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# SEASONAL CHANGES IN THE NUMBER OF SOME PHYSIOLOGICAL GROUPS OF HETEROTROPHIC BACTERIA IN WATER, SOIL AND PLANTS OF THE WETLANDS NEAR OLSZTYN

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# SEZONOWE ZMIANY LICZEBNOŚCI NIEKTÓRYCH GRUP FIZJOLOGICZNYCH BAKTERII HETEROTROFICZNYCH W WODZIE, GLEBIE I ROŚLINNOŚCI ŚRÓDLEŚNYCH OBSZARÓW BAGIENNYCH OKOLIC OLSZTYNA

Badano liczebność bakterii heterotroficznych i niektórych grup fizjologicznych tych drobnoustrojów (amylolitycznych, lipolitycznych, proteolitycznych, kazeolitycznych) w wodzie, glebie i turzycy błotnej (*Carex acutiformis* Ehrb.) jednego z większych obszarów bagiennych w rejonie leśniczówki Stary Dwór koło Olsztyna. Ogólna liczba bakterii heterotroficznych w wodzie badanego wetlandu nie przekraczała 2,3 x $10^5$ jtk $cm^{-3}$; na roślinach 3,4 x $10^9$ jtk $g_{sm.}^{-1}$ w części zanurzonej w wodzie i 1,6 x $10^9$ jtk $g_{sm.}^{-1}$ w części napowietrznej. W glebie i na powierzchni korzeni starszych odpowiednio 3,7 x $10^9$ i 1 x $10^9$ jtk $g_{sm.}^{-1}$, na korzeniach nowych 2,5 x $10^{10}$ jtk $g_{sm.}^{-1}$. Spośród badanych grup fizjologicznych bakterii heterotroficznych najliczniej reprezentowane były bakterie amylolityczne i kazeolityczne. Maksymalne ich liczebności na powierzchni zanurzonych w wodzie fragmentów roślin, w glebie i na korzeniach starych (ubiegłorocznych) osiągała czasami kilka, kilkadziesiąt i więcej milionów jtk $g_{sm.}^{-1}$. Bakterie lipolityczne i proteolityczne występowały w mniejszych ilościach; tylko sporadycznie osiągały wartości rzędu kilku, kilkudziesięciu milionów jtk $g_{sm.}^{-1}$. Wyniki badań sugerują, iż główna część procesów mikrobiologicznych przemian związków organicznych w wetlandzie odbywa się na styku woda – roślina – gleba.

## Summary

Counts of heterotrophic bacteria and some physiological groups of those microorganisms (amylolytic, lipolytic, proteolytic, caseolytic) were studied in water, soil and on sedge plants (*Carex acutiformis* Ehrb.) in one of larger wetlands near the forester's lodge Stary Dwór, near Olsztyn. The total count of heterotrophic bacteria in the water from the wetland did not overcome 2.3 x $10^5$ CFU $cm^{-3}$; on plants the respective counts were 3.4 x $10^9$ CFU $GDW^{-1}$on submerged parts and 1.6 x $10^9$ CFU $GDW^{-1}$on aerial leaves. In the soil and on the surface of older roots the counts of heterotrophic bacteria were, respectively, 3.7 x $10^9$ and 1 x $10^9$ CFU $GDW^{-1}$, whereas on new root the number of bacteria was 2.5 x $10^{10}$ CFU $GDW^{-1}$. Among the physiological groups of heterotrophic bacteria analyzed, amylolytic bacteria were the most numerous. Their maximum counts on the surface of submerged fragments of plants, in soil and on old (from the previous year) roots reached between less

than ten million, less than a hundred million and more units. Lipolytic and proteolytic bacteria were present in smaller numbers; they only sporadically reached between less than ten million or less than a hundred million CFU $GDW^{-1}$. The differences in the counts of the physiological groups of heterotrophic bacteria in water and on different plant fragments between the two studied sites were small and never exceeded one order of value. The results suggest that microbiological mineralization of organic contents in wetland occurs mostly at the borderline of water and plant phases.

## INTRODUCTION

One of the major functions of wetlands is reduction of organic and mineral pollutants which permeate from the catchment basin during spring thaws and/or storm waters. A significant role in those processes is played by bacteria in water, on plants and in the rhizosphere. In the Masurian Lake District, wetlands are an integral part of the natural landscape. Their proper activity is closely connected with a number of factors, including the biochemical activity of bacteria and other organisms populating water and soil, submerged parts of plants and their rhizosphere. The microbiological processes are fundamental when one considers productivity of certain areas. Those functions include decomposition of organic substances (carbohydrates, proteins, fats), their mineralization, degradation of organic waste such as hydrocarbons, nitrification and denitrification. Along with other soil, plant and hydrological parameters, microorganisms condition the proper functioning of a whole ecosystem [5]. Most wetland bacteria populate submerged parts of plants, where they create a microbiological biofilm consisting of various microorganisms, which possess different physiological properties and biochemical capabilities. It is claimed that those microorganisms contribute to the improvement of wetland water quality through mineralization of organic C and N compounds [1, 2, 12] and via transformation of mineral compounds to forms available to plants [5]. Emergent macrophytes release several biologically active compounds, which sustain the growth and biochemical activity of bacteria populating their surface. They are also important in preventing soil (bottom) erosion by blocking the flow of sediment and debris by stabilizing stream banks and wetland edges and by promoting infiltration [4]. The relevant literature on counts of wetland heterotrophic bacteria is fragmentary [6–9]. The present paper contains a more complex description of the dynamics of seasonal variations in heterotrophic bacterial counts in general and in the number of specific groups of bacteria which can decompose starch, protein (gelatin), casein as well as counts of lipolytic bacteria in water and on submerged and emergent parts of sedge and in the rhizosphere those plants. The material for the tests was collected from one of the largest wetland-type marshes near the town of Olsztyn.

