Benthic Bacteria of Lake Chełmżyńskie (Poland)

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Abstract

Research was conducted on the number, secondary production and physiological properties of benthic bacteria in the eutrophic Lake Chełmżyńskie. It was found that the number of psychrophilic bacteria (CFU) occurring in the bottom sediments of the studied lake and the rate of bacterial production (BP) were all characterized by a distinct seasonal variability and depended on the location of the study site and the type of bottom sediments (ANOVA, p-value <0.05). The maximum total number of bacteria (TNB), CFU and BP were observed in summer. Muddy sediments were characterized by a greater number of bacteria than sandy ones. A higher number of heterotrophic microorganisms and greater rate of secondary production were found at the sites located in the part of the studied waterbody near the town than at the sites located far from the town. The most numerous benthic bacteria were strains of hydrolyse fat, protein and cellulose. The least numerous were pectinolytic and chitinolytic bacteria.

Keywords: bacteriobenthos, bacterial production, freshwater lake, physiological properties

Introduction

Bottom sediments are an extremely important element of every aquatic ecosystem. They are where heterotrophic bacteria occur in the greatest numbers and hence are the place in which the processes of mineralization of organic matter take place the most intensively. The number of benthic bacteria can exceed the number of planktonic bacteria by as much as a few hundred times. The upper layer of sediments a few centimetres thick is particularly abundant in bacterial cells [1]. The number of microorganisms in the sediments decreases with depth as the available sources of carbon and nitrogen are exhausted and toxic products from metabolic processes are accumulated. The horizontal distribution of bacteria is to a significant degree conditioned by the nature of the bed and the type of bottom sediments [2]. Muddy sediments, being richer in organic matter, usually contain considerably more bacteria than sandy sediments [2]. As regards physiological and biochemical properties, benthic microflora displays greater activity in comparison with planktonic microflora [3]. However, knowledge concerning the ability of benthic bacteria to decompose high molecular compounds in an aquatic environment and the intensity of the processes conducted is still scarce and fragmentary. Thus, studying the processes and the physiological groups of bacteria, determining the number, biomass and growth dynamics and investigating the amount of secondary production of bacteria occurring in bottom sediments, will enable the role of heterotrophic benthic bacteria in freshwater waterbodies to be better understood. The aim of this paper was to determine these microbiological parameters characterizing the benthic bacteria population of Lake Chełmżyńskie.

Materials and Methods

Study Area

The research was conducted in the north-west part of Lake Chełmżyńskie near the town of Chełmża (sites I-V)

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and further from the town (sites VI-VIII) (Fig. 1). Sites I, II, III, V, VI, were muddy sediments; sites IV, VII, VIII were sandy.

The lake lies in the Chełmińsko-Dobrzyńskie Lake District at a distance of about 20 km from Toruń and is part of the Fryba-Vistula river basin. The surface area of the lake is about 271.1 ha, the volume of water is 16451.9 thousand m^3, maximum depth 27.1 m, and average depth about 6.0 m. Buildings from the town of Chełmża lie close to the northwestern shore. The shoreline of the lake is not varied. The land near the shore is mainly cultivated fields and meadows. Around the lake there are mostly flat banks, 60% of which give access for bathing. Thus the lake is used for recreation and watersports by the people of Chełmża, the surroundings and nearby Toruń.

Sampling

Samples of bottom sediments were collected in spring (07.05.2003), summer (10.07.2003) and autumn (09.10.2003).

Sediments from the lake bottom were collected using a tubular grab made by the authors with a diameter of 5 cm. The surface layer of the sediment (to a depth of 10 cm) was transferred aseptically to sterile glass jars and transported to the laboratory in a container with ice, inside which the temperature did not exceed +4°C. Immediately after collecting the bottom sediments, the subsamples indended to the determination of the total number of bacteria were preserved in formaldehyde, whose final concentration was 3%.

Estimating the Number and of Benthic Bacteria

The total number of benthic bacteria (TNB) in 1 cm^3 (dry mass) of bottom sediments was determined using a modified version of the direct counting method on membrane filters [4]. In order to separate bacterial cells from particles of sediments, the samples were shaken energetically in 90 ml of sterile diluter (buffer water after Doubner [5]) for 10 minutes, after which they were centrifuged at 750 x g (High Speed Centrifuge, Type 310, Mechanika Precyzyjna, Warsaw) [6]. After decanting the water from above the sediment, it was diluted 1000-fold and filtered through a membrane filter. The bacteria on the filter were dyed with reagent acridine orange.

