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### Biocenoza potoku wysokogórskiego pozostającego pod wpływem turystyki\*. 2. Bakterie jako wskaźnik zanieczyszczenia wody Rybiego Potoku

## Biocenosis of a high mountain stream under the influence of tourism. 2. Bacteria as an index of water pollution of the Rybi Potok stream

#### Wpłynęło 8 lipca 1976 r.

A b s t r a c t — The changes in the total number of heterotrophic bacteria and of physiological groups (i.e. proteolytic ammonifying, and denitrifying bacteria and of the *Escherichia coli* titre) were compared in the annual cycle in the water of the stream Rybi Potok in the pure sector and at the point of inflow of domestic sewage from the shelter at Lake Morskie Oko. On the basis of changes in the number of bacteria a logarithmic comparative coefficient (LCC) was calculated, this enabling two zones of water self-purification to be differentiated in a stream sector of about 3.2 kilometres in length.

Physiographical and chemical investigations on the composition of the water in the Tatra streams and lakes were carried out by Oleksyno-wa, Komornicki (1965), Pasternak (1965), Oleksynowa (1970), and Bombowna (1965, 1971). It was found that the chemical composition of the water depended on the geological structure of the catchment basin and its formation and, thus, on the climate and the rate of the current. In rocky areas waters poor in electrolytes have an oligotrophic character, which changes into mesotrophy in the areas of sedimentary rocks with an admixture of limes and dolomites (Pasternak 1971).

\* Praca wykonana w ramach problemu węzłowego PAN-27.

In the Tatra streams algological and zoological investigations were also carried out. A number of papers were concerned with the occurrence and zonal distribution of algae (Kawecka 1965, 1971, Kawecka et al. 1971), and the total number and taxonomic units of the fauna (Kownacki 1971, Kownacki, Kownacka 1971, Kownacka 1971) in the water of Tatra lakes and streams.

On the other hand, no results of any hydrobacteriological investigations have yet been published, only one paper having appeared on the bacteria settling the waters of the Zimna and Kasprowa Niżna caves (F is c h e r 1959). The author found variability in the occurrence of physiological groups in the bacterial community of water bodies in the caves. In an agar culture the number of bacteria from the water of the Zimna cave was 300 cells/ml, and from that of the Kasprowa Niżna cave 350 cells/ml.

In the years 1971—1972 the Laboratory of Water Biology of the Polish Academy of Sciences carried out complex investigations on the water of the stream Rybi Potok, including chemical (Bombówna 1977), algological (Kawecka 1977), and zoological analyses (Kownacki 1977). The present bacteriological study forms part of this investigation.

The post-glacial Rybi Potok valley lies in the High Tatras within the confines of the Tatra National Park. The stream flows out from Lake Morskie Oko (area 34.54 ha, depth 50.8 m) at an altitude of 1393 m above sea level, crossing the post-glacial terminal moraine. About 300 m from the point of outflow from the lake, the stream passes into two ponds, Rybie Stawki, 3 metres in depth, and flows into the River Białka Tatrzańska at an altitude of 1085 m. Almost along its whole length the stream is shaded by dense spruce forest.

The region of Lake Morskie Oko and the valley of the Rybi Potok are among the most frequented places in the Tatras, the tourists being attracted by the magnificent scenery and easy access by an asphalt road. On the terminal moraine which encloses the lake from the north a shelter is located, taking in well over a million tourists a year. Domestic sewage containing great amounts of organic matter is led from the shelter to a septic tank. Although the tank is periodically emptied, nevertheless some of the sewage gets into the Rybi Potok, bringing about its pollution.

The objective of the present study was to determine the degree of pollution of the stream Rybi Potok in the annual cycle and the effect of its self-purification in the longitudinal profile, in connection with the variable intensity of the tourist traffic.

