce F.M., Gregorauskiene V., Kahelin H., Kivisilla J., Klaver G., Klein H., Larson J., Lis J., Locutura J., Marsina K., Mjartanova H., Mouvet C., O'Connor P., Odor L., Ottonello G., Paukola T., Plant J.A., Reimann C., Schermann O., Siewers U., Steenfelt A., Van der Sluys J., De Vivo B., Williams L. (1998) FOREGS Geochemical Mapping Field Manual. Espoo, Finland, Geological Survey of Finland, 38pp. (Geological Survey of Finland Guide 47)
21. Savio D., Stadler P., Reischer G.H., Demeter K., Linke R.B., Blaschke A.P., Mach L.R., Kirschner A.K.T., Stadler H., Farnleitner A.H. (2019) Spring water of an alpine karst aquifer is dominated by a taxonomically stable but discharge-responsive bacterial community. Frontiers in microbiology, 10, 28.
22. Schwab V.F., Herrmann M., Roth V.N., Gleixner G., Lehmann R., Pohnert G., et al. (2017) Functional diversity of microbial communities in pristine aquifers inferred by PLFA- and sequencing-based approaches. Biogeosciences 14, 2697–2714.
23. Sinreich M., Pronk M., Kozel R. (2014) Microbiological monitoring and classification of karst springs. Environmental Earth Sciences, 71(2): 563-572.
24. Siwek J. (2004) Źródła w zlewniach Prądnika, Dłubni i Szreniawy: naturalne i antropogeniczne uwarunkowania jakości wód (Springs in the Prądnik, Dłubnia and Szreniawa catchments: natural and anthropogenic conditions for water quality). Kraków: Instytut Geografii Gospodarki Przestrzennej Uniwersytetu Jagiellońskiego, 98 pp. (in Polish)
25. Springer A. E., Stevens L.E., Anderson D.E., Parnell R.A., Kreamer D.K., Levin L.A., Flora S.P. (2008) A comprehensive springs classification system. Aridland springs in North America: ecology and conservation. University of Arizona Press and Arizona–Sonora Desert Museum, Tucson, 49-75.
26. Szczucińska A.M., Wasielewski H. (2013) Seasonal water temperature variability of springs from porous sediments in Gryżynka Valley, western Poland, Quaestiones Geographicae, 32 (3): 111-117.
27. Theil-Nielsen J., Søndergaard M. (1998) Bacterial carbon biomass calculated from biovolumes. Archiv für Hydrobiologie 141: 195-207.
28. Zieliński P., Jekatierynczuk-Rudczyk E., Puczko, K. (2020) Factors affecting the abundance and activity of the bacterioplankton in lowland forest springs in north-eastern Poland. Ecohydrology & Hydrobiology, 20(4): 675-686.