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Distribution of Epiphytic Bacteria on the Surface of Selected Species of Helophytes and Nimpheides from the Littoral Zone of the Southern Part of Jeziorak Lake in Poland

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Abstract

Research was carried out on the total number of bacteria and the number of heterotrophic epiphytic bacteria occurring on the surface of four selected species of freshwater macrophytes predominant in the littoral zone of the southern part of Lake Jeziorak. The highest numbers of bacteria inhabiting the studied plants were found on the surface of the stems of lesser reedmace, and the lowest numbers on the surface of leaves of floating pond-weed. It was shown that the number of epiphytic bacteria depends on the species of plant, which fragment of it, and the season in which the research is conducted. The results obtained are illustrated with photographs taken under a scanning electron microscope.

Keywords: heterotrophic bacteria, epiphytic bacteria, macrophytes.

Introduction

One of the most significant groups of microorganisms in water bodies, as far as numbers and importance are concerned, are bacteria inhabiting permanent abiotic and living substrates. They abundantly inhabit the surface of upper plants as well as sea and freshwater algae. Chan and McManus [4] state that the number of bacteria occurring on *Ascophyllum nodosum* algae and *Polysiphonia lanosa* algae is from 10^4 to 10^7 cells calculated per 1 g of dry mass of algae. Ramsay and Fry [16], in research on the number of bacteria on *Elodea canadensis* and *Chara vulgaris*, found that the numbers of bacteria on the mature, well-developed leaves of these plants was 1 - $10 \cdot 10^5$ and 15-$30 \cdot 10^5$ cells, respectively, per cm^2 surface of leaf. The above data concern the number of bacteria determined using the indirect method (culture - seedings on plates). Using the direct method of counting bacteria, Baker and Orr [2] found that the total number of epiphytic bacteria on the surface of *Ranunculus penicillatus* and *Veronica baccabunga* plants was higher, being 0.07 - $1.80 \cdot 10^7 \cdot cm^{-2}$ surface area and 0.03 - $1.50 \cdot 10^7 \cdot cm^{-2}$ surface area, respectively.

In the present paper, by determining the total number of bacteria and the number of heterotrophic epiphytic bacteria, the authors have tried to answer the following questions:

1. Is there a dependence between the number of epiphytic bacteria and individual species of macrophytes?
2. Can particular parts of plants significantly influence the development and number of bacteria?

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Materials and Methods

Study Area

Microbiological research was conducted in Moty Bay, situated in the southwestern part of Jeziorak Lake. This lake is located in the northeastern part of Poland within the Iława Lake District, and is part of the Drwęca-Vistula catchment area. It is a channel-type water body, oriented meridionally, formed during the last glaciation. Its 32.3 km^2 surface area makes it the sixth largest lake in Poland. The lake's maximum length is 27.45 km, and width 2.35 km. The average depth of the lake is about 4.3 m. Lake Jeziorak's shoreline is well-developed (with a factor of 6.6), with many bays, including the strongly eutrophic Moty Bay. The western shore of Jeziorak Lake is surrounded by a mixed pine-beech and deciduous forest, while the eastern shore, along the section lying 13 km to the north of Iława, borders on meadows and cultivated fields, and further away on a coniferous and mixed forest [3]. Jeziorak Lake is categorized as a eutrophic water body. Its water is yellowish green, its transparency is relatively poor, and its pH is alkaline.

Sampling

Hydromacrophytes from the group of helophytes and nympheids, predominant in the littoral zone of Moty Bay in Lake Jeziorak, were used for the studies. The helophytes studied included the common reed (*Phragmites australis* (Cav.) Trin. ex Steudel) and lesser reedmace (*Typha angustifolia L.*), and the nympheids included the yellow water-lily (*Nuphar luteum L.*) and floating pond-weed (*Potamogeton natans L.*). Common reed, lesser reedmace and yellow water-lily were collected from sites along the eastern shore of Moty Bay, while floating pond-weed was taken from the western shore (Fig. 1).

Fifteen-centimetre-long sections from the shoots of the common reed and lesser reedmace situated below the surface of the water and in the near-bottom layer (measuring from the rhizome) were taken for microbiological research, as well as whole leaf blades from floating pond-weed and yellow water-lily.

Plant material was put into sterile glass jars and transported to the laboratory, packed in ice to keep the temperature below +7°C. Not more than 6 hours passed between taking the samples and conducting the analyses.

Material for study was collected in 1997-2000, each time in spring, during the period of intensive growth of the young shoots; in summer, when the plants were flowering; and in autumn, when the plants were dying.

At the same time the samples were taken for microbiological tests, some physico-chemical properties of the water were determined *in situ*: temperature and oxygen content (determined using an oxygen meter DO_2 - Meter, Jenway - U.K., type 9071), water transparency (using a Secchi disc), the water factor (using an electron pH-meter N-5122 Mera-Elwro), light intensity in the water at a depth of about 20 and 100 cm (using a luxometer LX-204 Slandi) and electrolytic conductivity of the water (using a conductometer CM-204 Slandi).

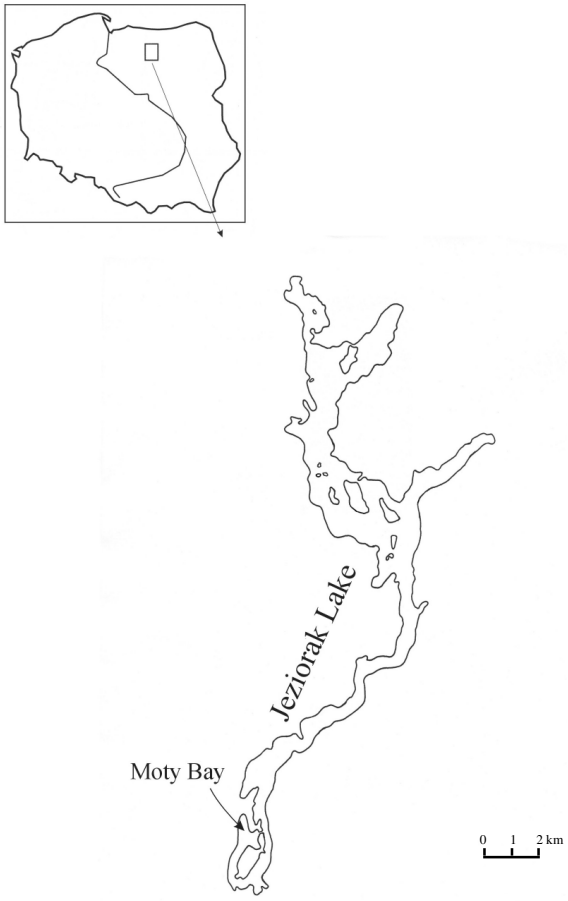

Fig. 1. Area of investigation in the southwestern part of Jeziorak Lake.