## MATERIALS AND METHODS

### *Study area*

The study covered the Lakeland area near the Stary Dwór forest's lodge. It is located among slopes afforested with pine and spruce in the upper parts and birch in the lower parts and with willow in coastal zone. The surface area of the Lakeland area covers approximately 0.2 $km^2$, with a channel (6 m in width and 4–5 m in depth) running thought in the center (a remnant of an old lake). The entire area is periodically flooded in spring and autumn, whereas in the summer the water count outside the channel zone falls to

a dozen cm or so. The flora is dominated by sedges (*Carex*) and rushes (*Juncus*) that form a permanent cover or isolated tufts surrounded by water.

***Materials***

The number of heterotrophic and other physiological groups of bacteria was determined in water of the littoral zone overgrown with dense phytomass and between tufts of plants emerging from water; on plants emerging from water – separately on submerged stem and aerial leaves; in the root system (rhizosphere) – on old (the previous year's) and new (the current year's) roots, as well as in soil.

***Collection of samples***

Samples of water and tufts of sedge (*Carex acutiformis* Ehrb.) including the soil and root system were taken in 1-month intervals, in the vegetative seasons from March to November of two following years. Water samples were collected from the depth of 0.3 m directly into sterile glass bottles. Site 1 was located 30 m from a forest tract leading along the edge of wetland on its eastern side. Sites 2 and 3 were located on the edge of wetland, on the northeastern side, 80 m from path leading to the Stary Dwór forest's lodge. Under laboratory conditions, the material collected was separated into parts of plant immersed in water (stems), part protruding from water (aerial leaf), previous-year roots (dead) and current year roots (live), and soil from the root system. Aseptically weighed 10 g of soil samples and particular fragments of plants were transferred into flask with 90 $cm^3$ of sterile physiological NaCl solution and shaken in a shaker for 30 min. The obtained suspension of soil and plants and roots was diluted and transferred in the volume of 1 $cm^3$ on respective media appropriate for individual physiological group of bacteria. Simultaneously weighed 10 g samples of soil and particular fragments of plants were dried at 105°C to measure the dry matter.

***Microbiological analyses***

Bacteriological analyses comprised the following:

1. total number (CFU $cm^{-3}$/CFU $GDW^{-1}$) of heterotrophic bacteria on TGY medium after 7 day incubation at 20°C;
2. the number (CFU $cm^{-3}$/CFU $GDW^{-1}$) of amylolytic bacteria on nutrient medium (broth-agar with addition of dissolved starch) after 7 day incubation at 20°C;
3. the number (CFU $cm^{-3}$/CFU $GDW^{-1}$) of proteolytic bacteria on nutrient medium (broth-agar with addition of gelatin) after 1, 2 and 3 day incubation at 20°C;
4. the number (CFU $cm^{-3}$/CFU $GDW^{-1}$) of caseolytic bacteria on nutrient medium (broth-agar with addition of 0.5% skim-milk by Frazier and Rapp [3] after 7 day incubation at 20°C;
5. the number (CFU $cm^{-3}$/CFU $GDW^{-1}$) of lipolytic bacteria on TGY medium with 1% addition of tributyrin after 7 day incubation at 20°C.

Total numbers of heterotrophic bacteria were determined as all growing colonies on TGY medium; amylolytic bacteria were determined as colonies with break zone after Lugol liquid treatment; caseolytic and lipolytic bacteria were counted as colonies with break zone.

***Statistical evaluation***

In order to estimate significance of differences in counts of studied groups of microorganisms in the wetland during the 2-year time of the study, a single and double factor analysis of variance (single factor' ANOVA and ANOVA for factors system) was conducted, verifying the hypothesis of the equality of means ($H_0$:$x_1 = x_2 = \ldots = x_5$) at the level of significance $\alpha = 0.05$, assuming that the variance for the numerousness of the bacteria groups under study are uniform. The Kruskal-Wallis test, which is a non-parametric equivalent of the analysis of variance, was applied too [15].

## RESULTS

This paper reports only the general fluctuations in the numbers of the studied groups of microorganisms depending on the time of sample collection and kind of samples; the detailed results can be obtained from the authors.

