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The effect of intensive fertilization of a bankside meadow on the activity of plankton bacteria in the River Nida (Southern Poland)*

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Abstract — The activity of mixed communities of aquatic bacteria was investigated and the process of bacterial bioaccumulation and destruction was quantitatively determined in the water of the River Nida, in a sector affected by surface run-off from a bankside meadow intensively fertilized in the form of ammonium nitrate. The successive doses of fertilizer did not cause significant changes in the bacterial biomass. The average amount of energy used by bacteria for bioaccumulation and biodegradation was similar at all stations while the direction of the energy flow varied.

Key words: river, micro-biological activity, bioaccumulation, biodegradation.

1. Introduction

Biological transformations affecting the state of an aquatic environment are determined by the process of primary production, destruction of organic matter, extracellular secretion of products by living organisms and, finally, by the inflow of pollution from the outside (Ohle 1972, Fogg 1975, Seki 1982, Starzecka, Trela 1982, Starzecka Mazurkiewicz 1986, Starzecka 1987).

Bacteria play the most important role in the heterotrophic processes of cycling of biological elements in the whole biosphere, and thus, also in waters (Kuznyetsov 1959, Sorokin 1978, Seki 1982). The determination of their activity is therefore an important indicator of the direction of processes involved in bioaccumulation and bodegradation, i.e., in the energy flow in the environment (Albright, Wentworth 1973, Donderski 1983, Starzecka, Mazurkiewicz 1986,

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S t a r z e c k a 1987). The aim of the present work was to investigate the activity of mixed communities of aquatic bacteria and quantitatively determine the bacterial bioaccumulation and destruction in the water of the River Nida in a sector affected by surface run-off from an intensively fertilized bankside meadow.

2. Study area

The investigation was carried out in the period May 1983 — May 1985 at three stations lying in a 600 m sector o the River Nida at Chroberz, above and below a 1 ha meadow which was intensively fertilized with ammonium nitrate in the amount of 240 N kg ha^{-1} year $^{-1}$, in three doses of 80 N kg ha^{-1} each. The Station 1 (control) was located above the meadow, Station 2 in its immediate neighborhood, and Station 3 — 200 m

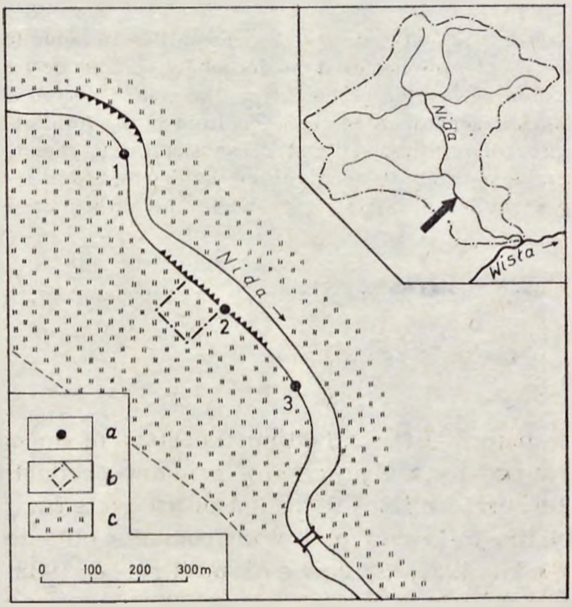

Fig. 1. Localization of sampling stations 1—3. a — stations; b — experimental meadow; c — meadows. Triangles — steep river banks

below the meadow (fig. 1). Near the banks typical (humic) alluvial pseudogleyed and gleyed heavy soils were found, formed from alluvia of the river.

On the basis of earlier studies the water of the River Nida at Chroberz could be classified as relatively pure (S t a r z e c k a et al. 1979). However, a marked eutrophication of this river sector has been shown by hydrochemical (B o m b ó w n a unpubl.), algological (K a w e c k a

1986, Bednarz unpubl.), and faunistic (Grabacka unpubl., Dumnicka unpubl.) investigations carried out in recent years. A description of the stations is given in Table I.

Table I. Description of the investigated stations

Stations	Character of the bottom	Depth of the river (m)	Current	Flow (m^3 sek^{-1}) min	Flow (m^3 sek^{-1}) max	Riverside	Remarks
1	muddy-sandy	0.2 - 0.7	slow medium	6.3	18.4	poorly developed shore-line	300 m above the meadow, the control station, tufts of fleshy filamentous alage in the spring and summer period, euglenins and green alagae
2	muddy	0.8 - 1.5	medium fast	6.3	18.4	high and steep bank	just below the meadow, algae as at the control station
3	sandy-muddy	0.4 - 0.8	medium	6.3	18.4	high bank	200 m below the meadow, in summer agglomerations of duckweed at the bank, in spring abundant development of green algae, Potamogeton appeared periodically

3. Material and method

The meadow was fertilized on April 12, June 15, September 10, 1983, April 18, July 7, September 6, 1984, and April 12, 1985.