Microscopic Observation of Bacteria Inhabiting the Surface of Plants

The occurrence of bacteria on the surface of plants was observed using a scanning electron microscope (Digital Scanning Microscope - DSM 940, Zeiss Jena). The plant material for observation was prepared according to a modified version of Baker's method [1]. Fresh plant fragments were cut into approximately 1 cm^2 pieces and preserved for 24 hours in 4% glutaraldehyde solution prepared in 0.1 M phosphate buffer with a pH of 7.2. Next these fragments were rinsed 4 times for 15 minutes in buffer solution and dehydrated in an ethanol series (10%, 25%, 50%, 75% and 90% for 20 minutes), after which they were left for 10-15 hours in 100% ethyl alcohol and dried to the critical point using Critical Point Dryer 010 (Balzers Union Ltd). The dried plant fragments were stuck to aluminium tripods and dusted with gold (Sputter Anlage für REM - Proben - SCD 030, Balzers Union Ltd). The finished preparations were observed with a scanning microscope, paying attention to the distribution of bacteria on the plants and their morphological differentiation.

Determination of the Total Number of Epiphytic Bacteria (TNB)

In order to determine the total number of epiphytic bacteria, 10.0 g of fresh mass from the epidermis of the common reed and lesser reedmace was collected. This material was covered with 90 cm^3 of sterile buffer water [6] and blended for 2 minutes in a homogeniser (Unipan type 392) at 4000 revolutions per minute. 5.0 g of the epidermis from the upper and lower surface of the leaves of floating pond-weed and yellow water-lily was taken with a sterile scalpel and homogenised as above in 45 cm^3 of sterile buffer water. 10-millilitre portions of plant homogenates were diluted at a ratio of 1:105, using buffer water as a dilutant [6], were preserved in formalin (the final concentration of formaldehyde in the samples was 0.7%) and were filtered initially through a 50 µm diameter nylon mesh in order to eliminate fragments of plant tissue (displaying autofluorescence). The total number of bacteria in the homogenates obtained was determined by directly counting bacteria on the membrane filters (AODC - acridine orange direct count) according to Zimmermann and Meyer-Reil [21] and the modification made by Zimmermann [20]. The results were calculated per 1 g of dry plant mass and 1 cm^2 of plant surface.

Determination of the Number of Heterotrophic Epiphytic Bacteria (CFU)

In order to determine the number of heterotrophic epiphytic bacteria (CFU - colony forming units), plant homogenates used as before were diluted ten times in sterile buffer water (most often at the ratio of $1:10^6$) and seeded on the surface of iron-peptone agar (IPA) according to Ferrer et al. [7]. All the seedings were prepared in five parallel replicates. The plates with the seedings were incubated for 10 days at temperature *in situ*. The results were calculated per 1.0 g of dry plant mass and per 1 cm^2 of plant surface. The surface area of the stem fragments was calculated according to the equation $P = 2\pi r \cdot h$, where $\pi = 3.14$, r is the average radius of the stems, and h is the average length of the stem fragments taken for research. The surface area of the leaves was measured by a pole planimeter (type PL1, PZO Warsaw).

Statistical Analysis of the Results

The results of the experiments were compiled statistically using "Statistics for Windows" version 5.1, 1996, StatSoft, Tulsa, Oklahoma, U.S.A. The significance of the differences between the number of bacteria occurring on different fragments of the same plant was estimated using Student test (t-test). The tests were estimated at a level of significance of $p \leq 0.05$. The influence of the fragment of the plant or the surface of the leaf (1), the species of plant (2), and the season (3) on the number of bacteria (data logarithmically transformed) was estimated using 3-way ANOVA. Average values were compared using the Newman-Keul multi-interval test.

Results

Physico-chemical Properties of the Water of Moty Bay in Jeziorak Lake

The changes of some physico-chemical parameters of the water of Moty Bay in Jeziorak Lake between May 1997 and October 2000 are presented in Table 1.

Temperature measurements showed that differences between the temperature of surface water and that at a depth of 1 metre in the littoral zone and pelagic zone were insignificant. A slightly higher water temperature was usually observed in the littoral zone. The highest water temperature was found in the surface layer of the littoral during summer, and the lowest in the same layer of the littoral zone in autumn.

Water transparency was measured with a Secchi disc and varied between 0.45 and 1.20 m during the study periods. On average, the greatest water transparency was observed in autumn, and the lowest in spring.

It follows from experiments on the content of oxygen dissolved in the water that its average concentration was generally higher in spring and autumn than in summer. The lowest oxygen content in the water was observed in summer in the surface layer of the littoral zone, and the maximum was in spring in the same water zone.

The average oxygen saturation of water in the study periods varied from 91.3% to 122.9%, and was always higher in spring and summer than in autumn. This pattern was observed in both the littoral and pelagic zones.

The electrolytic conductivity of the water in Moty Bay ranged from 319 to 420 $\mu S \cdot cm^{-1}$ during the study period. On average the lowest values of conductivity were observed in autumn, both in the waters of the pelagic zone and of the littoral zone. As a rule, water at a depth of 1 metre displayed greater electrolytic conductivity than surface water.

It follows from studies on the intensity of light in the water of Moty Bay that in surface water it was always at its highest in summer and at its lowest in autumn. Light reaching a depth of 1 metre in spring and summer was on average about 20 - 21 % of the intensity of light reaching a depth of 20 cm, while in autumn it was about 36 %.

The pH of the water of Moty Bay in Lake Jeziorak is alkaline. During the study period the pH value varied from 7.8 to 8.7.