***Number of bacteria in the water, sedge submerged stem and aerial leaf surfaces***

In the water from that wetland the counts of heterotrophic bacteria varied within three orders of value (from $2 \times 10^2$ to $2.32 \times 10^5$ CFU $cm^{-3}$) (Tab. 1, Fig. 1). Amylolytic, proteolytic and lipolytic bacteria reached at the most slightly above $1 \times 10^3$ (respectively 1050, 1330 and 1670). Caseolytic bacteria were the most numerous group of heterotrophic bacteria in the water (to $3 \times 10^4$ CFU $cm^{-3}$). In the particular months of the study period the differences (significant statistically) in the counts of bacteria determined on TGY medium from water samples at all sites were sometimes 10-fold large. Generally, in both years more bacteria were observed in autumn, less often – in spring or summer (Fig. 1). Amylolytic, proteolytic, caseolytic and lipolytic bacteria were present more frequently in water respectively: amylolytic bacteria – in May, July and August, proteolytic bacteria – in May and June, caseolytic bacteria – in April and May (Fig. 2).

On the surface of submerged parts of sedge, the number of heterotrophic bacteria determined on TGY medium during the whole period of study ranged within 4–5 orders of value (from $1.74 \times 10^5$ to $3.4 \times 10^9$ CFU $GDW^{-1}$) (Tab. 1). The smallest counts of those bacteria were determined in November and the highest – in September. The amounts of amylolytic, proteolytic and lipolytic bacteria varied from $3.3 \times 10^3$ to $5.3 \times 10^7$ CFU $GDW^{-1}$, while those of caseolytic bacteria ranged from $5.5 \times 10^5$ to $1.1 \times 10^9$ CFU $GDW^{-1}$ (Tab. 1). The differences in their counts between both sites (site 1 and 2) ranged within 1–3 orders of value. They were generally more numerous in the spring and summer seasons (Fig. 2).

On the surface of aerial leaf of sedge the number of heterotrophic bacteria (studied only at site 1) varied from $4.6 \times 10^4$ to $1.7 \times 10^9$ CFU $GDW^{-1}$ (Tab. 1). The number of those bacteria was more numerous in spring (Fig. 1). Amylolytic, proteolytic, caseolytic and lipolytic bacteria on aerial leaves of sedge were not assayed.

Table 1. Mean and range of heterotrophic bacteria and some of their physiological groups in the water, soil and sedge of the natural wetland near Olsztyn under two year study

| Microorganisms | Site | Wetland water | Soil | Sedge (*Carex acutiformis*) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Submerged stem | Aerial leaf | Old roots | New roots |
| | | CFU x $cm^{-3}$ | CFU x $10^3$ $GDW^{-1}$ | | | | |
| Heterotrophic bacteria | 1 | 33 x $10^3$<br>200–220 x $10^3$ | 248 x $10^3$<br>101–3.7 x $10^6$ | 193 x $10^3$<br>174–1.1 x $10^6$ | 175 x $10^3$<br>46–1.7 x $10^6$ | 25 x $10^3$<br>130–302 x $10^3$ | 3.7 x $10^6$<br>27–24.8 x $10^6$ |
| | 2 | 11 x $10^3$<br>600–29 x $10^3$ | 84 x $10^3$<br>68.7–1 x $10^6$ | 600 x $10^3$<br>2 x $10^3$–3.5 x $10^6$ | – | 127 x $10^3$<br>57–1 x $10^6$ | 2 x $10^6$<br>192–1.4 x $10^6$ |
| | 3 | 32 x $10^3$<br>650–232 x $10^3$ | – | – | – | – | – |
| Amylolytic bacteria | 1 | 680<br>45–1050 | 3.7 x $10^3$<br>167–22.9 x $10^3$ | 5.7 x $10^3$<br>223–10.6 x $10^3$ | – | 11.1 x $10^3$<br>11.1–75 x $10^3$ | – |
| | 2 | 351<br>55–740 | 4 x $10^3$<br>130–25 x $10^3$ | 24.2 x $10^3$<br>3.1 x $10^3$–5.1 x $10^3$ | – | 1.8 x $10^3$<br>72.7–8.3 x $10^3$ | – |
| Proteolytic bacteria | 1 | 268<br>6–630 | 531<br>0.3–2.7 x $10^3$ | 10.3 x $10^3$<br>3.3–53.1 x $10^3$ | – | 1.5 x $10^3$<br>1.1–24 x $10^3$ | – |
| | 2 | 235<br>3–1.3 x $10^3$ | 706<br>0.8–4 x $10^3$ | 8.9 x $10^3$<br>16.5–39.3 x $10^3$ | – | 531<br>2.5–10 x $10^3$ | – |
| Caseolytic bacteria | 1 | 5.8 x $10^3$<br>350–20 x $10^3$ | 400 x $10^3$<br>50–276 x $10^3$ | 220 x $10^3$<br>1 x $10^3$–1.1 x $10^6$ | – | 140 x $10^3$<br>66.7–960 x $10^3$ | – |
| | 2 | 4.9 x $10^3$<br>170–30 x $10^3$ | 260 x $10^3$<br>42–170 x $10^3$ | 200 x $10^3$<br>550–619 x $10^3$ | – | 100 x $10^3$<br>70–692 x $10^3$ | – |
| Lipolytic bacteria | 1 | 517<br>23–1.7 x $10^3$ | 1.8 x $10^3$<br>18.6–10.1 x $10^3$ | 3.8 x $10^3$<br>200–10 x $10^3$ | – | 2 x $10^3$<br>37.3–12.6 x $10^3$ | – |
| | 2 | 315<br>16–812 | 5.9 x $10^3$<br>6.7–33 x $10^3$ | 6.7 x $10^3$<br>190–16.9 x $10^3$ | – | 0.8 x $10^3$<br>6.4–5.1 x $10^3$ | – |