Among other organisms bacteria play the role of a water pollution index, since, as is known, their development is closely correlated with the presence of various types of organic substances. In order to determine the degree of pollution and to deliminate the zones of water self-purific-

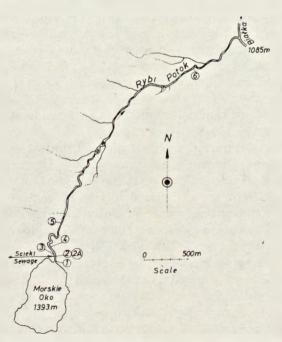
ation in the longitudinal profile of the stream, the quantitative changes of heterotrophic bacteria were used, several physiological groups being considered.

The Rybi Potok offered particularly good conditions for this type of investigation, as it was possible to characterize the quantitative relations of the identified groups of bacteria in its pure sector, with a natural undisturbed biocenosis, at the point of sewage discharge, and, finally, in the self-purification zone of the stream.

#### Method

The water samples were collected in sterile bottles, (100 ml in volume) with glass stoppers, directly by dipping the bottles in the water, at 6 stations, from May, 1971, to June, 1972, at 1.5-month intervals.

Station 1 lay 10—50 m from the outflow of the stream from the lake, above the sewage outlet, while, the other five stations lay below at a distance of 2—5 m (station 2), 15—30 m (station 3), 100—120 m (station 4), 500—550 m (station 5), and 3200—3250 m (station 6) from the sewage outlet (fig. 1).



Ryc. 1. Rozmieszczenie stanowisk poboru prób w Rybim Potoku Fig. 1. Distribution of sampling stations in the stream Rybi Potok The water samples were inoculated directly or after dilution on suitable media or substrata, in three parallel replications.

The determinations in the samples included: the total number of heterotrophic bacteria, plate method on normal  $2^{0}/_{0}$  agar, pH 7.2 at  $20^{\circ}$  after 7 days; number of proteolytic bacteria using the plate method on  $15^{0}/_{0}$ gelatine substratum, pH 7.6 at  $20^{\circ}$  after 2 days; ammonifying bacteria using the titre method on the medium with peptone water (R o d i n a 1968) at  $20^{\circ}$  after 7 days; denitrifying bacteria using the titre method, on the medium with glucose and KNO<sub>3</sub> (R o d i n a 1968), at  $20^{\circ}$  after 7 days; *Escherichia coli* titre on Kessler medium, pH 7.2 at  $44^{\circ}$  after 24 hrs, the identification being carried out on Endo substratum. The titre values of ammonifying and denitrifying bacteria were calculated into the number of bacteria in 1 ml water using Mc Crady's statistical tables.

#### Results

# 1. Comparison of the pure sector of the stream with the polluted one in the annual cycle

In the water flowing out of Lake Morskie Oko (station 1) the total number of heterotrophic bacteria was low at particular times of sampling and ranged from some scores to several hundred cells in 1 ml water, the maximum of 6000 cells/ml being noted only in October.

At this station no proteolytic bacteria were found in January, March, or May. In other periods they numbered from 10—20 cells in 1 ml water. At station 1 the maximum development of proteolytic bacteria was noted in the summer months (Table I).

The other bacteria from the investigated physiological groups, the ammonifying and denitrifying ones, also occurred in insignificant numbers. Ammonifying bacteria attained a maximum (250 cells/ml) in September and October, and the denitrifying bacteria in June (30 000 cells/ml), this number being exceptionally high as compared with that noted in other sampling periods (Table I). Throughout the year the *E. coli* titre was here over 5 (Table II).

At station 2 a rapid increase in the total number of heterotrophic bacteria as well as of the identified physiological groups was observed. In particular periods the number of bacteria increased 100—1000 times. The curves of the graph (fig. 2) show a significant difference between stations 1 and 2. At station 1 the curves illustrating the course of the quantitative annual variability in the investigated groups of bacteria, were single-peaked. At station 2 two peaks (a summer and a winter one)

Tabala I.	Zminny linzebności	. bakterii na badan	ych stancwiskach	w Rybim Potoku
	w poszezególnych t	erminach poboru pr	бЪ	

Table I.	Changes in the number of bacteria at the investigated stations in the Rybi
	Petok stress on particular dates of sampling