Microscope Observation of Epiphytic Bacteria

From the many photographs taken during the studies on the distribution of epiphytic bacteria on the surface of macrophytes, the most characteristic (typical) for a given group of plants were selected. Lesser reedmace is used as an example to show how the surfaces of helophytes are inhabited. A small number of bacterial cells are visible on young fragments of this plant in spring (photo 1 a). In summer (photo 1 b) and autumn (photo 1 c) a significant increase in the number of bacteria on the surface of the

plants was observed, with the bacterial cells tending to accumulate in depressions of the plant epidermis (photo 1 b).

Bacteria cells were distributed very unevenly on the leaves of nympheids. There were few bacteria dwelling on the upper surfaces of leaves, and they tended to occur near stomata (photo 2 a). On the lower surface of the leaves of the yellow water-lily, bacteria formed distinct concentrations near the abundant secretory hairs growing there (photo 2 b). The lower surfaces of the leaves of floating pond-weed were inhabited more evenly by bacteria than the lower surfaces of the leaves of the yellow water-lily. Particularly high populations of bacteria were observed on the leaves of floating pond-weed in autumn (photo 2 c).

Total Number of Epiphytic Bacteria (TNB)

The results of the research on the total number of epiphytic bacteria are presented in Table 2. It follows that the highest numbr of bacteria occurred on the surfaces of the stems of lesser reedmace. The average number of bacteria on this plant varied from $1.03 \cdot 10^{12}$ to $2.72 \cdot 10^{13}$ cells per g of dry plant mass, which corresponds to $1.55 \cdot 10^{9}$ - $3.90 \cdot 10^{10}$ cells per cm^2 of stem surface. It also follows that the total number of bacteria covering lesser reedmace grew from spring, reaching the maximum level in summer and decreasing in autumn. This phenomenon was observed on the upper and lower fragments of the plants. In spring and summer the near-bottom sections of the stems were inhabited by more bacteria calculated per g of dry plant mass than sections in the surface water layer. In autumn the opposite phenomenon was observed. The difference in the number of bacteria growing on the upper and lower sections of the plants during this period was statistically significant. The number of bacteria calculated per cm^2 of surface area proved to be higher in spring on the near-bottom sections of the plants, while in summer and autumn on the sections in the surface water layer.

The total number of bacteria occurring on the stems of the common reed also increased between spring and summer and decreased in autumn. However, it was on average about 18 to above 300 times lower than on the stems of lesser reedmace. The near-bottom sections of the stems were generally covered with more bacteria than the upper sections, with the exception of young plants collected in spring.

The total number of bacteria on the floating leaves of the yellow water-lily was on average between $1.50 \cdot 10^{9}$ and $1.18 \cdot 10^{12}$ cells per g of dry plant mass and between $1.30 \cdot 10^{5}$ and $8.80 \cdot 10^{7}$ cells per cm^2 of leaf surface area, and it was always higher on the lower side of the leaf blades than on the upper side. The differences in the number of bacteria growing on the upper and lower surfaces of the leaves were statistically significant ($p < 0.001$).

The total number of bacteria on the leaves of floating pond-weed formed a slightly different picture. During the period between spring and summer a significant fall in the number of bacterial cells was observed each time on this plant, both on the upper and on the lower surface of the leaves, while in the period from summer to autumn there

Table 1. Some physico-chemical properties of the water in Moty Bay in Jeziorak Lake (average values from 1997-2000).

Parameter	Site	Season		
		Spring	Summer	Autumn
Temperature[°C]	L_s	19.2 (16.5 - 24.7)	23.4 (21.4-25.9)	9.6 (7.0-12.2)
	L_{1m}	18.9 (16.2 - 24.4)	23.0 (20.6-25.7)	10.2 (8.1-12.4)
	P_s	18.4 (16.0 - 23.1)	22.9 (20.5-25.4)	9.9 (7.9-12.1)
	P_{1m}	18.2 (15.8 - 23.0)	22.3 (20.0-25.0)	9.9 (7.9-12.0)
Transparence[m]	L, P	0.61 (0.45 - 0.90)	0.74 (0.65-0.80)	0.98 (0.85-1.20)
Oxygen content [mg · dm^{-3}]	L_s	11.4 (10.3 - 12.6)	10.1 (8.3-12.2)	10.7 (9.7-11.4)
	L_{1m}	10.5 (9.6 - 11.5)	10.0 (8.9-12.4)	10.3 (9.6-11.4)
	P_s	9.9 (9.1 - 10.9)	10.0 (8.8-11.8)	10.2 (9.8-11.00)
	P_{1m}	11.0 (10.3 - 12.2)	9.9 (8.8-11.0)	10.4 (9.9-11.2)
Oxygen saturation of water [%]	L_s	122.9 (105.7 - 131.3)	118.1 (94.0-145.6)	94.9 (88.7-102.9)
	L_{1m}	110.7 (96.5 - 120.5)	116.4 (99.0-148.0)	92.5 (88.3-102.9)
	P_p	104.0 (94.5 - 114.3)	114.8 (101.2-140.8)	91.3 (87.4-99.3)
	P_{1m}	116.2 (103.5 - 125.6)	112.2 (101.4-126.7)	92.6 (88.3-101.1)
Electrolytic conductivity [µS · cm^{-1}]	L_s	359.8 (319 - 399)	363.8 (329-401)	335.3 (322-341)
	L_{1m}	371.8 (341 - 400)	368.5 (353-387)	359.8 (326-420)
	P_s	353.0 (327 - 398)	364.8 (336-391)	344.8 (335-356)
	P_{1m}	352.8 (325 - 405)	367.8 (338-388)	346.8 (320-370)
Light intensity [klx]	L_s, P_s	25.3 (10.5 - 64.7)	57.3 (45.2-74.1)	7.7 (6.6-8.6)
	L_{1m} ,P_{1m}	5.2 (1.1 - 15.0)	11.6 (6.2-14.5)	2.8 (1.5-4.5)
pH factor	L, P	8.3 (7.8 - 8.5)	8.4 (8.2-8.7)	8.2 (7.5-8.7)

Explanation: L - littoral; P - pelagial; L_s - surface water layer in the littoral zone; P_s - surface water layer in the pelagial zone; L_{1m} - water at a depth of 1 m in the littoral zone; P_{1m} - water at a depth of 1 m in the pelagial zone; the range of variability is given in parentheses.