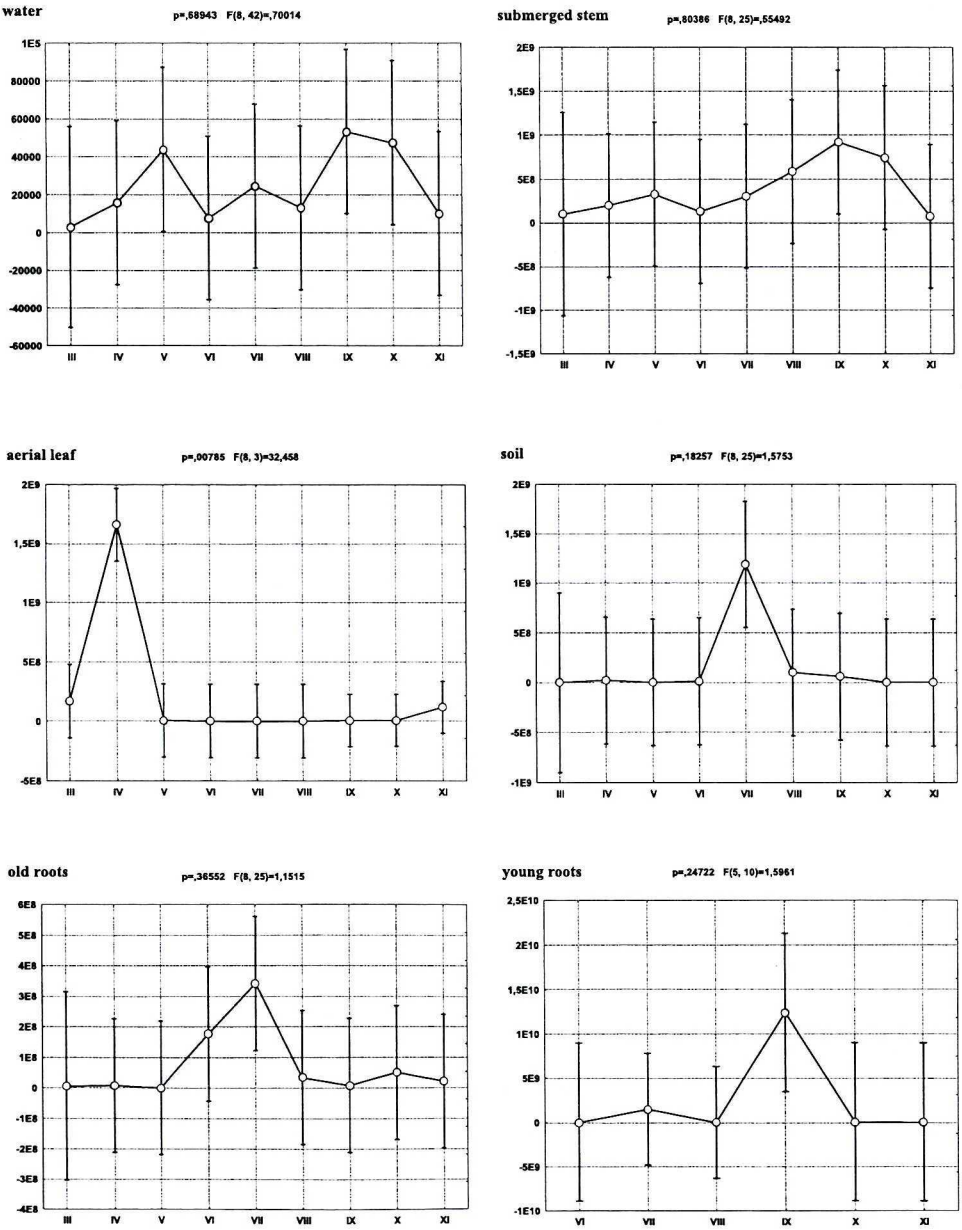
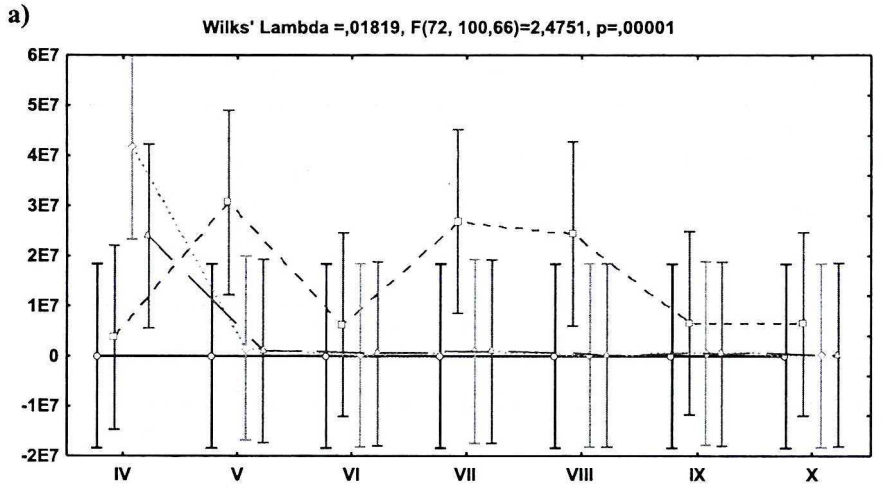
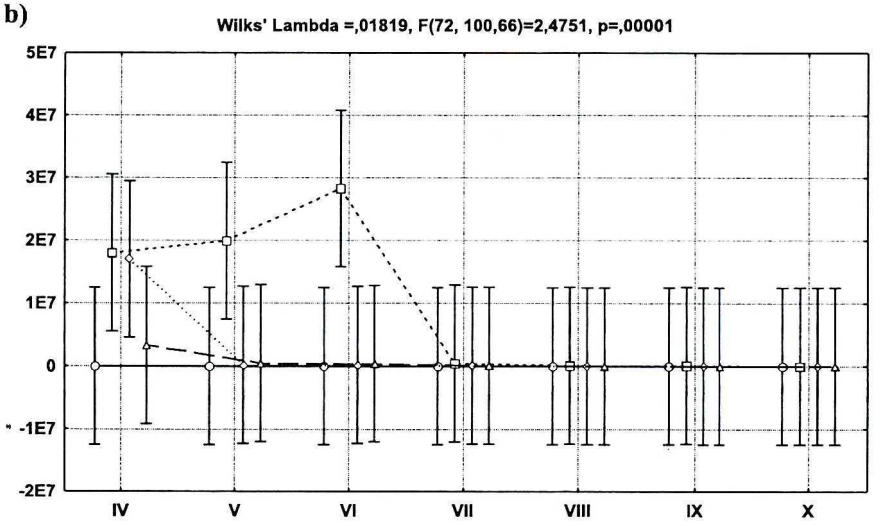