		No	Liczba komórek umber of cella f	w 1 ml wody in 1 ml of water	+
Data peboru prób Date of sampling	Stanowisko Station	Ogólna grupa bakterii hete- rotrofioznyoh General group of heterotro- phic bacteria	Bakterie pro- teolityczne Proteolytic bacteria	Barterie amo- nifikaoyjne Ammonifying hacteria	Bacterie de- nitryfikecyjne Denitrifying bacteria
27.V. 1971 r.	1 23 4 56	70 28 200 3 900 3 360 2 900 1 360	0 80 70 80 50 30	95 950 950 950 75 45	95 45 150 45 95 10
15.VII. 1971 r.	123456	670 39 850 6 900 650 1 100 410	340 5 580 2 650 470 890 310	25 7 500 4 500 400 9 500 450	12 1 500 4 500 95 95 75
2.IX. 1971 r.	1 2 7 4 5 6	505 800 000 270 000 3 400 1 200 15 300	250 69 250 47 700 680 120 2 420	250 95 000 1 600 1 500 750 4 500	45 15 000 15 000 950 4 500 750
23.X. 1971 r.	1 2 3 4 5 6	6 000 175 000 25 000 710 570 480	57 69 000 13 900 250 150 140	250 25 000 2 500 95 30 150	7 1 400 9 1 1
3.XII. 1971 r.	1 2 3 4 5 6	324 57 400 24 590 1 100 1 000 390	16 6 550 2 130 140 165 40	150 25 000 4 500 250 250 150	45 25 000 15 000 4 500 300 90
26.I. 1972 r.	1 2 3 4 5 6	114 100 800 54 000 7 100 555 128	0 2 600 790 72 38 9	15 25 000 4 500 200 <b>750</b> 25	950 95 000 9 000 150 160 140
29.III. 1972 г.	1 2 3 4 5 6	180 580 000 235 000 58 850 19 800 2 560	0 55 500 32 700 4 370 4 250 730	25 450 000 450 2 500 2 500 2 500 2 500	9 45 000 16 3 2 500 450
3.VI. 1972 r.	1 2 3 4 5 6	130 620 000 198 000 45 000 15 000 16 500	19 3 300 2 600 1 900 2 000 2 000	4 950 750 95 2 500 150	30 000 40 000 160 000 150 000 950 95

and a distinct decrease in late autumn (October, November) were noted in the numbers of bacteria. The intensity of the tourist traffic (curves a and b) throughout the year was also plotted on the above graphs at monthly intervals, taking into account overnight visitors or those stopping only briefly at the shelter (data supplied by Mgr Czesław Łapiński, the manager of the shelter at Lake Morskie Oko).

The curves show that the quantitative variations in the tourist traffic are remarkably similar to the changes in the total number of heterotrophic bacteria at station 2 (fig. 2 A). The quantitative changes of pro-

teolytic and ammonifying bacteria were similar to the changes in the total number of heterotrophic bacteria at this station. The small number of ammonifying bacteria coincided particularly well with the slack seasons and an increased number with the summer and winter maxima (fig. 2 C). The general character of changes in the number of proteolytic bacteria is similar, though the autumn decrease in their number was shifted more towards the winter months (fig. 2 B). Denitrifying bacteria showed similar seasonal changes at stations 1 and 2. Their numbers were

Tabela II. Zwiany miana E. ooli na badanyoh stanowiskach w Rybim Potoku w poszczególnych terminach poboru prób

Stanowisko		-	1971 r	1972 r.					
·Station	27.♥.	15. <b>∀II</b> .	2.IX.	23.X.	3.XII.	26.1.	29.III.	3 <b>.</b> ♥I.	
1	> 5	>5	> 5	>5	> 5	> 5	> 5	> 5	
2	2	10-1	10-2	10 <sup>-1</sup>	> 5	> 5	1	1	
3	2	10-1	10-2	10-1	> 5	> 5	5	1	
4	2	10 <sup>-1</sup>	10 <sup>-1</sup>	1	>5	> 5	1	2	
5	>5	10 <sup>-1</sup>	5	1	> 5	> 5	5	5	
6	> 5	1	> 5	1	> 5	> 5	2	5	