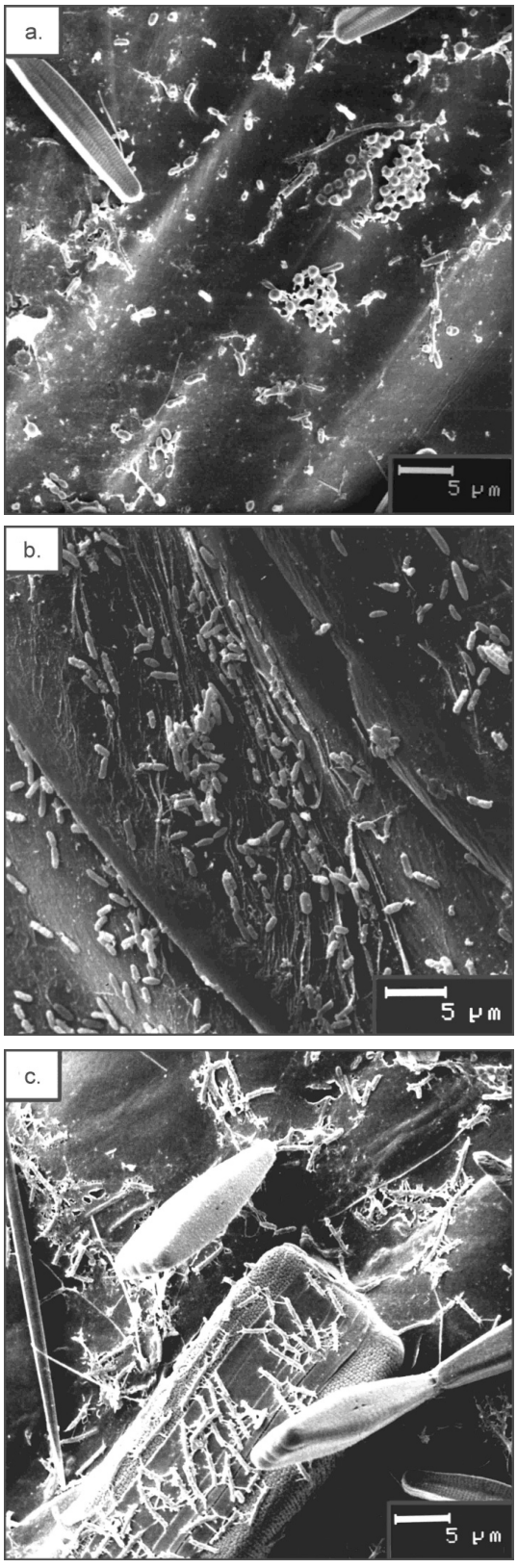

Photo 1. Bacteria on the surface of shoots of *T. angustifolia* in different seasons (SEM): a- spherical and cylindrical forms of bacteria and *Cymbella* type diatoma in spring (mag. 2000x); b - epiphytic microflora in summer - concentration of bacteria visible in depressions of the epidermis (mag. 2000x); c - cylindrical forms of bacteria and diatoma of the types *Fragilaria* and *Gomphonema* in autumn (mag. 2000x).

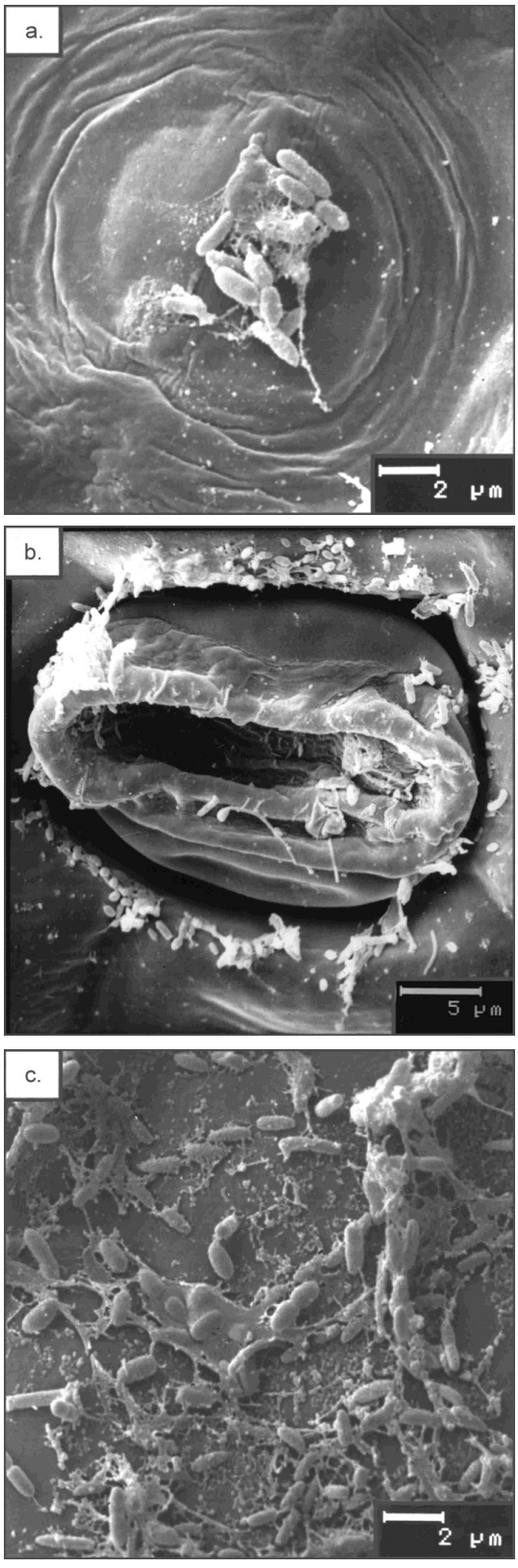

Photo 2. Distribution of bacteria on the leaves of nympheids (SEM): a - concentration of bacterial cells near stomata on the upper surface of a *N. luteum* leaf (mag. 5000x); b - accumulation of cells near secretory hairs on a leaf of *N. luteum* (mag. 3000x); c - bacterial cells and glycocalix produced by them on the lower surface of a leaf of *P. natans* (mag. 5000x).

was a distinct increase. As in the case of the yellow water-lily, the lower surfaces of the leaves were more heavily colonised by bacteria than the upper surfaces, and these differences were statistically significant.

