POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 32(3): 549–559, Y. 2017

DISTRIBUTION AND PHYSIOLOGICAL ACTIVITY OF HETEROTROPHIC BENTHIC BACTERIA IN LAKES WITH DIFFERENT TROPHIC CONDITIONS LOCATED IN THE BORY TUCHOLSKIE NATIONAL PARK (POLAND)*

Łukasz Kubera, Wojciech Donderski

Department of Microbiology
Kazimierz Wielki University in Bydgoszcz, Poland

K e y w o r d s: benthic bacteria, microbiological activity, bottom sediments, microbial ecology, lakes.

A b s t r a c t

Research on ecophysiology of benthic bacteria indicates that microbiological processes which occur in bottom sediments have a major influence on the development of water bodies. Organic matter accumulated on the bottom makes lakes shallower and accelerates their aging. Microbiological processes are particularly important in small lakes, which are a common element of the environment. This article presents the results of the investigation of the distribution of heterotrophic bacteria in the bottom sediments of four lakes with different trophic conditions in the Bory Tucholskie National Park and the relationship between the number of benthic bacteria and their physiological activity. The total number of benthic bacteria and the ability of isolated strains to carry out selected physiological processes were assessed by cultivating bacteria on appropriate substrates. We also determined the coefficient of physiological activity, which was then used for the correlation analysis and verification of the research hypothesis. The highest number of benthic bacteria was found in the eutrophic and dystrophic lakes. The lowest number was recorded in lake sediments of the mesotrophic lake, although at this site the studied strains were the most active. As a result, the correlation analysis confirmed the hypothesis that there is no relationship between the metabolic potential of benthic bacteria and their number in a sample.

Address: Łukasz Kubera, Kazimierz Wielki University, al. Powstańców Wielkopolskich 10, 85-090 Bydgoszcz, Poland, phone: +48 (52) 376 79 25, e-mail: kubera@ukw.edu.pl
* The research was financed by the National Science Center, based on DEC-2012/07/N/NZ9/02082 decision.

WYSTĘPOWANIE I AKTYWNOŚĆ FIZJOLOGICZNA HETEROTROFICZNYCH BAKTERII BENTOSOWYCH W ZRÓŻNICOWANYCH TROFICZNIE JEZIORACH PARKU NARODOWEGO „BORY TUCHOLSKIE"

Łukasz Kubera, Wojciech Donderski

Zakład Mikrobiologii
Uniwersytet Kazimierza Wielkiego w Bydgoszczy, Polska

Słowa kluczowe: bakterie bentosowe, aktywność mikrobiologiczna, osady denne, ekologia mikroorganizmów, jeziora.

Abstrakt

W badaniach nad ekofizjologią bakterii bentosowych prowadzonych na przestrzeni lat potwierdzono, że procesy mikrobiologiczne zachodzące w osadach dennych mają fundamentalny wpływ na kierunek kształtowania się zbiorników wodnych. Zdeponowane na ich dnie substancje organiczne przyczyniają się do wypłycania i starzenia się zbiorników jeziornych. Procesy te mają szczególne znaczenie w kontekście niewielkich jezior, które stanowią bardzo liczny i ważny komponent środowiska naturalnego.

W artykule zaprezentowano wyniki badań podjętych w celu ilościowej oceny występowania bakterii heterotroficznych w osadach zróżnicowanych troficznie jezior położonych w Parku Narodowym „Bory Tucholskie" oraz wpływu liczebności populacji bakteriobentosu na ich aktywność fizjologiczną. Ogólną liczbę bakterii bentosowych oraz zdolność wyizolowanych szczepów do przeprowadzenia wybranych procesów fizjologicznych oznaczono metodami hodowlanymi na podłożach testowych. Wyznaczono ponadto wskaźnik aktywności fizjologicznej, który umożliwił wykonanie analizy korelacji i weryfikację hipotezy badawczej. Na podstawie analiz największą liczebność bakterii bentosowych stwierdzono w jeziorze eutroficznym oraz dystroficznym. Najmniejszą liczbę bakterii odnotowano w osadach jeziora mezotroficznego, jednakże na stanowisku tym analizowane szczepy najintensywniej przeprowadzały procesy fizjologiczne. W konsekwencji w analizie korelacji potwierdzono hipotezę o braku zależności potencjału metabolicznego szczepów bakterii bentosowych od ich liczebności w badanej próbie.

