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The Role of Bacterioplankton and Aquatic Macrophytes in Autopurification of Hydroecosystems Polluted with Phosphorus

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Abstract: The problem of anthropogenic pollution of the freshwater ecosystems with inorganic phosphorus becomes an actual. Bacterioplankton and macrophytes were shown to play a significant role in the utilization of phosphates in the ecosystems but their particular roles remain unclear. This study simulates different biotopes - with and without macrophytes (*Typha angustipholia*). It was found that a number and a structure of bacterioplankton is changed. In the biotope with higher water plants, there are conditions favorable for growth of polyphosphate-accumulating bacteria. These bacteria accumulate polyphosphates and start playing a significant role in the preservation of this element within the planktonic community of the ecosystem.

Key words: Phosphorus · Autopurification · Ecosystem · Macrophyte · Bacterioplankton

INTRODUCTION

The problem of anthropogenic pollution of the freshwater ecosystems with inorganic phosphorus becomes an actual due to a range of reasons. Phosphorus has a highest coefficient of bioaccumulation. While entering into waters it provokes a rapid growth of blue-green algae. This may results in depletion of oxygen, making anaerobic conditions and accumulation of biotoxins. Therefore, elimination of inorganic phosphorus from natural water is of great importance.

Microorganisms are considered the main participants in the elimination of phosphorus from the polluted ecosystems. The element is able to accumulate within bacterial cells as granules of volutin presenting itself a reserve of phosphorus and energy. These granules were described in a range of microbes [1-6]. The accumulation of polyphosphate polymers allows microorganisms to adapt to stress conditions of the environment. Intracellular polyphosphates are also connected with other physiological processes like mobility, biofilm formation, competence and virulence [5, 7].

Macrophytes, being the important components of ecosystems, play a key role in consumption and accumulation of the biogenic elements [8, 9]. It is known that nitrogen and phosphorus are able to accumulate within plant tissues [10-12]. However, the communicative links with bacterioplankton and participation in the circulation of biogens (including phosphorus) remain poorly investigated except for a few studies [13] where the role of submerged macrophytes in the modification of algae and bacteria communities was presented.

For small and shallow reservoirs of the Middle Russia, it is characteristic to observe the introduction of allochtonous organic compounds and a great diversity of macrophytes. An annual production of macrophytes may be equal or exceed production of phytoplankton [8]. Therefore, in those hydroecosystems interactions between macrophytes and bacterioplankton seems to play a crucial role in the processes of circulation of biogenic elements. The detection of role of each participant of this interaction presents an important task.

The aim of the present work was to investigate the role of bacterioplankton in the autopurification processes in model mesocosms with natural water, hydrobionts and macrophytes (*Typha angustipholia*) in a seasonal dynamics.

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MATERIALS AND METHODS

Investigations were performed during a vegetation period from June until October of 2009 in conditions of mesocosms located at the territory of the State Budgetary Establishment Research Institute for Problems of Ecology and Mineral Wealth Use.

For simulation mesocosms, 50 L aquaria were used. At the bottom of the aquaria, a special natural ground (5 cm in depth) from Sredniy Kaban Lake was placed. The experimental aquaria were fullfilled with 30 L of natural water from the same lake. 2 types of biotopes were simulated – overgrown (with curtains of *Typha angustipholia*) and open biotope (without the macrophytes). Both of them were stored for 20 days for adaptation without any influence. After that, mineral salt - Na_3PO_4 was put into the water in concentrations of 2 mg/L and 20 mg/L that corresponds to 1 maximum allowable concentration (MAC) and 10 MAC according to standards for waters of fish-household and drinking utilization. In each biotope, 3 variant were simulated: 1. control - natural water without any supplements; 2. experiment 1 - 1 MAC; 2. experiment 2 – natural water + 10 MAC.

Water samples were isolated before the salt addition and then after every 2 weeks for 4 months (July-October). The total amount of bacterioplankton and polyphosphate-accumulating bacteria was detected via a direct counting at the membrane filters using the MBI-4 microscope. For this purpose, water was filtrated through "Vladipor" filters (Vladimir, Russia) with the size of pores of 0.2 mcm. Then, the filters were stained with toluidine blue to obtain metachromatic stain of volutin granules. General calculation of amount of bacteria and bacteria with volutin granules was made. At the each filter, more than 500 cells in 10 areas of vision were calculated and more than 100 cells were measured. Bacteria sizes were detected with linear ocular micrometer. Bacterial volumes, biomass and carbon content were calculated according to the standard methods [14]. Hydrochemical analysis of natural water was also performed.