Water samples were collected from midstream at a distance of about 0.5 m from the bank, into sterile bottles, on 24 dates (May 16, June 13, July 18, August 15, September 28, October 24, December 7, 1983, January 16, February 20, March 13, April 16, May 14, June 11, July 16, August 13, September 10, October 15, November 14, December 10, 1984, January 22, March 6, March 27, April 16, May 13, 1985). The activity of mixed bacteria communities was determined in the samples, using a GXI 610 E oxygen electrode. The activity was measured on the basis of oxygen losses in 24-hour cultures carried out at $\pm 22°C$ in natural (not enriched) water and in water with an addition of asparagine (100 μM dm^{-3}). Before the culture was started the water was filtered through a 0.25 μ plankton net in order to remove phyto- and zooplankton and pured into 250 cm^3 flasks.

The production of bacterial biomass (C mg dm^{-3} water 24 h^{-1}) was computed from the amount of oxygen assimilated by bacteria during the culture (Sorokin, Kadota 1972).

The content of biomass of heterotrophic bacteria in the water was calculated according to the Kuznyetsov and Romanyenko formula (1963) modified by Starzecka (1987).

The Schrödinger coefficient, expressing the ratio of respiration to biomass in bacteria (Odum 1982), and the coefficient of the heterotrophic activity, which described the ratio of production of bacterial biomass in the water enriched with asparagine to the production of bacterial biomass in natural water, were also calculated (Godlewska-Lipowa 1974, Starzecka, Mazurkiewicz 1986).

4. Results

4.1. Production of bacterial biomass

Production of bacterial biomass in natural (not enriched) water was characterized by uniform values and similar seasonal changes above (Station 1) and below (Stations 2 and 3) the fertilized meadow.

The range of variation of the micro-biological production was similar at all stations. It was 0.21 C mg dm^{-3} 24 h^{-1} at Station 1, and slightly smaller at Stations 2 and 3, reaching 0.19 and 0.18 C mg dm^{-3} 24 h^{-1}, respectively (fig. 2A). A distinctly greater production of bacterial biomass in comparison with the other two stations was found in summer at Station 2 (Table II).

Table II. Production of bacteria biomass in C mg dm^{-3} 24 h^{-1} (mean of seasons). K - natural water without substrate; A - water with 100 µM dm^{-3} of asparagin

Stations	Spring		Summer		Autumn		Winter	
	X	A	X	A	K	A	X	A
1	0.095	0.619	0.118	0.852	0.065	0.525	0.080	0.458
2	0.090	0.607	0.342	0.848	0.060	0.373	0.108	0.513
3	0.076	0.592	0.089	0.852	0.060	0.565	0.090	0.504

In the period of investigation the average production was uniform at all three stations, i.e., 0.09 C mg dm^{-3} 24 h^{-1} above the meadow and in its immediate neighbourhood and 0.08 C mg dm^{-3} 24 h^{-1} at Station 3 (fig. 2A).

In the water samples enriched with asparagine the production of bacterial biomass was more abundant than in the natural water. The obtained values were uniform at all 3 stations both in the entire period of the investigation and in the separate seasons, especially in spring and summer (fig. 2B, Table II).

Also, it should be noted that in periods after the successive doses of nitrogen no significant changes in the abundance of production of bacterial biomass were observed in the natural water or in that enriched with asparagine (fig. 2).