Introduction

Heterotrophic bacteria constitute one of the largest and most important groups of microorganisms in aquatic environments. Due to their different physiological properties and ability to degrade organic compounds, they play a key role in self-purification of lakes. Their number, growth and changes in population size and structure reflect the current condition of water bodies (DONDERSKI and KALWASIŃSKA 2003). Bottom sediments make an integral part of these ecosystems, offering habitat for many aquatic organisms (MADEYSKI and TARNAWSKI 2006). Being an important source of nutrients, they participate in regulating the biogeochemical cycle in the environment (KALINOWSKI and ZAŁĘSKA-RADZIWIŁŁ 2009). Researchers around the world have attempted to define the role of benthic bacteria in degradation and transformation of organic matter (MUDRYK et al. 2000, KREVS et al. 2006, SUSLOVA et al. 2009, KIERSZTYN et al. 2012). On the one hand, there is a widely held belief that the content and availability of organic nutrients have a major impact on the distribution of bacterial populations in aquatic environment (KALINOWSKA et al. 2013). CHRÓST and SIUDA (2006) proved that higher numbers of aquatic bacteria are found in hypertrophic and eutrophic lakes, and lower, in oligotrophic. On the other hand, it has been argued that the trophy of a particular water body has little influence on the number of bacteria in bottom sediments. Since the exoenzymes of bacteria display different activity, the rate and type of degradation in sediments are lake-specific (WOBUS et al. 2003, MIELNIK 2005).

The existing information gap and little scientific concern for the problem inspired the authors to undertake a study whose main objective was to determine the relationship between the number and physiological activity of benthic bacteria in lakes of different trophic conditions. Taking into account current scientific reports and our own observations we put forward a hypothesis that the relationship of this kind does not exist.

Materials snd Methods

Object of the study

The study was conducted in four lakes with different trophic conditions, located in the Bory Tucholskie National Park. The trophic status of each lake was established by park authorities. Three of these lakes are legally protected. The morphometric parameters and the location of research sites are presented in Table 1.

Table 1

Selected parameters of the research sites

Lake name	Number of site	Trophic level	Area [ha]	Depth [m]	Location
Mielnica	I	Eutrophic	11.3	1.0	N 53°48.396’ E 017°31.171’
Olbrachta*	II	Mesotrohic	2.46	3.5	N 53°48.289’ E 017°31.717’
Głuche*	III	Oligotrophic	3.3	8.0	N 53°49.265’ E 017°32.635’
Rybie Oko*	IV	Dystrophic	0.2	3.5	N 53°48.828’ E 017°32.315’

* protected lakes

Sampling

Samples were collected from a surface layer of bottom sediments (up to 10 cm deep), using a tube water sampler, from the deepest part of the studied lakes. All samples were transported to the laboratory in sterile glass containers at ± 6°C, and examined immediately. The research was conducted in a seasonal cycle from summer 2014 to spring 2015. Triplicate sediment samples were collected at each research site. In situ physico-chemical parameters of water collected from above the bottom sediments were also measured at all research sites (Table 2). There were measured parameters such as temperature, pH, electrolytic conductivity and oxygen concentration.

Table 2

Correlations between physico-chemical parameters of water collected from above the bottom sediments and the number of heterotrophic benthic bacteria

Parameter	Correlation (r)	*p*
Temperature [°C]	-0.73	> 0.05
pH	0.2	
Electrolytic conductivity [μS cm^{-1}]	0.44	
Oxygen concentration [mg dm^{-3}]	0.63	

Determining the number of heterotrophic bacteria

We determined the total number of heterotrophic bacteria (CFU) in the samples of sediment using the spread plate method according to *Jakość wody*... PN-EN ISO 8199:2010. Before inoculation we diluted the samples in sterile peptone saline (0.85% aqueous NaCl solution with 1 g of peptone/l). Next, we prepared inoculations of 100 fl of each sample on sterile nutrient agar (three parallel repetitions). The plates were incubated at +20°C for 72 hours. After that we counted grown colonies using the formula from the above ISO standard, and calculated the results per gram of dry mass. Some colonies (pure cultures) were preserved for further analysis. We isolated of 25 strains.