As follows from the 3-way ANOVA (Table 3), the species of plant, its fragment, and the time of year in which material was collected for research all had a statistically significant influence on the total number of epiphytic bacteria inhabiting all of the macrophytes under investigation. It follows from statistical analysis that the species of plant had the greatest influence [$F_{(3,288)}$ 3104.02 ($p<0.0001$)] along with the time of year [$F_{(8,288)}$ 574.86 ($p<0.0001$)]. The studied section of the plant or the surface of the leaf from which material was taken for study had less influence on the number of bacteria [$F_{(1,288)}$ 416.35 ($p<0.0001$)]. It was found that statistically significantly more bacteria occurred on the lower (near-bottom) fragments of plants and on the lower surfaces of leaves (average $\log_{10}$ of the total number of bacteria = 12.94) than on the upper fragments and surfaces of leaves (average $\log_{10}$ of the total number of bacteria = 12.62). The most epiphytic bacteria occurred on the surface of lesser reedmace, and the least on the surface of the leaves of floating pond-weed and the yellow water-lily. The average log10 of the total number of epiphytic bacteria for the species of plants under investigation decreased in the following order: lesser reedmace - 14.06 > common reed - 12.56 > yellow water-lily - 12.31 > floating pond-weed - 12.19. Epiphytic bacteria occurred in the greatest numbers on the surfaces of all the studied plants in the summer and autumn of 1998 (average $\log_{10}$ of the total number of bacteria was, respectively, 13.79 and 13.26). Statistically the least bacteria occurred on the surfaces of plants in the spring of 1999 (average $\log_{10}$ of the total number of bacteria =11.94).

Table 2. Total number of epiphytic bacteria (TNB) of macrophytes in Moty Bay.

Season	TNB	Species and fragment of plant							
		CR_u	CR_l	LR_u	LR_l	WL_u	WL_l	PW_u	PW_l
Spring 1998	TNB·10^{10}·g^{-1}dry plant mass	8.13	** 5.01	249.11	- 297.12	0.32	- 0.58	6.30	- 8.92
	TNB·10^{6}·cm^{-2}	66.02	** 39.15	3739.16	** 5578.70	0.27	- 0.49	5.62	- 7.96
Summer 1998	TNB·10^{10}·g^{-1}dry plant mass	12.62	*** 40.73	4861.12	- 7344.10	71.63	*** 281.20	0.54	** 1.90
	TNB·10^{6}·cm^{-2}	160.92	*** 1337.36	165842.27	- 105150.42	53.56	*** 210.24	0.30	** 1.06
Autumn 1998	TNB·10^{10}·g^{-1}dry plant mass	1.44	*** 4.55	658.47	** 433.15	1.19	*** 8.03	22.85	- 36.85
	TNB·10^{6}·cm^{-2}	115.88	- 119.41	21209.04	*** 8182.92	1.53	*** 10.30	53.76	- 86.70
Spring 1999	TNB·10^{10}·g^{-1}dry plant mass	0.80	- 0.47	41.10	- 50.90	0.03	*** 0.10	0.30	** 0.85
	TNB·10^{6}·cm^{-2}	6.50	- 3.67	616.91	* 955.69	0.02	*** 0.08	0.27	** 0.76
Summer 1999	TNB·10^{10}·g^{-1}dry plant mass	3.71	* 5.26	433.19	** 700.96	8.56	*** 57.55	0.02	*** 0.19
	TNB·10^{6}·cm^{-2}	47.31	*** 172.71	14778.78	** 10036.12	6.40	*** 43.03	0.01	*** 0.10
Autumn 1999	TNB·10^{10}·g^{-1}dry plant mass	0.15	** 0.34	39.45	* 25.26	5.17	*** 26.17	2.01	** 4.86
	TNB·10^{6}·cm^{-2}	12.07	- 8.92	1270.67	*** 477.20	6.63	*** 33.57	4.73	** 11.44
Spring 2000	TNB·10^{10}·g^{-1}dry plant mass	8.32	- 7.94	18.66	- 20.61	0.110.09	*** 0.43	3.25	- 4.65
	TNB·10^{6}·cm^{-2}	67.56	- 62.05	280.09	** 386.97		*** 0.36	2.90	- 4.15
Summer 2000	TNB·10^{10}·g^{-1}dry plant mass	30.65	- 33.14	98.01	- 122.55	1.98	*** 14.16	0.13	*** 1.57
	TNB·10^{6}·cm^{-2}	390.83	*** 1088.15	3343.72	** 1754.63	1.48	*** 10.59	0.07	*** 0.87
Autumn 2000	TNB·10^{10}·g^{-1}dry plant mass	2.23	* 3.02	3.46	*** 1.93	0.16	*** 0.70	1.72	** 3.14
	TNB·10^{6}·cm^{-2}	179.45	*** 79.25	111.44	*** 36.46	0.20	*** 0.89	4.05	** 7.39
3 - year average:									
Spring	TNB·10^{10}·g^{-1}dry plant mass	5.75	4.47	102.96	122.88	0.15	0.37	3.28	4.81
	TNB·10^{6}·cm^{-2}	46.69	34.96	1545.39	2307.12	0.13	0.311	2.93	4.29
Summer	TNB·10^{10}·g^{-1}dry plant mass	15.66	26.38	1797.44	2722.54	27.39	17.64	0.23	1.22
	TNB·10^{6}·cm^{-2}	199.69	866.07	61321.59	38980.39	20.48	87.95	0.13	0.68
Autumn	TNB·10^{10}·g^{-1}dry plant mass	1.27	2.64	233.79	153.45	2.17	11.63	8.86	14.95
	TNB·10^{6}·cm^{-2}	102.47	69.19	7530.38	2898.86	2.79	14.92	20.85	35.18

Explanation: Species and fragment of plant: CR_u - upper sections of the common reed; CR_l - lower sections of the common reed; LR_u - upper sections of lesser reedmace; LR_l - lower sections of lesser reedmace; WL_u - upper surfaces of leaves of yellow water-lily; WL_l - lower surfaces of leaves of yellow water-lily; PW_u - upper surfaces of leaves of floating pond-weed; PW_l - lower surfaces of leaves of floating pond-weed; comparison of the number of bacteria on upper and lower fragments of plants using the Student test:
* significance level $0.01<P<0.05$, ** significance level $0.001<P<0.01$, *** significance level $P<0.001$ - difference insignificant statistically.