Table II. Changes in the E. coli titre at the investigated stations in the Rybi Potok stream on particular dates of sampling

small in the summer months but larger in winter and spring (fig. 2 D). In spite of such great seasonal changes, the stimulating effect of the development of bacteria was manifested in a 100—1000 times increase in the number of denitrifyers at station 2. The variation in the *E. coli* rods was distinctly visible, particularly in summer, when from an initial titre value of over 5 at station 1 they attained the value of  $10^{-1}$  and  $10^{-2}$  at station 2 (Table II).

#### 2. The process of water self-purification in the Rybi Potok

The number of bacteria at the pure station 1 was regarded as the point of reference in determining the degree of water self-purification in the longitudinal profile of the stream, the logarithmic comparative coefficient (LCC) being calculated for the successive stations 2, 3, 4, 5, and 6 on the basis of the formula:

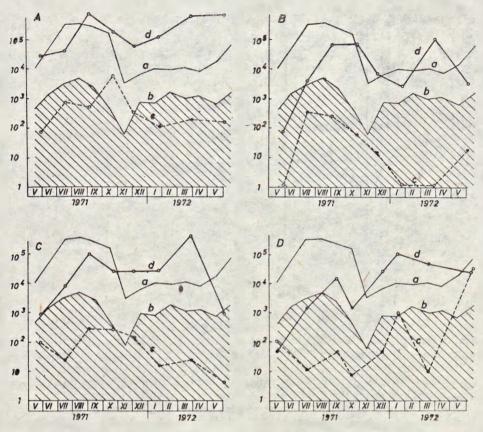
LCC<sub>1</sub> = 0; LCC<sub>2</sub> = log 
$$\frac{b_2}{a}$$
; LCC<sub>3</sub> = log  $\frac{b_3}{a}$ ; ... LCC<sub>6</sub> = log  $\frac{b_6}{a}$ 

where  $\alpha$  — number of bacteria at station 1,

 $b_2$ ,  $b_3$ , ...  $b_6$  — the number of bacteria at stations 2, 3 ... 6. The above formula indicates that the coefficient defines the difference between the

- Tabela III. Zmiany legarytmioznego współczynnika porównawczego (LWF) na stanowiskach wyznaczonych poniżej depływu ścieków w poszczególnych terminach poboru prób. Jako punkt edniesienia przyjęto lg liczby bakterii na stanowiska 1 (czystym)
- Table III. Changes in the logarythmic comparative coefficient (LCC) at stations below the sewage discharge on particular dates of sampling. The logarythm of the number of bacteria at the clean station 1 was accepted as the point of reference