Physiological properties

To assess the ability of benthic bacteria to degrade selected organic compounds we inoculated them on media enriched with suitable organic polymers. We used of 25 strains from each research site. To examine the degradation of each compound, we supplemented the media accordingly with tributyrin (fats), casein (proteins), and starch, colloidal chitin, and carboxymethylcellulose (sugars). After the incubation, some plates were covered with suitable reagents in order to read the results. The result was considered positive when there was a hydrolysis zone around the colony. The following were also tested: the ability of the studied strains to ammonify, to reduce nitrats to nitrites, and to produce hydrogen sulphide (LALKE-PORCZYK and DONDERSKI 2005).

Coefficient of physiological activity

Based on the results of physiological processes we determined the coefficient of physiological activity of heterotrophic bacteria (FA_{HB}) according to the formula proposed by DAHLBÄCK et al. (1982):

$$FA_{HB} = \frac{\sum_{i=1}^{n} a_i}{n}$$

where:
a_i – the percentage of strains exhibiting specific physiological properties
n – number of physiological tests.

Statistical

STATISTICA 12 software was used for statistical analysis of the results. The analysis of the differences between the sets of data and the comparison of independent factors was prepared using ANOVA analysis of variance or a non-parametric test (Kruskal-Wallis). Tukey test (post-hoc) was used to determine the differences within the sets of data. Pearson correlation coefficient was determined in order to analyze the relationship between the obtained values. All statistical tests were carried out at $p < 0.05$.

Results

The number of heterotrophic bacteria in the bottom sediments of the investigated lakes is presented in Figure 1. The highest average number of benthic bacteria was recorded at research site I, i.e. in the eutrophic lake ($1.61 \cdot 10^6$ CFU g^{-1} dry weight) and site IV, i.e. in the dystrophic lake ($1.51 \cdot 10^6$ CFU g^{-1} dry weight). The number of benthic bacteria was considerably lower at the sites located in the meso- and the oligotrophic lake. Benthic bacteria were the most numerous in summer ($2.45 \cdot 10^6$ CFU g^{-1} dry weight on average). Taking into account an average of all investigated lakes, the lowest number of these bacteria was noted in winter ($0.35 \cdot 10^6$ CFU g^{-1} dry weight on

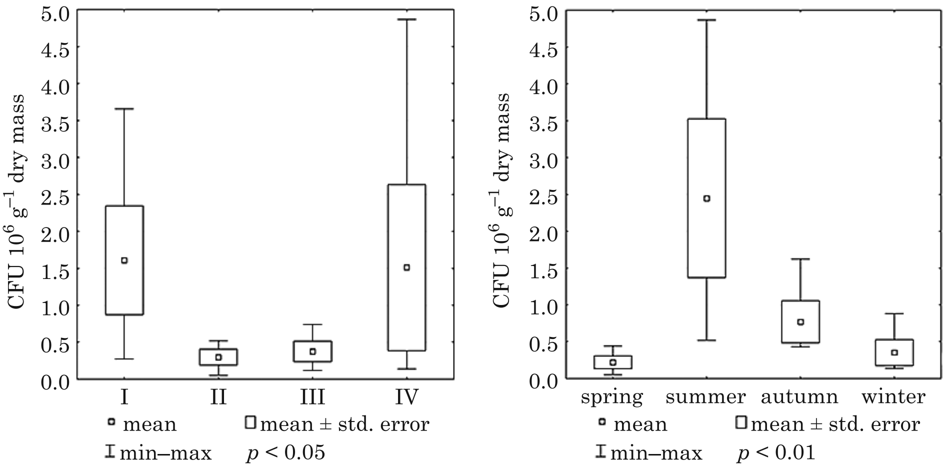

Fig. 1. Number of heterotrophic benthic bacteria at the 4 research sites in all seasons and in the research seasons at all sites: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake

average). The analysis of variance revealed statistically significant differences in the number of benthic bacteria between the research sites and seasons ($p < 0.05$). Table 3 show that differences in the number of benthic bacteria between lakes was mainly statistically insignificant. In an annual cycle the temperature and the number of benthic bacteria had the strongest negative correlation (r = -0.7), compared to the remaining physico-chemical parameters of water from above the sediment) However, this result was not statistically significant (Table 2). Table 4 presents the percentage of benthic strains involved in particular physiological processes. Some repeatibility of the results was observed for ammonification: all studied strains had this ability. Regardless of the season and research site, only a small percentage of the strains had the ability to depolimerize chitin. The other physiological processes had different intensity. On the basis of the presented results we determined the coefficient of physiological activity of heterotrophic bacteria. Figure 2 shows that the highest activity of benthic strains was recorded at research site II, i.e. in the mesotrophic lake, the lowest, in the lake with a low content of organic substances (site III). Moreover, the only statistically significant differences ($p < 0.05$) were noted between these two sites. The physiological activity of the bacteria was not significantly correlated with the season. The highest was observed in autumn, and slightly lower, in spring and summer. In winter physiological activity of the analyzed strains decreased considerably. On the basis of the above results we determined the correlation between the number and activity of heterotrophic benthic bacteria. The low correlation coefficient together with the lack of statistical significance suggests that the metabolic potential of the studied bacteria does not depend on their number in the sample (Figure 3).

Table 3
Statistical differences in the number of heterotrophic benthic bacteria between the groups of data

	I	II	III	IV	Spring	Summer	Autumn	Winter
I								
II	*							
III	ns	ns						
IV	ns	ns	ns					
Spring								
Summer					**			
Autumn					*	ns		
Winter					ns	**	ns	

Explanation: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake
ns – not significant; * $p < 0.05$; ** $p < 0.01$

Table 4
Physiological properties of benthic bacteria: *P* – proteolytic; *L* – lipolytic; *A* – amylolytic; *C* – cellulolytic; Ch – chitinolytic; Am – ammonification; Ni – nitrates reduction; H_2S – hydrogen sulfide production

Season	Sites	Physiological group of bacterial strains [%]							
		P	*L*	*A*	*C*	Ch	Am	Ni	H_2S
Spring	I	36	32	40	44	8	100	44	68
	II	76	80	72	64	4	100	20	16
	III	36	44	32	36	16	100	24	64
	IV	44	60	52	28	0	100	88	56
Summer	I	36	40	40	44	8	100	48	64
	II	72	76	76	68	4	100	16	20
	III	32	40	36	36	12	100	24	68
	IV	52	84	52	32	4	100	56	40
Autumn	I	32	72	44	44	8	100	56	68
	II	88	88	80	72	8	100	12	36
	III	32	40	36	36	8	100	24	68
	IV	52	100	56	32	4	100	28	24
Winter	I	16	20	36	24	0	100	16	40
	II	72	64	68	52	4	100	8	28
	III	24	32	32	20	4	100	0	20
	IV	48	56	36	20	0	100	0	64

Explanation: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake

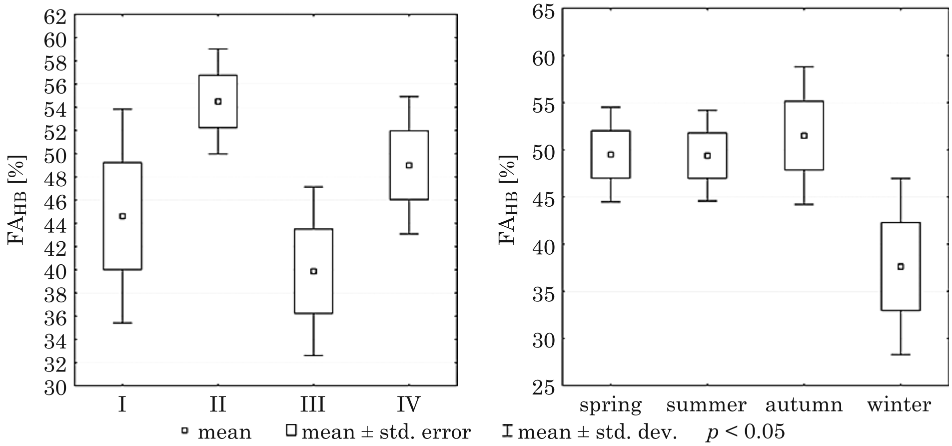

Fig. 2. Physiological activity of heterotrophic benthic bacteria: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake

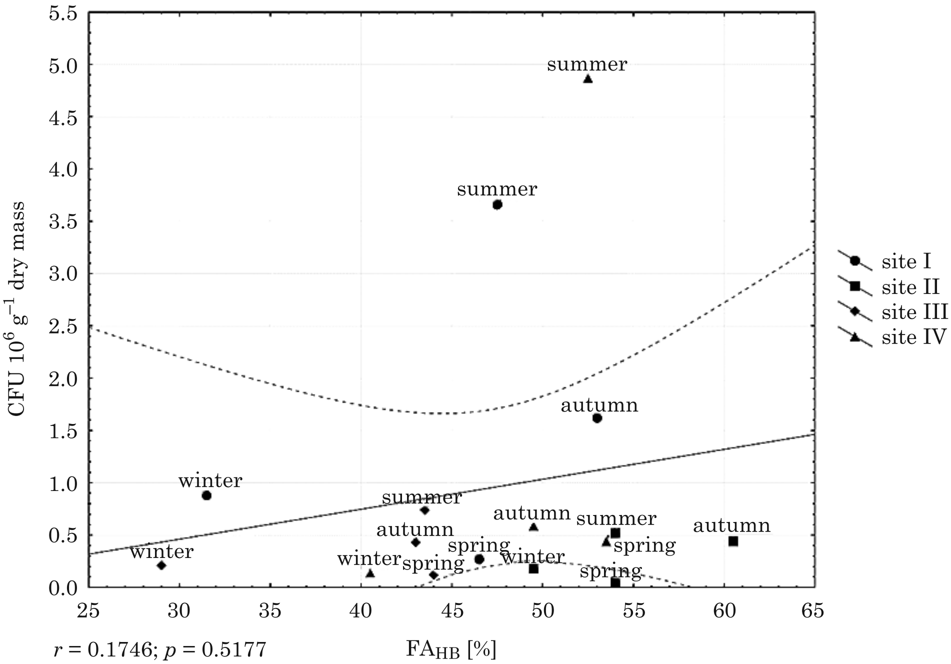

Fig. 3. Correlation between physiological activity and the number of heterotrophic benthic bacteria: site I – eutrophic lake, site II – mesotrohic lake, site III – oligotrophic lake, site IV – dystrophic lake