Number of Heterotrophic Epiphytic Bacteria (CFU)

The results of the research on the number of heterotrophic epiphytic bacteria are presented in Table 4. It follows from them that the number of these bacteria varied and depended on the species of plant, the studied part and the time of year. The most heterotrophic bacteria were found on the surface of the stems of lesser reedmace, and the least on the surface of the leaves of floating pond-weed.

On the stems of the common reed, the number of heterotrophic bacteria increased from spring to summer and decreased in autumn. The near-bottom sections of the stems were colonised by a greater number of bacteria (on average from $3.79 \cdot 10^7$ to $1.13 \cdot 10^9$ cells $\cdot g^{-1}$ of dry plant mass) than the sections in the surface water layer (on average from $9.10 \cdot 10^6$ to $4.95 \cdot 10^8$ cells $\cdot g^{-1}$ of dry plant mass). It follows from the results of the Student test that the differences in the number of bacteria on both fragments of the plant were statistically significant, with the exception of the results obtained in autumn concerning the number of bacteria calculated per cm^2 of plant surface area (Table 4).

On the surface of the stems of lesser reedmace, the number of heterotrophic bacteria also increased from spring to summer, after which it fell significantly. In spring and summer more bacteria occurred on the sections of the stems growing in near-bottom water (on average from $3.81 \cdot 10^9$ to $5.87 \cdot 10^9$ cells $\cdot g^{-1}$ of dry plant mass), whereas in autumn

Table 3. 3-way ANOVA test comparing the influence of the fragment of the plant or the surface of the leaf (1), species of plant (2) and the season (3) on the total number of epiphytic bacteria calculated per 1 g of dry plant mass.

Factor	Variance	df @	Parameter F	p (significance)
Fragment of plant or leaf surface (+)	9.0246	1	416.3490	< 0.0001
Plant species (+)	67.28164	3	3104.0210	< 0.0001
Season (+)	12.4605	8	574.8610	< 0.0001
Fragment of plant or leaf surface * plant species (+)	1.9600	3	90.4230	< 0.0001
Fragment of plant or leaf surface * season (+)	0.0973	8	12.7760	< 0.0001
Plant species * season (+)	0.2769	24	317.1120	< 0.0001
Fragment of plant or leaf surface * plant species * season (+)	6.8736	24	6.1200	< 0.0001
Intragroup variability	0.0217	288	-----	-----

Comparison of averages (Newman - Keul test of multiple differences, $p \leq 0.05$)

1. Fragment of plant or leaf surface

upper	lower
12.6210 a	12.9376 b

2. Plant species

common reed	lesser reedmace	yellow water - lily	floating pond - weed
12.5587 c	14.0559 d	12.3100 b	12.1926 a

3. Season

spring 1998	summer 1998	autumn 1998
12.9044 e	13.7935 g	13.2581 f
spring 1999	summer 1999	autumn 1999
11.9402 a	12.8663 e	12.5904 d
spring 2000	summer 2000	autumn 2000
12.5215 c	12.9677 e	12.1716 b

Explanation: @ - degree of freedom number; (+) - factor having a significant influence ($p \leq 0.05$); a, b, ...g - statistically homogeneous group of averages ordered from the lowest (a) to the highest (g), average values denoted with the same letter are not statistically different ($p \leq 0.05$)

in surface water (on average from $1.28 \cdot 10^9$ cells $\cdot g^{-1}$ of dry plant mass). It was observed, moreover, that a tendency for the number of bacteria to increase per g of dry plant mass did not always correlate to an increase in the number of bacteria calculated per unit of surface area. This phenomenon was noted in the summer of 1997, when the number of bacteria calculated per g of dry plant mass on the near-bottom sections of lesser reedmace increased relative to the spring sample, but fell when calculated per cm^2 of surface area. The differences in the number of bacteria between the sections growing in the surface and near-bottom water layers were generally highly statistically significant.

The number of heterotrophic bacteria on the surface of the leaves of the yellow water-lily was on average from $2.70 \cdot 10^6$ to $1.04 \cdot 10^9$ cells per g of dry plant mass on the upper surfaces, and from $4.70 \cdot 10^6$ to $2.92 \cdot 10^9$ cells per g of dry plant mass on lower surfaces in direct contact with the water surface. The number of bacteria on the upper and lower surfaces of the leaf blades increased several times between spring and summer, and then decreased significantly in autumn. The number of heterotrophic bacteria on the lower surfaces of the leaves was higher than on the upper surfaces in each of the studied seasons. These differences were statistically significant, with the exception of young leaves collected in early spring, where the differences in the number of bacteria calculated per g of dry plant mass and cm^2 of plant surface were statistically insignificant.

The number of heterotrophic bacteria on the leaves of floating pond-weed was at its lowest in spring, as on the surfaces of other plants. During the period from spring the number of bacteria increased, reaching its maximum in autumn. This rule applied to the upper and lower surfaces of the leaves. It follows from the research that, as for the yellow water-lily, significant differences occurred in the number of bateria inhabiting the upper and lower surfaces of leaves. The upper surfaces were less well inhabited by bacteria than the lower leaves, and these differences were statistically significant.

The results of the 3-way ANOVA (Table 5), concerning the influence of the fragment of the plant or the surface of the leaf, the species of plant and the season on the number of heterotrophic bacteria calculated per g of dry plant mass, show that the number of heterotrophic epiphytic bacteria inhabiting the surface of the plants being studied (the common reed, lesser reedmace, the yellow water-lily and floating pond-weed) was very much dependent on all of these factors. Statistically significantly more bacteria occurred on the sections of plants growing in near-bottom water and on the lower surfaces of leaves (average $\log_{10}$ of the number of bacteria = 9.25) than on those growing in surface water (average $\log_{10}$ of the number of bacteria = 8.96). It follows from these data that statistically significantly the most bacteria occurred on the surface of lesser reedmace (average $\log_{10}$ of the number of bacteria = 10.00), and the least on the surface of the yellow water-lily (average $\log_{10}$ of the number of bacteria = 8.77).