Fig. 1. Expected marginal means (some are not estimated) of heterotrophic bacteria (CFU $cm^{-3}$/CFU $GDW^{-1}$) in different kinds of samples collected during different months of study (factor); decomposition of effective hypotheses; vertical columns mean 0.95 confidence intervals

a)


Wilks' Lambda =,01819, F(72, 100,66)=2,4751, p=,00001
6E7
5E7
4E7
3E7
2E7
1E7
0
-1E7
-2E7
IV
V
VI
VII
VIII
IX
X

b)


Wilks' Lambda =,01819, F(72, 100,66)=2,4751, p=,00001
5E7
4E7
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0
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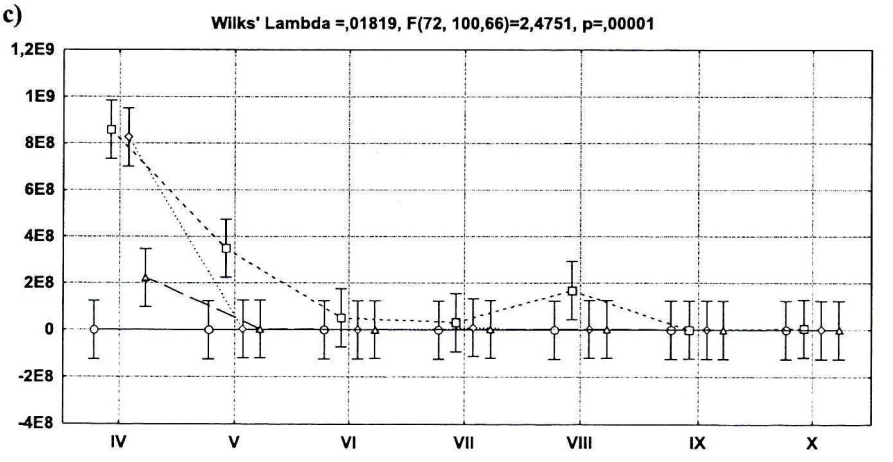
-2E7
IV
V
VI
VII
VIII
IX
X

c)


Wilks' Lambda =,01819, F(72, 100,66)=2,4751, p=,00001
1,2E9
1E9
8E8
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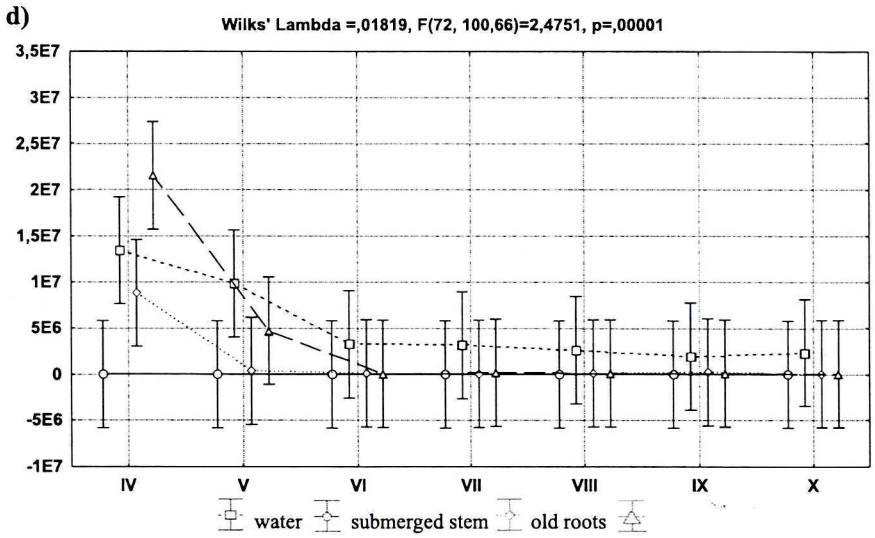