										Bakt	erie	~ 1	Baotería											
	Ogólna grupa heterotrofów General group of heterotrophe						proteolityczne - proteolythio						smonifikacyjne - ammonifying					denitryfikacyjne - denitrifying						
Data poboru prób	St	anow	sko -	Stati	lon		Sta	anowie	sko -	Stati	on		St	tanow	isko -	Stati	on		Star	ow1sko	- Sta	tion		
Date of eampling	Lg z liczby bakterii w 1 ml wedy		L	WP			Ig z liczby bakterii w 1 ml wody			LWI			Lg z liczby bakterii w 1 ml wody			LWI			Lg z liczby bakterii w 1 ml wody			LWP		
Log from the number of besteria in 1 ml of wate		imber of LCC		number of			Log from the LCC number of becteria in 1 ml of water					Log from the number of becteria in 1 ml of water			r c c	3								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
27. 1. 1971	1.84	12.60	1.74	1.68	1.61	1.28	0	1.90	1.84	1.90	1.69	1.47	1.97	1,00	1.00	1.00	-0.10	-0.32	1.97	-0.32	0.19	-0.32	0.00	-0.97
15.VII.1971	2.82	1.77	1.01	-0.01	0,21	-0.21	2.53	1.21	0.89	0.14	0.41	-0.04	1.39	2.47	2.25	1.20	2.57	1.25	1.07	2.09	2.57	0.89	0.89	0.79
2. IX. 1971	2.70	3.19	2.72	2.82	0.37	1.48	2.39	2.44	2.28	0.43	-0.98	0.98	2.39	2.57	0.80	0.77	0.47	1.25	1.65	2.52	2.52	1.32	2.00	1.22
23.X.1971	3.77	1.46	0.61	-0.92	-1.02	-1.09	1.75	3.08	2.38	0.64	0.42	0.39	2.39	2.00	1.00	-0.42	-0.92	0.22	0.84	2.30	0	-0.10	-0.84	-0.84
3.XII.1971	2.51	2.24	1.88	0.53	0.48	0.09	1.20	2.61	2.12	0.94	0.01	0.39	2.17	2.22	1.47	0.22	0.22	0.00	1.65	1.74	2.52	2.00	0.82	0.30
26.I.1972	2.05	2.94	2.67	1.79	0.68	0.05	0	3.41	2.89	1.85	1.57	0.95	1.17	3.22	2.47	1.12	1.69	0.22	2.97	1.00	-0.02	-0.80	-0.77	-0.83
29.III.1972	2.25	3.50	3.11	2.51	2.04	1.15	0	4.74	4.50	3.67	3.39	2.86	1.39	4.25	1.25	. 2.00	2.00	2.00	0.95	3.69	0.24	-0.47	2.44	1.69
3.VI.1972	2.11	3.67	3.18	2.53	2.06	2.25	1.27	2.23	2.13	2.00	2.02	2.02	0.60	2.37	2.27	1.37	2.79	1.57	4-47	0.12	0.72	0.69	-1.49	-2.49
Srednia Mean	2.50	2.67	2.11	1.11	0.80	0.62	-1+14	2.70	2.37	1.44	1.15	1.12	1.68	2.51	1.56	0.90	1.09	0.77	1.94	1.64	1.09	0.42	0.38	-0.14



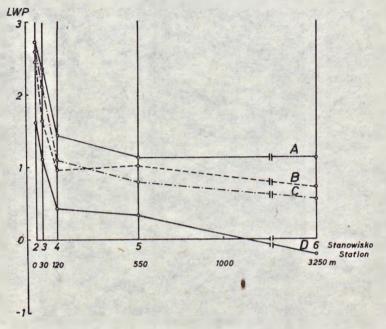
Ryc. 2. Sezonowe zmiany liczebności bakterii i ruchu turystycznego. A — ogólna grupa bakterii heterotroficznych; B — bakterie proteolityczne; C — bakterie amonifikacyjne; D — bakterie denitryfikacyjne. Liczba turystów w przeliczeniu na jeden miesiąc: a turyści chwilowo zatrzymujący się w schronisku; b — turyści nocujący w schronisku. Liczba bakterii na 1 ml wody: c — na stanowisku 1 (czyste); d — na stanowisku 2 (w bezpośrednim sąsiedztwie dopływu ścieków)

Fig. 2. Seasonal changes in the numbers of bacteria and in tourism. A — general group of heterotrophic bacteria; B — proteolytic bacteria; C — ammonifying bacteria; D denitrifying bacteria. Number of tourists calculated for 1 month: a — tourists visiting the shelter briefly; b — overnight tourists. Number of bacteria in 1 ml of water: c at the pure station 1; d — at station 2 (next to the sewage outlet)

logarithm from the number of bacteria at a polluted station and that from the number of bacteria at the pure station. The coefficient was determined for particular sampling periods and for all the investigated groups of bacteria (Table III).

Figure 3 presents the arithmetic mean for individual stations, calculated from the sum of coefficients (LCC) from the whole period of measurements for a given group of bacteria. The shape of the curves in the graph shows that two sectors may be distinguished in the stream.