Table 4. Number of heterotrophic epiphytic bacteria (CFU) of macrophytes in Moty Bay.

Season	CFU	Species and fragment of plant											
		CR_u		CR_l	LR_u		LR_l	WL_u		WL_l	PW_u		PW_l
Spring 1997	CFU·10⁷· g⁻¹ dry plant mass	1.73	*	2.71	313.66	***	715.00	0.13	-	0.21	0.94	***	4.95
	CFU·10⁴·cm⁻²	1.40	*	2.12	470.81	***	1342.48	0.01	-	0.02	0.08	***	0.44
Summer 1997	CFU·10⁷· g⁻¹ dry plant mass	9.35	**	54.65	631.76	*	806.54	122.11	***	428.10	2.47	***	7.17
	CFU·10⁴·cm⁻²	11.92	***	179.44	2155.32	***	1154.78	9.13	***	32.01	0.14	***	0.40
Autumn 1997	CFU·10⁷· g⁻¹ dry plant mass	1.56	**	6.47	252.67	**	124.63	4.18	**	10.12	114.92	*	212.30
	CFU·10⁴·cm⁻²	12.55	-	16.98	813.84	**	235.45	0.54	**	1.30	27.04	*	49.95
Spring 1998	CFU·10⁷· g⁻¹ dry plant mass	6.41	**	10.52	27.50	*	46.44	0.40	-	0.73	0.22	*	1.13
	CFU·10⁴·cm⁻²	5.21	**	8.22	41.28	**	87.20	0.03	-	0.06	0.02	*	0.10
Summer 1998	CFU·10⁷· g⁻¹ dry plant mass	89.71	*	172.10	346.91	-	367.30	86.70	*	156.80	1.39	-	2.44
	CFU·10⁴·cm⁻²	114.39	**	565.09	1183.52	***	525.89	6.48	*	11.72	0.08	-	0.14
Autumn 1998	CFU·10⁷· g⁻¹ dry plant mass	0.26	*	1.11	4.42	**	0.52	2.11	**	7.39	94.30	**	156.26
	CFU·10⁴·cm⁻²	2.09	-	2.91	14.24	***	0.98	0.27	**	0.95	22.19	**	36.77
2 - year average:													
Spring	CFU·10⁷· g⁻¹ dry plant mass	4.07		6.62	170.58		380.72	0.27		0.47	0.58		3.04
	CFU·10⁴·cm⁻²	3.31		5.17	256.05		714.84	0.02		0.04	0.05		0.27
Summer	CFU·10⁷· g⁻¹ dry plant mass	49.53		113.38	489.34		586.92	104.41		292.45	1.93		4.81
	CFU·10⁴·cm⁻²	63.16		372.27	1669.42		840.34	7.81		21.87	0.11		0.27
Autumn	CFU·10⁷· g⁻¹ dry plant mass	0.91		3.79	128.55		62.58	3.15		8.76	104.61		184.28
	CFU·10⁴·cm⁻²	7.32		9.95	414.04		118.22	0.41		1.13	24.62		43.36

Explanation: Species and fragment of plant: CR_u, CR_l, LR_u, LR_l, WL_u, WL_l, PW_u, PW_l - see Table 2; comparison of the number of bacteria on the upper and lower fragments of the plants using the Student test - see Table 2.

Statistically significantly the least bacteria during the study period was noted in spring (in 1998 the average log10 of the number of bacteria = 8.43). Heterotrophic epiphytic bacteria grew on the surface of plants in the greatest numbers in summer (in 1997 and 1998 the average log10 of the number of bacteria was 9.80 and 9.73, respectively). Figure 2 presents the dependence between the total number of bacteria and the number of heterotrophic epiphytic bacteria. It was found that a great positive correlation existed between these two values, and its coefficient was 0.6298.

Discussion

Microorganisms are one of the most important groups of organisms that take part in the processes of the circulation of matter and energy in nature. Among them, heterotrophic bacteria play the most important role. The organic substances broken down by them are utilised in the processes of building structural and reserve components of cells, and are also a source of energy, which is indispensable

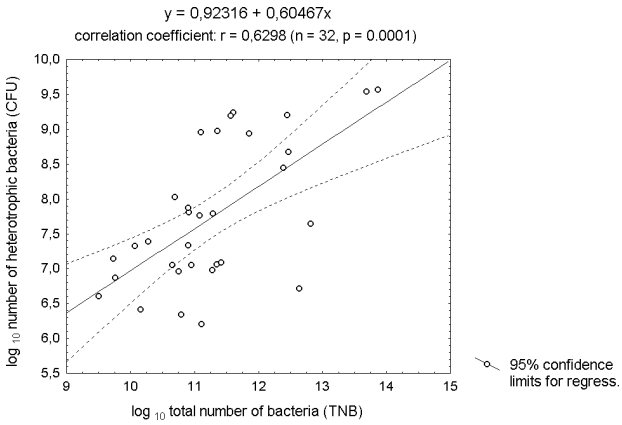

Fig. 2. Dependence between the total number of bacteria and the number of heterotrophic epiphytic bacteria in 1998 (data after logarythmic transformation).

Table 5. 3-way ANOVA test comparing the influence of the fragment of the plant or the surface of the leaf (1), species of plant (2) and the season (3) on the number of heterotrophic epiphytic bacteria calculated per 1 g of dry plant mass.

Factor	Variance	df @	Parameter F	p (significance)
Fragment of plant or leaf surface (+)	3.0678	1	144.4678	< 0.0001
Plant species (+)	12.8910	3	607.0674	< 0.0001
Season (+)	9.0462	5	426.0043	< 0.0001
Fragment of plant or leaf surface * plant species (+)	0.6133	3	28.8800	< 0.0001
Fragment of plant or leaf surface * season (+)	0.0973	5	4.5820	0.0009
Plant species * season (+)	5.8572	15	275.8311	< 0.0001
Fragment of plant or leaf surface * plant species * season (+)	0.1606	15	7.5623	< 0.0001
Intragroup variability	0.0212	96	-----	-----

Comparison of averages (Newman - Keul test of multiple differences, $p \leq 0.05$)

1. Fragment of plant or leaf surface

upper	lower
9625 a	9.2544b

2. Plant species

common reed	lesser reedmace	yellow water - lily	floating pond - weed
8.8097 b	10.0049 d	8.7720 a	8.8473 c

3. Season

spring 1997	summer 1997	autumn 1997
8.6302 b	9.7956 d	9.4336 c

spring 1998	summer 1998	autumn 1998
8.4322 a	9.7322 d	8.6271b

Explanation: @, (+), a, b, ...d - see Table 3.

for carrying out vital functions [17]. In water bodies, organic material, both autochtonic and allochtonic in origin, undergoes biodegradation and transformation with the participation of bacteria. Apart from planktonic and benthonic bacteria, epiphytic bacteria also played an important role in the degradation of organic substances in water bodies, and they were the object of the research in the present paper.