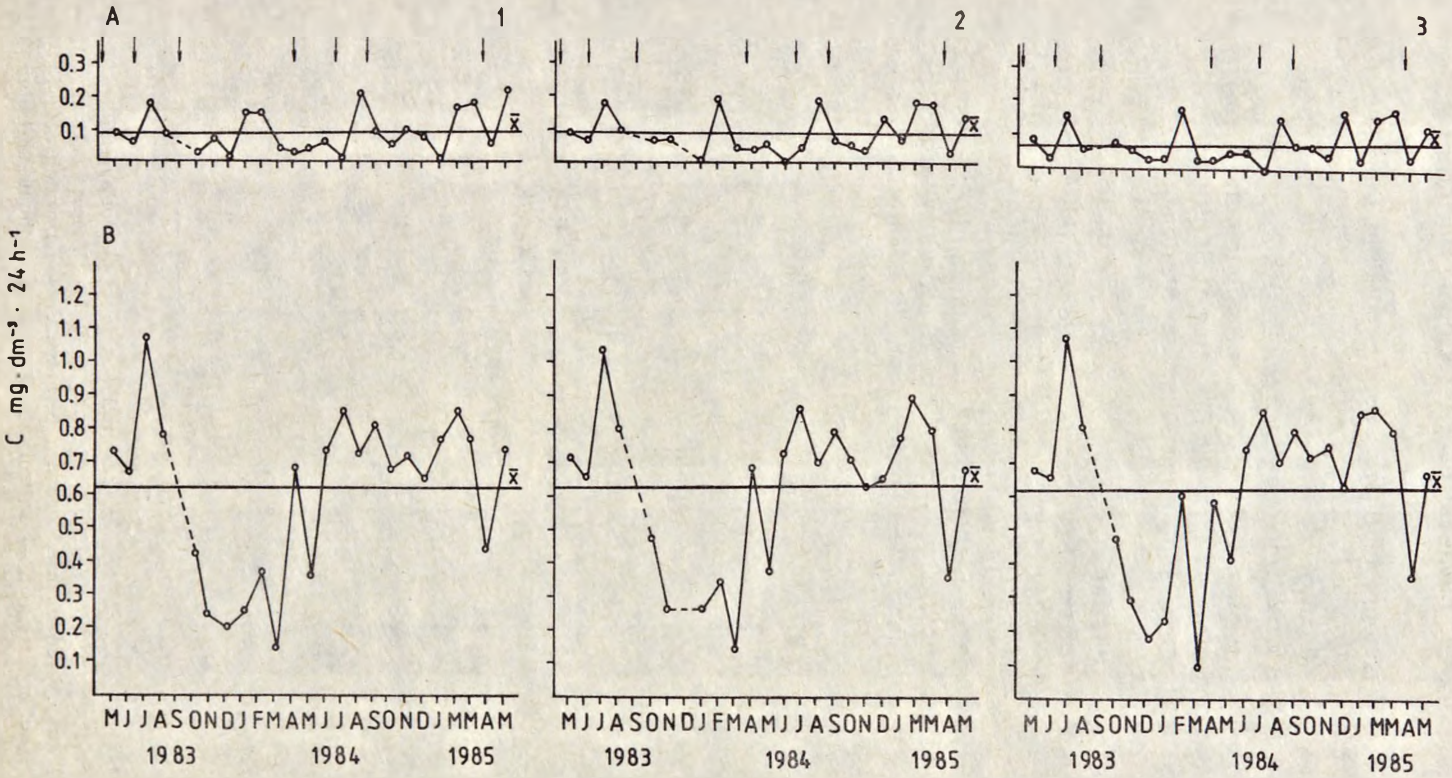

Fig. 2. Changes in the production of bacteria biomass in the years 1983—1985 at Stations 1—3. A — natural water without substrate, B — water with 100 μM dm^{-3} of asparagin; $\bar{x}$ — mean of the investigation period; y — time-limit of fertilizing

4.2. Energy flow at the level of heterotrophic bacteria

The largest amount of energy accumulated in the cells of bacteria was found at Station 3. In the investigated period the average bioaccumulation was 2.00 cal dm^{-3} of water and exceeded that at Station 1 1.2 times and at Station 2 1.7 times (fig. 3).

In the period of investigation the average amount of energy utilized for the respiration of bacteria was the same at Stations 1 and 2 (3.89 cal dm^{-3} 24 h^{-1}) and slightly smaller at Station 3 (3.38 cal dm^{-3} 24 h^{-1}) (fig. 3).

The average total amount of energy utilized for bioaccumulation and biodegradation during 24 hours was similar in the whole investigated river sector and reached 5.49, 5.09 and 5.38 cal dm^{-3} of water at Stations 1, 2 and 3, respectively.

The Schrödinger R/B coefficient confirmed the least energy consumption per biomass unit (62.83%) at Station 3, a slightly larger one (70.86%) at Station 1, and the greatest consumption (76.42%) at Station 2. The A/K