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Ryc. 3. Średnie roczne wartości logarytmicznego współczynnika porównawczego (LWP) w podłużnym profilu Rybiego Potoku. A — ogólna grupa bakterii heterotoficznych; B — bakterie proteolityczne; C — bakterie amonifikacyjne; D — bakterie denitryfikacyjne Fig. 3. Mean annual values of the logarithmic comparative coefficient (LCC) in the longitudinal section of the Rybi Potok. A — general group of heterotrophic bacteria; B — proteolytic bacteria; C — ammonifying bacteria; D — denitrifying bacteria

In the first, between stations 2 and 4, about 120 m in length, a very rapid decrease was noted in the numbers of the identified bacteria, while in the second, between stations 4 and 6, about 3200 m in length, a much slower decrease was noted.

It should be mentioned that in a few cases an increase in the number of bacteria was noted at station 6. This was observed in September 1971 and in June 1972, when for almost all physiological groups much greater or similar numbers were reported as compared with station 5 (Table I). Moreover, in October 1971 the number of ammonifying bacteria was similarly higher at station 6. Nevertheless, the mean percentage decrease in the number of bacteria between stations 5 and 6 (calculated without the above mentioned periods) amounted to  $50^{0}/_{0}$  of proteolytic bacteria, to about  $60^{0}/_{0}$  of the total number of heterotrophic and ammonifying bacteria, and  $80^{0}/_{0}$  for the denitrifying bacteria.

As compared with the point of sewage discharge (station 2), at a distance of over 3 km, at station 6, the mean annual decrease in the number of bacteria in the water was considerable and amounted to about 2 orders of magnitude for the total number of heterotrophic bacteria,

1.74 for the ammonifying ones, 1.58 for the proteolytic, and 1.50 for the denitrifying bacteria.

The equilibrium in the stream, upset by the inflow of sewage, over a distance of about 3.2 kilometres, does not regain its initial state. The logarithmic comparative coefficient allows the conclusion that at station 6 the annual mean number of bacteria is maintained at a level higher than the initial value by a 1.12 order of magnitude for the proteolytic bacteria, by 0.72 for the ammonifyers and by 0.60 for the total group of heterotrophic bacteria. Only the annual mean number of denitrifyers slightly decreases below the initial value, attaining a negative LCC value of -0.14.

#### Discussion

Numerous investigations show that the development of heterotrophic bacteria directly depends on the concentration of organic substances in the water. These organic substances may be of natural origin, resulting from the metabolism or seasonal decay of aquatic organisms. Such sources of organic matter, together with annual thermic variations and other factors of ecological character, limit the seasonal changes in the numbers of bacteria in natural environments. Rainfall should be added to the natural factors periodically modifying the variability of the bacterial flora, since it either dilutes the autochtonous organic substances or brings in allochtonous substances with floods (G o d l e w s k a - L i p o w a et al. 1974, K l i m o w i c z 1973, G e r b a et al. 1973).

In surface waters the most drastic upsetting of the equilibrium of the trophic state is of antropogeneous character, revealed in the inflow of industrial and domestic sewage. The latter is rich in final products of human metabolism, food scraps from restaurants, and cleaning agents (among them detergents). It is assumed that the kind and content of domestic sewage is of homogeneous character:  $80-90^{0}/_{0}$  of total nitrogen is urine (Goering 1972); besides, there are aminoacids, proteins and products of their decomposition, uric acid, free amids, and also nucleic acids. These constituents form a rich substratum for the development of bacteria. The prevalence of certain compounds over others temporarily favours the development of specific groups of bacteria, whose dominant role in the environment will be taken over by other groups of organisms when the suitable substratum is depleted.

In our investigations, over a distance of anywhere from 10—20 metres the environmental conditions in the Rybi Potok differed diametrically: station 1, under the influence of natural factors modifying the trophism of the water, and station 2 under antropogeneous pressure. On the basis of the classification elaborated by C a b e j s z e k et al. (1957) station 1 may be regarded as belonging to pure waters. The small number of bacteria shows relatively insignificant changes throughout the year, with a maximum at the end of the vegetation season, when the concentration of organic substances grows in consequence of an increased decay of aquatic organisms and above-ground parts of land plants. On the other hand, station 2, characterized by great numbers of bacteria, may be classified among strongly polluted waters (C a b e j s z e k et al. 1957), since the numbers of bacteria increased by 2—3 orders of magnitude as compared with station 1.