The number of heterotrophic bacteria (CFU) in the sediments was determined using the spread plates method, using iron-peptone agar as the medium [7]. Seedings were carried out in 3 parallel repetitions. The bacterial colonies that grew up were counted after 3 and 10 days of incubation at a temperature of 20°C. The result was calculated per 1 cm^3 (dry mass) of bottom sediments.

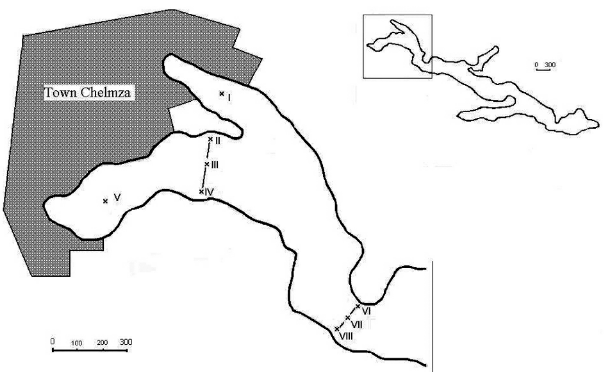

Fig. 1. Outline of Lake Chełmżyńskie.
I - VIII - research sites.

Bacterial Production

Secondary production of bacteria in bottom sediments was determined using Fuhrman and Azam's method [8, 9] measuring the rate of incorporation of radioactive thymidine [3H-methyl thymidine] (Amersham, 60Ci/mmol specific activity) into bacterial DNA. In order to separate bacterial cells from particles of bottom sediments, the sediments were shaken with diluter as above and then the whole sample was centrifuged at 750 x g (High Speed Centrifuge, Type 310, Mechanika Precyzyjna, Warsaw). Radioactive thymidine with a final concentration of 15-20 nmol/l was added to the liquid above the sediment and it was incubated for 30 minutes at a temperature of 20°C. The amount of thymidine incorporated by the bacteria in the test tubes was calculated with the aid of a Liquid Scintillation Counter, Wallac 1409 and converted to the rate of cell division using the conversion factor 1.24 x 10^9 cells/nmol [10]. Bacterial production was expressed as the amount of organic carbon in the biomass of bacterial cells [11].

Isolation of Bacterial Strains

After incubation and counting of the bacterial colonies which had grown on the plates, 25 of each bacterial strain, randomly chosen, were transplanted each time and from each site, and transferred to semi-liquid iron-peptone medium (5g agar/l) and incubated for 6 days at a temperature of 20°C. After checking the purity of the bacterial cultures in the slides coloured with the Gram method, the strains were preserved in the refrigerator at a temperature of +4°C for further studies. Every two months these strains were transplanted onto fresh iron-peptone medium.

Physiological Properties

The physiological properties of benthic bacteria were determined by seeding isolated strains on a series of test media containing the appropriate substrate. The ability of bacteria to hydrolyze protein, urea, starch, fat, DNA, pectin, cellulose and chitin was taken into account in the research. The media used in the tests were prepared after Donderski and Strzelczyk [3] and Donderski [12].

Statistical Analyses

Statistical analyses were done using program STATISTICA 5'97 for Windows. The main analytical method was multifactor analysis of variance ANOVA, which enabled us to compare three independent factors influencing bacterial abundance and secondary production: season, location and type of the bottom sediments of Lake Chełmżyńskie.

Results

Table 1 shows the results of the research on the total number of bacteria (TNB) in the bottom sediments of Lake Chełmżyńskie and the number of heterotrophic bacteria (CFU). It follows from the tests carried out that the total number of benthic bacteria varied between 0.07 and $12.90 \cdot 10^9$ cells. cm^{-3} (dry mass), while the number of CFU oscillated between 0.07 and $4.72 \cdot 10^6$ cells $\cdot$ cm^{-3} (dry mass). The values of TNB and CFU were all characterized by seasonal variability and depended on the type of bottom sediments (ANOVA, p-value <0.05, Table 2). The number of heterotrophic bacteria (CFU) were significantly different at the sites situated near the town and far from the town (ANOVA, p-value 0.000132, Table 2). The differences between total number of benthic bacteria (TNB) of Lake Chełmżyńskie at the research sites were not significantly different (ANOVA, p-value 0.140566, Table 2).

The maximum number of benthic bacteria (TNB and CFU) was observed in summer, the minimum in autumn. Muddy sediments (sites I, II, III, V, VI, Figure 1) were characterized by a greater number of bacteria than sandy sediments (sites IV, VII, VIII) with value of significance level lower than 0.05 (Table 2).