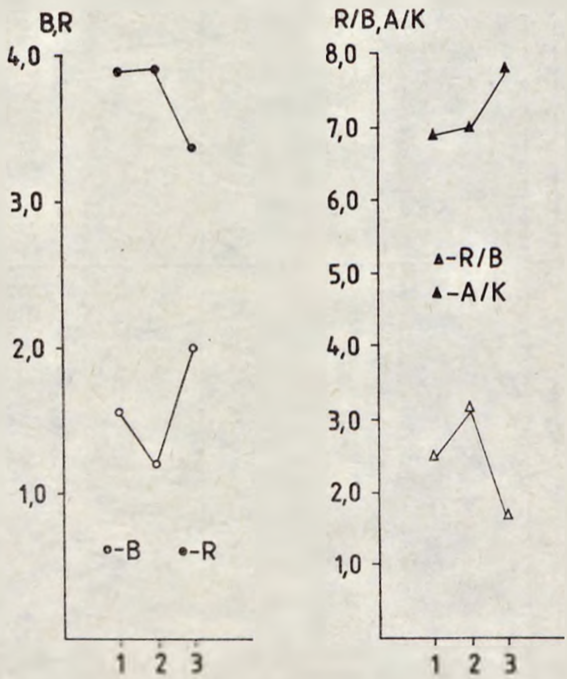

Fig. 3. Mean content of biomass, respiration rate of bacteria, and coefficients R/B and A/K in the period 1983—1985 at Stations 1—3. B — biomass cal dm^{-3}; R — respiration cal dm^{-3}. Production of bacteria biomass: A — in water with 100 μM dm^{-3} of asparagin; K — control in natural water without substrate

coefficient determined in relation to asparagine tended to an increase in the potential activity of the bacterial micro-flora at Stations 2 and 3 (fig. 3).

5. Discussion

The uniform level of production of bacterial biomass in the investigated sector of the River Nida shows that the run-off from the meadow where intensive fertilization ammonium nitrate was applied did not significantly affect the metabolic processes of heterotrophic bacteria. This

Table III. Chemical properties of water of the River Nida. Mean value for the investigation period January 1984 to May 1985 after Bombówna (unpubl.)

Parameter		Stations 1	2	3
Turbidity	SiO_2 mg dm^{-3}	34.8	41.2	36.4
Conductivity	uS ^{o}C	427	430	432
Dissolved oxygen	O_2 mg dm^{-3}	7.82	7.16	7.28
Oxidability	O_2 mg dm^{-3}	6.81	7.18	6.59
BOD_5	O_2 mg dm^{-3}	6.00	5.66	5.71
Mineral nitrogen	N_{min} mg dm^{-3}	2.65	2.68	2.74
Mineral phosphate	P_{min} mg dm^{-3}	1.15	1.18	1.21

suggests that in the 600 m sector of the river there occurred a similar content of biogenes and organic substances which were readily subjected to bacterial axidation. Below the meadow a slightly larger content of organic matter and mineral nutrients was found (Table III), this being expressed by a more abundant development o fgroups of bacteria involved in the conversion of nitrogen and non-nitrogen compounds (S t a r z e c k a unpubl.). Nevertheless, it did not visibly affect the general metabolic activity of mixed communities of aquatic bacteria able to assimilate various substrates. The movement of water in which the bacteria floated, and, thus, the very short time in which they remained in higher concentrations of assimilable substances, might also account for the observed phenomenon. Similarly, W i l l i a m s and C r a w f o r d (1983) found that the concentration of nitrogen nutrients did not significantly affect the activity of bacteria in the environment of a peat bog.

An increase in the production of bacterial biomass in the summer periods was associated with a higher temperature of the water, this unquestionably having a significant effect on the enzymatic kinetics of bacteria and, hence, on their growth and development, i.e., on their activity (B o t t 1975, C y p r y k - O s s o w s k a 1981). In the early spring periods an increased production of biomass may be associated with more abundant amounts of nutrients in the environment, owing to increased surface run-off brought about by the thaw occurring at that time. A similar dependence connected with the inflow of allochtonous organic matter to the water and pollution from the melting snow were observed by Zmysłowska and Sobierajska (1977).

A much greater production of mixed communities of bacteria in the water enriched with asparagine is in accordance with numerous data from the literature, which suggested that an increased concentration of the substrate was followed by an increase in the metabolic activity (Tiwari, Mishra 1982). This also confirms the rapid mineralization of the easily accessible substrate, asparagine, which for bacteria is a source of nitrogen or of carbon and nitrogen jointly (Donderski, Głażewska 1984), and points to the absence of any differences in the metabolic activity of plankton bacteria above and below the fertilized meadow where in the presence of asparagine the production of biomass was uniform (means for the experimental periods).

The average total amount of energy utilized by bacteria for bioaccumulation and biodegradation during 24 hours was similar at all stations, while the direction of energy flow was variable. In the immediate neighbourhood of the meadow bacteria used the most energy, i.e., 76.42% for biodegradation and 1/3 of this amount (23.60%) for bioaccumulation. At the other stations the amount of energy accumulated in the bacterial biomass was greater and the difference in relation to the amount used for biodegradation reduced only by half. However, disregarding the amount of energy released from the environment, both above and below the meadow, the prevalence of biodegradation processes confirms the pronounced eutrophication of the investigated sector of the River Nida, found in earlier studies (Kawecka 1986, Starzecka, Mazurkiewicz 1986).