Discussion

Due to physico-chemical and biological factors water bodies undergo dynamic changes. Microbiological processes observed in bottom sediments play a key role in shaping these aquatic ecosystems (KALWASIŃSKA and DONDERSKI 2005). Abundance is one of the basic parameters characterizing the microbial population in the natural environment. It may determine the fertility of the given ecosystem (i.e its trophic status) to a large extent. This observation is reflected in the results of this study, as we recorded the highest number of heterotrophic bacteria in the sediments of the eutrophic lake. Much lower numbers were recorded in the meso- and the oligotrophic lake. PORTER et al. (2004) also confirmed a relationship between the total number of planktonic bacteria and the trophic status of lakes. A high number of benthic bacteria was noted in the bottom sediments of a dystrophic lake with low pH owed to the presence of humic acids. Low water pH normally limits bacterial growth in aquatic environments. This observation was confirmed by LINDSTRÖM (2000) investigating five lakes with different trophic conditions in Sweden. He recorded higher bacterioplankton biomass in lakes with higher pH values. On the other hand, humic acids are susceptible to microbiological degradation, and can stimulate the activity of heterotrophic bacteria (MUNSTER et al. 1992). Organic polymers of high molecular weight such as proteins, fats and carbohydrates are common in aquatic systems. These compounds cannot be directly assimilated by microbial cells. In order to depolymerize macromolecular compounds and to release monomers ready for assimilation, bacteria regulate the synthesis and activity of specific hydrolytic exoenzymes (UNANUE 1999). Our research into the microbial decomposition of organic compounds indicates that lipolytic bacteria were dominant in the sediments of the investigated lakes. On average 58% of strains had the ability to hydrolyze tributyrin. Lipid compounds constitute one of the dominant fractions of organic matter in aquatic environments. They offer an important energy substrate for bacteriocenosis. According to many authors, dead and alive zooplankton and phytoplankton are the main source of lipid compounds in both saltwater and freshwater ecosystems (ARTS et al. 1992, GAŠPAROVIĆ et al. 2007). Chitinolytic bacteria were the least numerous among bacteria hydrolyzing organic compounds in the bottom sediments of the studied lakes. Their average share in the studied isolates was only 5.75%. POULICEK and JEUNIAUX (1991) noted that chitin level is lower in the sediments than in water of lakes. This may account for a small percentage of strains capable of degrading this polysaccharide in the studied sediments. Similar results were also obtained by SWIONTEK-BRZEZINSKA et al. (2008), who analyzed the activity of chitynolitic strains in lake Chełmżyńskie. Among the bacteria isolated from this water body they noted 11–19% of chitinase-producing strains. Only 3–8% of benthic strains had this ability. Many capacities of bacteria to carry out physiological processes may determine the ecological success of water bodies. The highest physiological activity was observed among strains isolated from the sediments of the mesotrophic lake (54.5%), the lowest, in the oligotrophic lake (39.9%). The latter result can be caused by the low content of nutrients which could be transformed microbiologically. Our results correspond with those obtained by LALKE-PORCZYK et al. (2004), who recorded 40–49% physiological activity of epiphytic bacteria in Lake Chełmżyńskie. The highest FAHB cofficient was noted in spring. The studied benthic strains were more active in autumn (51.5%) than in spring (49.7%).

Conclusions

As has been already mentioned, the availability of substrates determines to a large extent the development of aquatic microbiomes. However, bacterial activity depends on other factors as well. Our results indicate that throphic conditions influence the physiological activity of bacteria: statistically significant differences were recorded between the mesotrophic and the oligotrophic lake. There was no correlation between physiological activity of benthic bacteria and their number in a sample.

Due to the fact that a range of physico-chemical and biological factors make water bodies one of the most dynamically changing natural environments, our results may not be valid for all lake ecosystems. However, they led to positive verification of the initial hypothesis.

Acknowledgements

Special thanks to Joanna Studzińska for her help in planning and conducting laboratory analyzes.

Translated by DOROTA SOBIERAJSKA

Accepted for print 5.09.2017

References

ARTS M.T., EVANS M.S., ROBARTS R.D. 1992. *Seasonal patterns of total and energy reserve lipids of dominant zooplanktonic crustaceans from a hyper-eutrophic lake*. Oecologia, 90(4): 560–571.

CHRÓST R.J., SIUDA W. 2006. *Microbial production, utilization, and enzymatic degradation of organic matter in the upper trophogenic layer in the pelagial zone of lakes along a eutrophication gradient*. Limnol. Oceanogr., 51: 749–762.

DAHLBÄCK B., GUNNARSSON L.Å.H., HERMANSSON M., KJELLEBERG S. 1982. *Microbial investigations of surface microlayers, water column, ice and sediment in the Arctic Ocean*. Mar. Ecol. Prog. Ser., 9: 101–109.

DONDERSKI W., KALWASIŃSKA A. 2003. *Occurrence and physiological properties of bacterioplankton of Lake Chełmżyńskie (Poland)*. Pol. J. Environ. Stud., 12(3): 287–295.

GAŠPAROVIĆ B., PLAVŠIĆ M., ĆOSOVIĆ B., SALIOT A. 2007. *Organic matter characterization in the sea surface microlayers in the subarctic Norwegian fjords region*. Mar. Chem., 105(1–2): 1–14.

Jakość wody. Ogólne wytyczne oznaczania liczby bakterii metodą hodowli. PN-EN ISO 8199:2010.

KALINOWSKA K., GUŚPIEL A., KIERSZTYN B., CHRÓST R.J. 2013. *Factors controlling bacteria and protists in selected Mazurian eutrophic lakes (North-Eastern Poland) during spring*. Aquatic Biosystems, (9): 9.