Fig. 2. Expected marginal means (some are not estimated) of a) amylolytic, b) proteolytic, c) caseolytic and d) lipolytic bacteria in different kinds of samples (factor) collected during whole time of study; decomposition of effective hypotheses; vertical columns mean 0.95 confidence intervals

***Number of bacteria in the soil, rizosphere and sedge roots***

In the soil from the root system of sedge, the number of heterotrophic bacteria assayed throughout the whole time of studies ranged within 5 orders of value (from $6.9 \times 10^4$ to $3.7 \times 10^9$ CFU $GDW^{-1}$) (Tab. 1). At both sites they were more numerous in July. Amylolytic ($1.3 \times 10^5 - 2.5 \times 10^7$ CFU $GDW^{-1}$), proteolytic ($2.8 \times 10^2 - 4 \times 10^6$ CFU $GDW^{-1}$), caseolytic ($4.2 \times 10^4 - 2.8 \times 10^8$ CFU $GDW^{-1}$) and lipolytic bacteria ($6.7 \times 10^3 - 3.3 \times 10^7$ CFU $GDW^{-1}$) assayed at both sites were more numerous in spring (Fig. 2). In summer and autumn their number decreased. The differences in the counts of those bacteria at both sites did not exceed 1–2 orders of value.

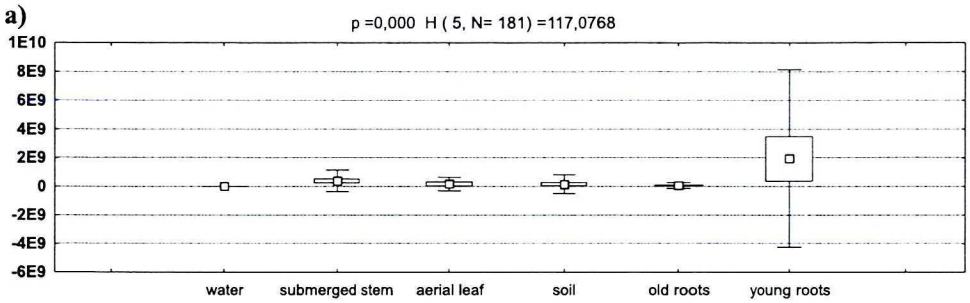
On the surface of old (the previous year's) roots as well as in root system of sedge, the count of heterotrophic bacteria varied from $5.7 \times 10^4$ to $1 \times 10^9$ CFU $GDW^{-1}$ (Tab. 1). The differences in their counts on the surface of old roots between both sites (site 1 and 2) ranged within 1–2 orders of value. They were generally more numerous in July. The numbers of amylolytic, proteolytic, caseolytic and lipolytic bacteria did not exceed respectively $7.5 \times 10^7$, $2.4 \times 10^7$, $9.6 \times 10^8$ and $1.3 \times 10^7$ CFU $GDW^{-1}$ (Tab. 1). Their number was more numerous in spring (Fig. 2).

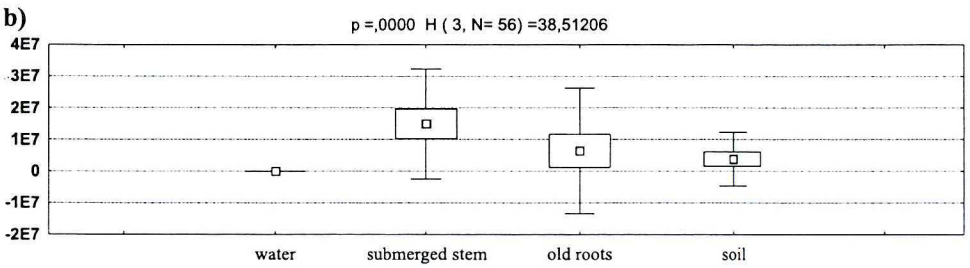
On the surface of new (the present year's) roots, the count of heterotrophic bacteria varied from $2.7 \times 10^4$ to $2.48 \times 10^{10}$ CFU $GDW^{-1}$ (Tab. 1). In annual cycle, they were most numerous in September relatively to the whole period of studies (Fig. 1). Assays of the counts of amylolytic, proteolytic, caseolytic and lipolytic bacteria on new roots were not performed.