It follows from the research conducted on the number of epiphytic bacteria that the total number of bacteria and the number of heterotrophic epiphytic bacteria displayed a distinct seasonal variability. The total number of bacteria inhabiting the underwater sections of the stems of the common reed and lesser reedmace, and the surfaces of the leaves of the yellow water-lily was at its highest in summer. A fall in the total number of bacteria on the surface of the leaves of floating pond-weed was observed from spring to summer, and a considerable increase was observed in autumn.

Niewolak [14] and Olah [15] explain the summer maximum of the number of bacteria on certain plants by citing the increased amount of organic substances secreted by the plants and the increase in the temperature of the water. On the other hand, the fall in the total number of bacteria observed in summer on the surfaces of other macrophytes may be caused due to the excretion of antibacterial substances by plants or by algae inhabiting them, or due to the excessive exposure of plant surfaces to sunlight. The results obtained in this paper of the research on the total number of epiphytic bacteria occurring on the surface of the leaves of the yellow water-lily and floating pond-weed are basically in accordance with the results given by Hossel and Baker [9] for two freshwater plants: *Rorippa nasturtium-aquaticum* and *Lemna minor*, and with the data given by Baker and Orr [2] for *Ranunculus penicillatus* and *Veronica beccabunga*. However, it follows from this paper that the surfaces of lesser reedmace and the common reed were inhabited by a considerably more numerous population of bacteria. In the literature, though, there is a lack of more detailed data concerning the total number of epiphytic bacteria calculated per gram of dry plant material. This limits to a considerable degree the possibility of conducting a comparison and a broader discussion on the results obtained in this paper.

The number of heterotrophic bacteria occurring on the surfaces of the plants under investigation underwent similar seasonal changes to the total number of bacteria, as was mentioned earlier. The results obtained were on average about 1000 times lower when calculated per cm^2 of surface area and per g of dry plant mass than the total number of bacteria on the same plants. Our data are higher than the results described by Conover and Sieburth [5], who also used the breeding method and found between $2.0 \cdot 10^1$ and $2.0 \cdot 10^5$ bacterial cells to be present calculated per g of dry plant mass of *Sargassum natans*.

It follows from the research in this paper and data in the literature that bacteria are unevenly distributed on the surface of plants. Hossel and Baker [10] write that the total number of bacteria on very young, underwater leaf apices of *Ranunculus penicillatus* was $3.90 \cdot 10^4$ cells per cm^2, and on older ones it rose to $6.01 \cdot 10^6$ cells per cm^2. The number heterotrophic epiphytic bacteria was $3.10 \cdot 10^4$ cells per cm^2 and $1.18 \cdot 10^5$ cells per cm^2, respectively. Baker and Orr [2] write that the number of bacteria on the leaves of *Veronica beccabunga* near the growth apex of the plant is distinctly lower than that on the leaves growing on the lower part of the plant. This probably results from the fact that the young, growing shoots do not provide favourable conditions for periphyton to settle on and inhabit their surfaces [13]. According to Sieburth [18] macrophytes secrete substances during their growth period that render the growth and breeding of bacteria impossible. The youngest, apical parts of the thallus of higher algae are also devoid of bacteria, according to Sieburth [19]. This uneven distribution of bacteria on the surface of plants was also observed in this paper. Older, near-bottom sections of the stems of the common reed were usually inhabited by more heterotrophic bacteria than the upper sections of the stems. The near-bottom fragments of lesser reedmace were colonised in spring and summer by a greater population of bacteria than the fragments growing in the surface water layer. In autumn, however, a higher number of bacteria was observed on the fragments growing in the surface water layer.

It is generally known that the number of planktonic bacteria in the above-sediment water layer is usually higher in comparison with the layers of water higher up. Hence bacteria inhabit the surfaces of stems growing in the above-sediment water layer more quickly and in greater numbers. The decreasing number of bacteria on, for example, the lower sections of lesser reedmace in autumn may be linked with the beginning of the process of the outer layers of this plant dying and decaying and with whole outer fragments of the plant falling off together with the microorganisms inhabiting them, which was observed during the research.

Distinct differences in the total number of bacteria and heterotrophic bacteria were also observed between the upper and lower surfaces of leaves of the yellow water-lily and floating pond-weed. It follows from the research that these differences were on the whole small in spring on very young leaves and increased with the age of the plants. The weak development of bacterial microflora on the upper side of leaf blades floating on the surface of the water was certainly influenced by strong radiation from the sun, including ultraviolet radiation, and considerable changes in temperature linked with the evaporation of water on the surface of the leaves; on the other hand, the development of microflora on the bottom side of the leaves was probably favoured by the permanent contact with the water, its stable temperature and the lack of direct sunlight, and also the greater secretion of nutritive substances by the many secretory hairs present here.

The uneven distribution of bacteria on the surface of leaves was confirmed during research for this paper on the yellow water-lily and floating pond-weed using scanning microscopy. Bacterial cells on the upper surfaces of the leaves of the yellow water-lily usually accumulated near

stomata. On the lower surfaces of the leaves bacteria were abundant near secretory hairs. Stomata allow the plant to exchange gases between the atmosphere and intercellular spaces of the leaf [12]. There is a lack of information in the literature, however, on the subject of metabolic secretions. It should be assumed that the secretion of substances through stomata can contribute to the development of more numerous microorganisms in their vicinity. The second element on the surface of leaves is the secretory hairs, very common in the plant world. It follows from the literature that they can secrete different products to the external environment, including oils or irritating substances [8, 11, 12]. The substances secreted by them undoubtedly serve as nutritive substrates for the bacteria.

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