KALINOWSKI R., ZAŁĘSKA-RADZIWIŁŁ M. 2009. *Method of developing the sediment quality guidelines based on ecotoxicological assays*. Ochrona Środowiska i Zasobów Naturalnych, 40: 549–560.

KALWASIŃSKA A., DONDERSKI W. 2005. *Benthic bacteria of Lake Chełmżyńskie (Poland)*. Pol. J. Environ. Stud., 14(6): 761–766.

KIERSZTYN B., SIUDA W., CHRÓST R.J. 2012. *Persistence of bacterial proteolytic enzymes in lake ecosystems*. FEMS Microbiol. Ecol., 80: 124–134.

KREVS A., KUCINSKIENE A., PASKAUSKAS R. 2006. *Microbial destruction of organic matter in the bottom sediments of lithuania lakes*. Microbiology, 75(2): 213–218.

LALKE-PORCZYK E., DONDERSKI W. 2005. *The role of bacteria growing on the root system of the common reed (Phragmites australis [Cav.] Trin. ex Steudel) in the metabolism of organic compounds*. Pol. J. Environ. Stud., 14(1): 57–64.

LALKE-PORCZYK E., DONDERSKI W., MAŁECKA M. 2004. *Physiological properties and taxonomy of epiphytic bacteria in Chełmżyńskie lake in Poland*. Pol. J. Environ. Stud., 13(5): 543–549.

LINDSTRÖM E.S. 2000. *Bacterioplankton community composition in five lakes differing in trophic status and humic content*. Microb. Ecol., 40: 104–113.

MADEYSKI M., TARNAWSKI M. 2006. *Evaluation of the ecological state of bottom sedimentsin chosen small water reservoirs*. Infrastruktura i Ekologia Terenów Wiejskich, 4(3): 107–116.

MILENIK L. 2005. *Influence of the use of a drainage basin on physical and chemical properties of bottom sediments of lakes*. Inżynieria Rolnicza, 4(64): 31–36.

MUDRYK Z.J., PODGÓRSKA B., BOLAŁEK J. 2000. *The occurrence and activity of sulphate-reducing bacteria in the bottom sediments of the Gulf of Gdańsk*. Oceanologia, 42(1): 105–117.

MÜNSTER U., NURMINEN J., EINIÖ P., OVERBECK J. 1992. *Extracellular enzymes in a small polyhumic lake: origin, distribution and activities*. Hydrobiol., (243/244): 47–59.

PORTER J., MORRIS S.A., PICKUP R.W. 2004. *Effect of trophic status on the culturability and activity of bacteria from a range of lakes in the English Lake District*. Appl. Environ. Microbiol., 70: 2072–2078.

POULICEK M., JEUNIAUX C. 1991. *Chitin biodegradation in marine environments. An experimental approach*. Biochem. Sys. Ecol., 19(5): 385–394.

SUSLOVA M.U., PARVENOVA V.V., ZIBOROVA G.A., FEDOTOV A.P. 2009. *Distribution of strains of spore-forming bacteria of the genus bacillus in the bottom sediments of lake Khubsugul in northern Mongolia as an indication of paleoclimate*. Biology Bulletin, 36(6): 634–638.

SWIONTEK-BRZEZINSKA M., LALKE-PORCZYK E., DONDERSKI W. 2008. *Utilization of shrimp waste as a respiration substrate by planktonic and benthic microorganisms*. Pol. J. Environ. Stud., 17(2): 273–282.

UNANUE M., AYO B., AGIS M., SLEZAK D., HERNDL G.J., IRIBERRI J. 1999. *Ectoenzymatic activity and uptake of monomers in marine bacterioplankton described by a biphasic kinetic model*. Microb. Ecol., 37: 36–48.

WOBUS A., BLEUL C., MAASSEN S., SCHEERER C., SCHUPPLER M., JACOBS E., RÖSKE I. 2003. *Microbial diversity and functional characterization of sediments from reservoirs of different trophic state*. FEMS Microbiology Ecology, 46: 331–347.