At station 2 the decisive factor in the inflow of organic substances is domestic sewage which seep into the stream from the septic tank at the shelter, while the amount of discharged sewage depends on the number of tourists. Hence, at station 2 a marked decrease in the number of bacteria is noted in October and November, when fewer tourists come to Lake Morskie Oko. In these months the number of bacteria increases at station 1. As was already mentioned, this is brought about by an increase in the content of organic matter in the water as a result of the mass decay of plant and animal organisms, in this period.

The differences between these two stations are most pronounced in the total number of heterotrophic bacteria. This group includes various types of physiological bacteria. Depending on the thermic conditions of summer or winter, a decisive role in the environment may be played by psychrophilous and mesophilous species. At both stations this was manifested in relatively small differences between summer and winter in the numbers of this group of bacteria.

The situation is different in the case of *E. coli* rods, which are a small group with specific habitat requirements. A decrease in the titre to  $10^{-1}$  and  $10^{-2}$  (station 2) coincides with the summer tourist maximum and indicates considerable pollution of water with excrements. There is no such dependence in the months of winter tourist maximum but this does not suggest any lesser pollution of the stream by excrements, but is the result of strong thermic inhibition of the development of *E. coli* rods. It was reported that the maximum development of these bacteria occurred in the periods of the greatest tourist traffic (Gerba et al. 1973). However, the optimum for *E. coli* rods is attained in the warmest months (Klimowicz 1973), hence the thermic conditions should be regarded as the basic limiting factor.

Seasonal changes of temperature are also revealed in the relation to other physiological groups of bacteria, especially at station 1, where in winter the number of proteolytic bacteria decreases by over 2 orders of magnitude, and of ammonifying bacteria by 1 order of magnitude, while the number if denitrifyers distinctly increases in the winter months. In

these cases the thermic factor inhibits or stimulates the development of the investigated groups of bacteria either directly or indirectly through other physiological groups which carry out the preliminary decomposition of the medium.

At station 1 the influence of natural factors is manifested in one annual peak in the number of bacteria, suggesting the optimum development conditions for the separate groups of the investigated bacteria in a given season of the year. Two periods of maximum increases in the number of the investigated groups of bacteria suggest different factors contributing to this development at station 2 during the year. The coincidence of maximum developments with the intensity of tourist traffic shows that this development depends on the amount of domestic sewage reaching the stream.

Another problem in the present study was the biological self-purification of the water in the Rybi Potok. On the basis of the results obtained, two zones were differentiated, determined according the rate of decrease in the LCC value in the investigated stream sector of about 3.2 kilometres.

The question arises as to the degree in which the decreasing number of bacteria in the longitudinal profile of the stream, in the individual zones, indicates a decrease in the level of organic compounds by means of biological self-purification processes, and as to what is the share of mechanical mixing of sewage in the strong current of the stream. It is difficult to answer this unequivocally. In the vicinity of the sewage outlet the numbers of bacteria were high. The fungus Leptomitus lacteus occurred in masses, the filamentous bacteria Sphaerotilus natans being also fairly frequent. The blue-green alga Phormidium favosum and the diatom Nitzschia palea, typical for polluted environments, developed in great quantities (Kawecka 1977). In the successive stations of the first zone, in a relatively short sector (about 120 m), a considerable decrease in the numbers of bacterial flora was observed. At the same time, the fungus Leptomitus lacteus disappeared below station 3. Considering the above-mentioned facts, one may assume with great probability that in the first zone of self-purification the main role is played by a good mixing of sewage in the strong current of the stream, while in the second zone the mixing of sewage in the current is less important, hence the share of the biological factors increases. This is manifested in the proportionally slower decrease in the numbers of bacteria along the course of the stream.