The research concerning the rate of secondary production of bacteria occurring in the bottom sediments of Lake Chełmżyńskie shows that the bottom sediments of the part of the lake near the town were characterized by higher values of secondary production than sediments in the part far from town (ANOVA, p-value 0.000055, Tables 1, 2). The highest value of secondary production was observed in summer at site I, in the part of the waterbody near the town, and the lowest in spring at site VIII, outside the town (Table1). There were significant differences between the rates of secondary production at muddy and sundy types of bottom sediments (ANOVA, p-value 0.000529, Table 2). Higher values of secondary produc-

Table 1. Number of heterotrophic bacteria (CFU), total number of bacteria (TNB) and bacterial production (BP) in bottom sediments of Lake Chełmżyńskie. Average values from three (*) or thirty (**) measurements.

Date of Sampling	Site	CFU* x 10^6 cells $\cdot$ cm^{-3} (d.m.)	TNB** x 10^9 cells $\cdot$ cm^{-3} (d.m.)	BP* μgC $\cdot$ cm^{-3} $\cdot$ d^{-1} (d.m.)
Spring (07.05.02)	I	1.70	8.80	2.87
	II	0.88	4.82	3.12
	III	1.28	4.92	3.15
	IV	0.17	1.44	0.61
	V	2.67	5.59	3.75
	VI	0.62	6.50	1.34
	VII	0.12	0.96	1.74
	VIII	0.14	0.89	0.31
Summer (19.08.02)	I	1.44	9.67	5.29
	II	4.72	5.60	3.09
	III	2.80	9.75	1.39
	IV	0.49	1.33	2.18
	V	2.78	12.90	4.89
	VI	1.23	4.28	2.27
	VII	0.20	2.78	0.50
	VIII	0.24	4.51	1.35
Autumn (09.10.02)	I	1.22	1.64	1.56
	II	0.72	0.64	3.10
	III	1.32	0.44	1.77
	IV	0.64	0.12	2.67
	V	1.10	0.57	4.93
	VI	0.49	0.39	1.31
	VII	0.07	0.07	2.44
	VIII	0.07	0.11	2.34

Explanatons: d. m. – dry mass

Table 2. Analysis of variance (ANOVA) of total number of bacteria (TNB), number of heterotrophic bacteria (CFU) and rate of bacterial production (BP) according to the season of the year, location of the research sites and type of the bottom sediments.

	TNB				CFU				BP			
Source of the variability	df	variation	F ratio	p - level	df	variation	F ratio	p - level	df	variation	F ratio	p - level
Season of the year	2	90.7893	43.81422	0.000000	2	2.19582	5.76921	0.005109	2	5.16196	6.13393	0,003769
Location of the research sites	1	4.6215	2.23032	0.140566	1	6.35613	16.69982	0.000132	1	15.86481	18.85207	0.000055
Type of the bottom sediments	1	142.3441	68.69419	0.000000	1	14.25383	37.44987	0.000000	1	11.29191	13.41812	0.000529
Within groups (error)	60	2.072141			60	0.380611			60	0.920792		

Explanations: df – number of independent variables, F ratio – among – groups variance/within – groups variance, p-level – significance level.

tion of bacteriobenthos were found at muddy sites and lower ones at sandy sites.

The research on the physiological properties of the benthic bacteria of Lake Chełmżyńskie (Fig. 2) shows that the most numerous strains of bacteriobenthos in this lake were those of hydrolyse cellulose (48.5-71.7%), protein (38.4-68.4%) and fat (32.4-71.0%). Also numerous, particularly in spring, were bacteria that hydrolyse deoxyribonucleic acid (3.5-63.2%), ureolytic bacteria (13.0-63.5%) and amylolytic bacteria (18.0-59.0%). The least numerous were pectinolytic bacteria (1.0-2.5%) and chitinolytic bacteria (2.5-5.0%).

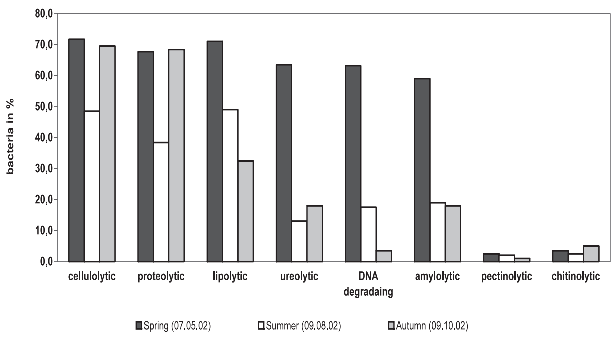

Fig. 2. Physiological properties of benthic bacteria of Lake Chełmżyńskie.

Discussion

Heterotrophic bacteria are one of the most important groups of microorganisms active in the decomposition processes of organic matter in waterbodies. Bottom sediments are the zone where these organisms occur particularly abundantly.