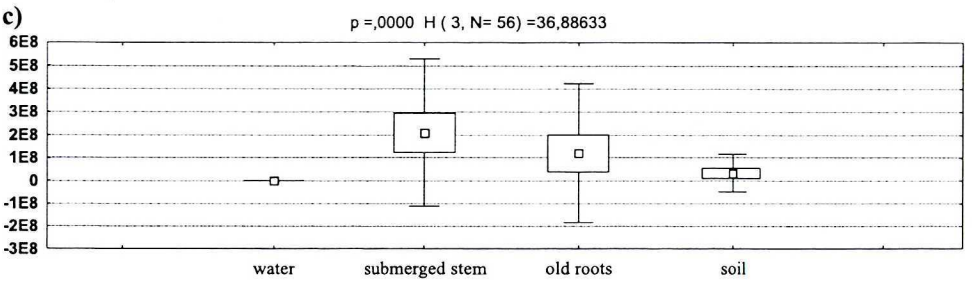
***Differences of studied bacteria numbers in the analyzed samples***

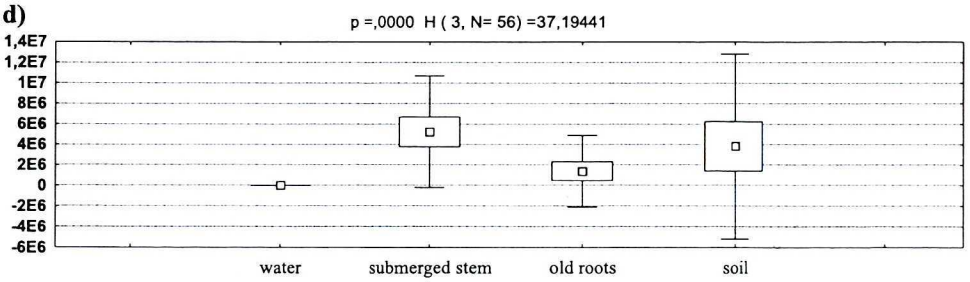
In the particular kind of samples collected during whole time of study, the differences in the counts of studied groups of microorganisms were statistically significant (Fig. 3).

The numbers of heterotrophic bacteria were higher in new roots and submerged stem samples. Amylolytic, proteolytic and lipolytic bacteria were more numerous in submerged stem and old roots, while caseolytic bacteria in submerged stem and soil samples. All groups of determined bacteria were generally less abundant in water.



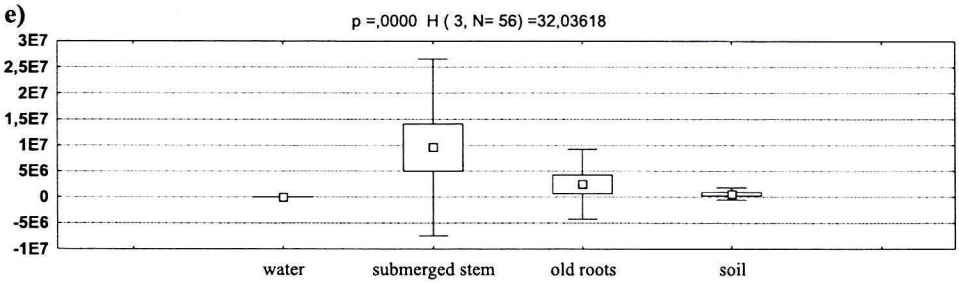



Fig. 3. Average numbers (± standard deviation and ± random mean square-RMS) of a) heterotrophic, b) amylolytic, c) proteolytic, d) caseolytic and e) lipolytic bacteria (CFU $cm^{-3}$/CFU $GWD^{-1}$) in different kinds of samples collected during whole time of study; independent variable (assembling): kind of sample; ANOVA test of Kruskal-Wallis' ranges

## DISCUSSION

The number of heterotrophic bacteria isolated from the surface of submerged parts of sedge and from the soil of the plants' root system in the wetland near the forester's lodge Stary Dwór near Olsztyn determined on TGY medium was comparable with the data reported by Ocevski [11] for the same groups of microorganisms and biotopes of plants like *Phragmites communis* Trin. ex Steudel., *Scirpus lacustris* L., *Polygonum amphibium* L., *Nuphar luteum* L. in the littoral zone of Ohrid Lake, assayed in the spring and summer of 1961. Differences in the counts of those microorganisms determined in the water, periphyton and rhizosphere of sedge between the two years' study may have been due to the weather conditions – the first year was cold and rainy, whereas the second year had a hot summer and little rainfall. Much sunshine recorded in the second year of the study could have restricted the development of heterotrophic bacteria, especially that in water and on the surface of emergent parts of plants. An increase in the number of heterotrophic bacteria from spring to summer could be attributed to increased photosynthesis by sedge and the release of organic forms of carbon to the environment. Increasing counts of heterotrophic bacteria from May to July were also observed by Ocevski [11] in the rhizoplane and rhizosphere of *Phragmites communis*, *Scirpus lacustris*, *Polygonum amphibium* and *Nuphar luteum* growing in the littoral zone of Ohrid Lake in Macedonia. According to that author, it was due to an increase in the water temperature from *ca* 14°C in May to slightly above 22°C in July. In summer, the growth of plants and photosynthesis processes as well as release of oxygen in the root system of plants may have conditioned the rise in the counts of particular heterotrophic bacteria on the surface of plants and in the rhizosphere. Among those epiphytic bacteria, amylolytic ones seem to be essential. Starch is an important storage material in many plants and is found mainly in inland water plants. Being a polysaccharide composed of amylose and amylopectins, it is hydrolyzed by various bacteria and fungi. Maltose (disaccharide), which appears as a product of this hydrolysis, is then hydrolyzed to glucose. Under aerobic conditions, its degradation occurs quite rapidly and is conducted by different species of bacteria which belong to the genera *Pseudomonas* and *Bacillus*, by *Actinomyces* and by higher fungi. Under anaerobic conditions (in bottom sediments), its degradation is actively assisted by saccharolytic