Using class index for water quality (RosHydroMet), a condition of hydroecosystem of the model mesocosm was assessed using a number of bacterioplankton (Table 1).

Paired t-tests were used for statistical analysis; $p<0.05$ was considered to indicate significance; data are presented as mean ± SD. The correlation analysis was also applied.

Table 1: Classification of water quality according to RosHydroMet index

Class of water quality	Level of water pollution	Total amount of bacteria, 10^6 cell/mL
1	Very pure	< 0,5
2	Pure	0,5-1,0
3	Slightly polluted	1,1-3,0
4	Polluted	3,1-5,0
5	Dirty	5,1-10,0
6	Very dirty	> 10,0

RESULTS

In the control variant of the open biotope, there was a synchronous increasing of number and biomass in a seasonal dynamics with a maximum in October 12; in the overgrown biotopes, there was no a seasonal dynamics - peaks in August 7 and 24 and in October 12 were marked. Number and biomass were higher in the open biotope in comparison with the overgrown one (Figs. 1&2).

Addition of phosphates into the open biotopes resulted in increasing of a total number of bacteria and their biomass in comparison with control (Figs. 1&2). Autopurification on bacterioplankton was absent during a period of observations. Depending on a time of water sampling, waters were characterized as slightly vicious, very vicious and dirty.

In the overgrown biotopes (Figs. 1&2), there was decreasing a number and biomass of bacteria 2 months after addition of 1 MAC of phosphates; in other words, a process of autopurification was detected. The analogous process was found in the control biotope with higher water plants. Waters were characterized as pure or slightly vicious depending on a time of sampling.

By the end of the vegetation period, there was an increasing of a number of bacteria in the overgrown biotopes that is probably connected with death of the higher water plants and phytoplankton.

Addition of phosphates in 1 and 10 MAC resulted in increasing volutin-containing bacteria in each biotopes (Fig. 3). In the control biotopes, there was a low amount of polyphosphate-accumulating bacteria (up to 7% of a total number) while after addition of 1 MAC of the compound the percent of the bacteria reached 20%. After addition of 10 MAC, in the open biotope, the percent of the bacteria was 12% while in the overgrown - 24%. The percent of polyphosphate-accumulating bacteria was about the same during all time of the experiment but sometimes in the overgrown biotopes the percent of the bacteria was higher despite a low concentration of phosphates (Fig. 3). It was detected that the concentration of total phosphorus was reducing,

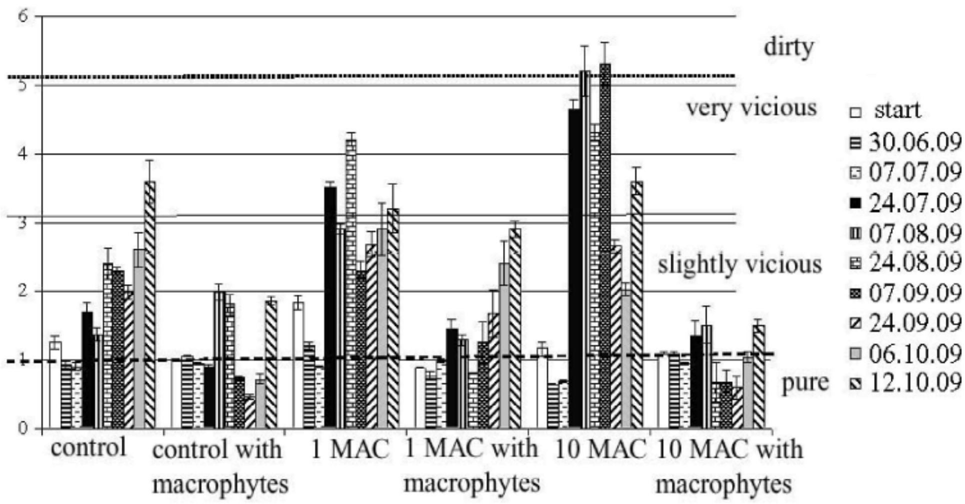

Fig. 1: A total number of bacterioplankton in a seasonal dynamics. Axis Y presents this parameter in cell per mL x 10^6