The summer maximum of the total number and the number of heterotrophic benthic bacteria (CFU) that was found during this research is probably connected with the enrichment of the bottom sediments in autochthonous nutrients, whose source is the decaying bodies of phyto- and zooplankton, which breed abundantly during summer in the water of this eutrophic lake. The higher the temperature of the water, the greater discharge of allochthonous pollution due to increased tourist traffic during this period and the use of the waterbody for recreational purposes are all undoubtedly favourable factors.

On the other hand, the decreasing number of benthic bacteria observed in autumn is probably caused by the low water temperature affecting the breeding rate of bacteria and reducing the supply of easily absorbed nutrients coming from decaying organisms.

The muddy sediments of Lake Chełmżyńskie, containing more organic matter, were characterized by a greater number of benthic microorganisms in comparison with sandy sediments, which is in accordance with observations by Niewolak [13,14] and Donderski and Strzelczyk [3].

A higher number of heterotrophic bacteria was found in the bottom sediments at sites in the part of the waterbody near the town compared with those further away from the town because the former part is more exposed to the influx of anthropogenic pollution, which is the main source of over-fertility of the waters of Lake Chełmżyńskie.

The amount of secondary production of the waterbody is also connected with the degree of eutrophication of the water. The estimation of its amount is of key significance to the determination of the role of microorganisms in the aquatic ecosystem. However, research on this issue concerning the bottom sediments of lakes is still relatively scarce. It follows from research carried out for this paper that higher secondary production of bacteria is characteristic of sites located in the part of the lake directly adjacent to the town compared with those far from the town, less subjected to the effects of urbanization. The highest values of secondary production were found in summer at muddy sites, the lowest in autumn at sandy sites, which has a direct connection with the number of benthic bacteria population in given periods.

Heterotrophic bacteria in waterbodies are not a homogeneous group of micro-organisms but a population of very different physiological groups with varied biochemical properties [3,12,15,16]. In this paper the ability of benthic bacteria to decompose some high molecular substances occurring in natural waters was studied. The results obtained confirmed the dominance of the most common physiological groups of microorganisms. The occurrence among benthic bacteria of large numbers of cellulolytic, proteolytic, lipolytic bacteria and bacteria that hydrolyse starch, urea or deoxyribonucleic acid can be explained by the fact that a large proportion of these compounds reach the bottom sediments before being decomposed in the water. This phenomenon concerns mainly shallow waterbodies, of which the studied lake is one.

The most numerous of the bacteria isolated from the bottom sediments of Lake Chełmżyńskie were strains that decompose cellulose. According to Zdanowski [17], environmental factors like temperature, pH of the water and the degree of eutrophication of the waterbody affect the degradation of cellulose. In our climatic zone the degradation of cellulose takes place most intensively in higher temperatures from May to October, and the optimum pH for the process of decomposing cellulose is a neutral or slightly alkaline factor. Nitrates also have a particular influence on the development of this group of bacteria. The higher the concentration of nitrates in the water, the higher the number of bacteria that decompose cellulose [18]. The relatively high temperature of the water when the samples were collected from the studied waterbody, its pH factor (7.7-9.2) and its eutrophic character can explain the abundant occurrence of cellulolytic bacteria there.

Apart from cellulose, other high molecular compounds readily decomposed in Lake Chełmżyńskie were protein and fat, which is in accordance with Donderski's and Strzelczyk's observations [3].

The high molecular compounds least readily decomposed by bacteriobenthos in the studied waterbody were pectin and chitin. According to Donderski and Strzelczyk [3], the higher number of bacteria capable of hydrolyzing chitin, pectin, cellulose and fat in the water than in the bottom sediments of lakes studied by him suggests that the majority of these substances are metabolized by bacteria in the water first, helped by the greater oxygenation and higher temperature of the water than the bottom sediments. Mudryk et al. [19] explain the lower frequency of pectinolytic and chitinolytic bacteria in Lake Gardno, which they studied, by the fact that the microbiological depolymerisation of these compounds requires the synergistic activity of many hydrolytic enzymes produced mainly by fungi and actinomycetes.

Conclusions

1. The number of psychrophilic bacteria (CFU) occurring in the bottom sediments of the studied lake and the rate of bacterial production (BP) were all characterized by a distinct seasonal variability and depended on the location of the study site and the type of bottom sediments.
2. The maximum total number of bacteria (TNB), CFU and BP were observed in summer.
3. Muddy sediments were characterized by a greater number of bacteria than sandy ones.
4. A higher number of heterotrophic microorganisms and greater rate of secondary production were found at the sites located in the part of the studied waterbody near the town than at the sites located far from the town.
5. The most numerous benthic bacteria were strains that hydrolyze fat, protein and cellulose. The least numerous were pectinolytic and chitinolytic bacteria.

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