species of bacteria of the genus *Clostridium* [13]. Lalke-Porczyk and Donderski [7] reported that saccharolytic bacteria could constitute up to 50% of populations of heterotrophic bacteria in the root system of the common reed (*Phragmites australis* (Cav.) Trin. ex Steudel). The same authors claimed in another paper that 10–71% of epiphytic bacteria and 23–42% of bacterioplankton assayed in Moty Bay of Jeziorak Lake were capable of hydrolyzing starch [6, 7]. Those bacteria occurred in highest numbers on the surface of common reed and reed-mace. The authors point to the seasonal fluctuation of this characteristic among heterotrophic bacteria – those bacteria were more numerous in spring and autumn (epiphytic bacteria) or in spring and summer (bacterioplankton). Results of our studies suggest a rather small contribution (0.2–28.4%) of this type of bacteria in the wetland water compared to the count of heterotrophic bacteria determined on TGY medium. However, their percentage on the surface of submerged fragments of sedge and in the roots of this plant was much higher. As in the study conducted by Lalke-Porczyk and Donderski [7], amylolytic bacterioplankton (in water) occurred in higher numbers in spring and summer while epiphytic bacteria (on the surface of sedge and in its roots) were more numerous in spring and autumn.

The presence of considerable numbers of proteolytic bacteria in water and submerged parts of sedge plants and in the root system and rhizosphere of those plants investigated in the wetland near Olsztyn can be a proof of intensive processes of mineralization of organic nitrogen compounds in that region, especially in spring, when those bacteria reached the peak counts. Most of those bacteria belong to typical psychrophilic organisms and low water temperatures in early spring do not limit their growth and development. The main limiting factor as regards their multiplication and development is the availability of organic nitrogen compounds. And those seem to have occurred in sufficient amounts in the wetland, especially after the wintertime, when many plant and animal organisms in the forest catchment basins had died. It is likely that during the spring thaws many of those dead organisms reach the wetland with runoff waters from the forested hills around; some can also be of endogenous origin.

Lipolytic bacteria play an important role in the biodegradation of organic substance in water reservoirs. However, in the water of the wetland we analyzed, they made up a rather small proportion of the total heterotrophic bacteria (0.04–15.1%). Mudryk and Skórczewski [10] confirmed lipolytic ability of 88% of heterotrophic bacteria isolated from the water of Gardno Lake; Lalke-Porczyk [6] reports that 60–96% of bacterioplankton isolated from Moty Bay of Jeziorak Lake showed high ability to decompose lipids. Differences in the percentage of lipolytic bacteria determined in the water of the wetland analyzed by us as well as those reported by other authors may be due not only to the different types of the water bodies studied but also, and perhaps mainly, to the different techniques applied for counting bacteria. In the present paper, the count of heterotrophic bacteria was determined on a medium with tryptone, glucose and yeast extract diluted to 1:8 original Difco medium [14]; the number of lipolytic bacteria was counted on the same medium with tributyrine, by counting the colonies which decomposed tributyrine. The authors mentioned earlier in our article determined the count of heterotrophic bacteria on an iron-peptone medium according to Ferrer *et al.* [9]; the percentage of lipolytic bacteria on the same medium with addition of tributyrine or on a CPS medium according to Jones, with addition of tributyrine [10]. Such differences in the methods applied (different media) could explain (next to some other factors) large differences in the percent contribution

of lipolytic bacteria to the whole population of heterotrophic bacteria isolated from the phyllosphere and rhizosphere of sedge assayed in our study versus the phyllosphere and rhizosphere of other plants analyzed by Lalke-Porczyk and Donderski [9] in water of Moty Bay of Jeziorak Lake.

## CONCLUSIONS

Differences in the number of heterotrophic bacteria and some other physiological groups of those microorganisms (amylolytic, proteolytic, caseolytic and lipolytic) in the analyzed water of the wetland during two consecutive years could have also been caused by the differences in the weather conditions, such as temperature, sunshine, developmental stages and death of plant organisms (phytoplankton) and animals, increased or/and decreased photosynthesis carried out by higher plants.

Fluctuations in the counts of heterotrophic bacteria which were epiphytes on submerged parts and aerial leaf of sedge or in the soil and rhizosphere of this plant may have been due to changes in the intensity of photosynthesis, which in turn entailed varied release of biologically active substances such as amino acids, carbohydrates or oxygen in the root system. The latter may have resulted in a higher count of heterotrophic bacteria on live roots of sedge. Periodically determined counts of heterotrophic bacteria within $10^9$–$10^{10}$ and more as well as considerably large amounts of amylolytic bacteria, which decomposed proteins, and lipolytic bacteria on submerged parts of mud sedge and in the soil of the root system and on roots of this plant suggest an important role of this microflora in the cycle of carbon and nitrogen in the wetland covered by our study. Mineralization of organic contents produced in situ or delivered from outside sources during spring thaws and/or atmospheric precipitation occur mostly at the borderline of water and plant phases.

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