It should be stressed that the greater utilization of energy per biomass unit, found at Stations 2 (the greatest R/B coefficient) suggests that the inflow of nutrients from the meadow might have contributed to a situation in which the amount of produced energy exceeded the biosynthetic demand in greater measure than at Stations 1 and 3. Thus, at Station 2 the energy scattering exceeded that found at the other two stations. This is in accord with the data reported by Tempest and Neijssel (1978), who show that growth is not directly associated with energy production and, in conditions of an excessive inflow of nutrients, the metabolic mechanism of micro-organisms can reach a high rate of energy production and maintain this rate independently of the energetic demands of biosynthesis.

The fact cannot be ignored that at Station 2 the bioenergetic relations also reflect the eco-physiological reactions of soil bacteria which have entered the river with surface run-off from the intensively fertilized meadow, i.e., from an environment of a very large content of both nutrients and micro-flora. On the other hand, the possible effect of the differentiated hydrological conditions at the stations should be taken into account. At Station 2 the greater depth of the river and faster water current may also contribute to the deformation of relations between the bioaccumulation and respiration of bacteria. As was found at Station 3 at a distance of 200 m below the fertilized meadow, bioenergetic reactions were already shifted (as at Station 1) in the direction of biosynthesis, as was shown by the reduced value of the R/B coefficient. At Station 3 the A/K coefficient was higher by only 0.86 and 0.75 in relation to Stations 1 and 2, respectively, and indicated the uniform trophic level of the aquatic environment above and below the meadow.

This shows that the more pronounced differences in the bio-energetic processes of bacteria, found in the closest neighbourhood of the meadow (Station 2), rapidly disappear. Hence, in the investigated 600 m river sector there occurs a certain equilibrium which was not upset by surface run-off from the intensively fertilized meadow. It would indicate that the run-off from the meadow constituted an ecological factor whose value was much lower than the threshold value which might have disturbed the state of equilibrium in the investigated biological systems.

The presented results and their interpretation may be referred only to lotic water ecosystems and to the properties characteristic for the investigated sector of the River Nida with regard to its trophy, environmental conditions, and current velocity.

6. Polish summary

Wpływ intensywnego nawożenia przybrzeżnej łąki na aktywność bakterii planktonowych rzeki Nidy (Polska Południowa)

W okresie od maja 1983 do maja 1985 przeprowadzono badania nad aktywnością mieszanych zespołów bakterii wodnych na trzech stanowiskach usytuowanych na rzece Nidzie, w miejscowości Chroberz, w sąsiedztwie łąki o powierzchni 1 ha, intensywnie nawożonej saletrą amonową w ilości 240 N kg ha^{-1} rok^{-1} w trzech dawkach po 80 N kg ha^{-1} (ryc. 1, tabela I).

Produkcja biomasy bakteryjnej na badanych stanowiskach była wyrównana i w okresach po zastosowaniu kolejnych dawek nawozowych nie stwierdzono istotnego wpływu na wielkość produkcji biomasy bakteryjnej w wodzie naturalnej, jak i wzbogaconej (ryc. 2A, 2B, tabela II).

Średnia całkowita ilość energii zużywana przez bakterie w ciągu 24 godzin na bioakumulację i biodegradację była podobna na wszystkich stanowiskach, natomiast kierunek przepływu energii był różny. W bezpośrednim sąsiedztwie łąki bakterie zużywały najwięcej (76,42%) energii na biodegradację materii organicznej i 3-krotnie mniej (22,60%) na bioakumulację. Na pozostałych stanowiskach ilość energii zakumulowanej w biomasie bakterii była większa, a różnica w stosunku do ilości energii zużywanej na biodegradację tylko 2-krotnie mniejsza (ryc. 3). Bez względu na ilość

energii uwalnianej ze środowiska, zarówno powyżej, jak i poniżej łąki, przewaga procesów biodegradacji potwierdza wykazane wcześniej znaczne zeutrofizowanie badanego odcinka Nidy (tabela III).

Nie można wykluczyć faktu, że stosunki bioenergetyczne na stanowisku 2 odzwierciedlają również ekofizjologiczne reakcje bakterii glebowych, które dostały się do rzeki ze spływami z łąki intensywnie nawożonej, czyli środowiska zasobnego w substancje pokarmowe i mikroflorę.

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