It should be stressed that the mean annual value of the logarithmic comparative coefficient (LCC) obtained from all sampling periods, suggests that after passing two zones of self-purification (i.e. at a distance of about 3.2 km from the source of sewage) the effect of the self-purification differed with regard to the individual physiological groups

of bacteria. Thus, as compared with the pure sector (station 1) the weakest self-purification was noted with proteolytic bacteria (LCC = = 1.12), while it was somewhat better for ammonifying bacteria (LCC = 0.72) and the total number of heterotrophic bacteria (LCC = 0.60). It was only with denitrifying bacteria that the coefficient value was negative (LCC = -0.14), this indicating a slightly smaller number of cells of these bacteria and thus better self-purification at station 6.

On the basis of quantitative changes of the bacterial flora we may conclude that domestic sewage brings about a disturbance of the natural biocenosis of the Rybi Potok. However, owing to the biochemical action of bacteria with the concomitant favourable conditions of oxygenation from 6.7 to 12.3 mg  $O_2/l$  of water (Bombówna 1976), the intense processes of self-purification overcome the noxious effect of domestic sewage in a short sector of the stream.

#### STRESZCZENIE

W latach 1971—1972 przeprowadzono badania wody Rybiego Potoku wypływającego z oligotroficznego jeziora Morskie Oko w Tatrach Wysokich. Określono zmiany ogólnej liczby bakterii heterotroficznych i kilku grup fizjologicznych (bakterii proteolitycznych, amonifikacyjnych i denitryfikacyjnych) oraz miano *Escherichia coli* w podłużnym profilu potoku na 6 stanowiskach, w odstępach 1,5-miesięcznych.

W bezpośrednim sąsiedztwie potoku zlokalizowane jest schronisko, które obsługuje ponad 1 200 000 turystów rocznie. W zależności od natężenia ruchu turystycznego dopływające ścieki powodują większe lub mniejsze zanieczyszczenie wody potoku. Stwierdzono, że w porównaniu z odcinkiem czystym (powyżej ujścia ścieków), w miejscu dopływu ścieków liczebność bakterii wzrasta od 100 do 1000 razy, a miano *E. coli* z wartości wyższej od 5 osiąga wartość  $10^{-1}$  do  $10^{-2}$  w okresie letnim.

Czysty odcinek potoku charakteryzuje się w zasadzie jednym rocznym maksimum liczebności bakterii. Dla poszczególnych fizjologicznych grup bakterii maksima przypadają w różnych okresach roku i są uwarunkowane optymalnym układem czynników naturalnych.

Poniżej ujścia ścieków dla wszystkich badanych grup bakterii stwierdzono dwa roczne maksima rozwoju przypadające na okresy wzmożonej frekwencji turystów (lato, zima). Natomiast wyraźne obniżenie liczebności bakterii przypada na martwy sezon turystyczny, tj. koniec października i listopad.

W oparciu o logarytmiczny współczynnik porównawczy (LWP) stwierdzono, że na około 3 km odcinku w wyniku procesów samooczyszczania się wody potoku ogólna liczba bakterii heterotroficznych spada średnio o 2 rzędy wielkości. Spadek liczebności pozostałych oznaczanych fizjologicznych grup bakterii jest mniejszy i mieści się w granicach od 1,74 do 1,50 rzędu wielkości.

Należy podkreślić, że przeciętna roczna liczebność bakterii w końcowym odcinku potoku jest wyższa w porównaniu z odcinkiem czystym od 1,12 do 0,60 rzędu wielkości, poza bakteriami denitryfikacyjnymi, dla których LWP jest niższy i równy -0,14.

Na podstawie przeprowadzonych badań można stwierdzić, że koncentracja ruchu turystycznego w rejonie Morskiego Oka wpływa w istotny sposób na zachwianie biologicznej równowagi w Rybim Potoku. Jednak na stosunkowo krótkim, ponad 3 km odcinku, dzięki aktywnym procesom samooczyszczania wpływ ten jest w znacznym stopniu niwelowany.

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