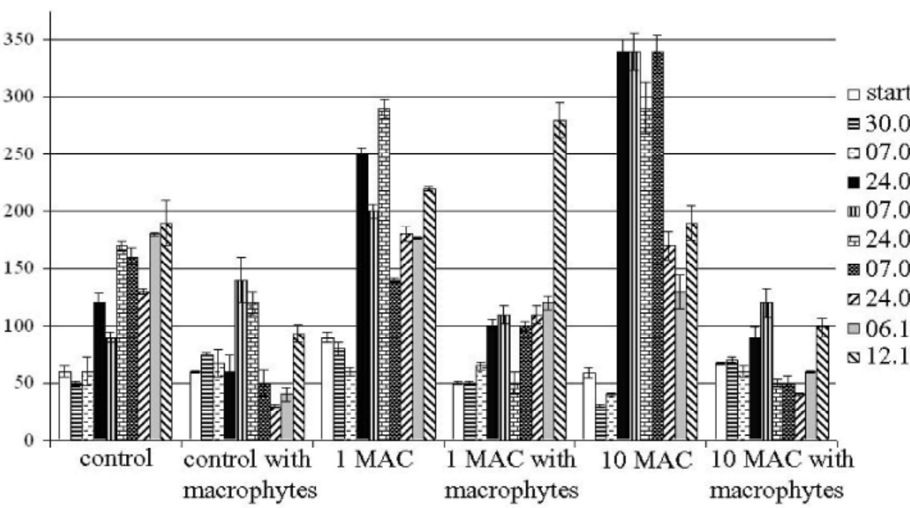

Fig. 2: Biomass of bacterioplankton in a seasonal dynamics. Axis Y presents this parameter in mcg C per L

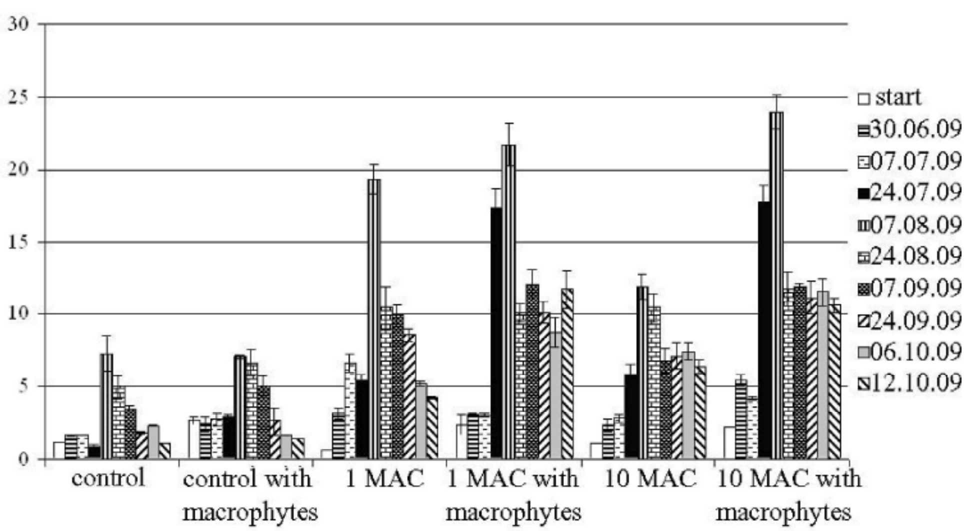

Fig. 3: The percent of polyphosphate-accumulating bacteria from a total number of bacterioplankton (axis Y, %)

Table 2: Data of hydrochemical analysis from various types of biotopes. Note: the amount of phosphates is presented as mg per cubic decimeter

			Open biotope						Overgrown biotope				
Date	Tap water	Initial water	Control	1 MAC	10 MAC	NP 5:1	NP 20:1	Initial water	Control	1 MAC	10 MAC	NP 5:1	NP 20:1
2.06.	0,1	0,065						0,05					
22.06.		0,182						0,05					
30.06.			0,08	0,57	5,05	1,96	0,68		0,102	0,05	3,9	0,68	0,31
7.07.			0,05	0,237	1,99	0,374	0,259		0,139	0,05	0,86	0,156	0,105
20.07.			0,301	0,44	1,63	1,86	0,624		0,05	0,05	0,634	0,75	0,05
24.08.			0,57	2,2	1,42	1,53	0,82		0,05	0,05	0,05	0,1	0,05
23.09.			0,36	2,18	3,16	1,07	0,34		0,05	0,05	0,05	0,05	0,05
13.10.			1,86	1,66	9,95	9,56	0,89		0,05	0,05	0,05	0,05	0,05

reached control values during a small period and was conserved by the end of the experiment (Table 2).

It should be noted here that increasing of total number of bacterioplankton and polyphosphate-accumulating bacteria was detected in the end of July and in the beginning of August, i.e. there was a seasonal features in alterations of structure and number of bacterioplankton.

The correlation analysis showed that a number of polyphosphate-accumulating bacteria had a significant positive link with a total number of bacterioplankton (correlation coefficient - 0.9, $p<0.05$).

DISCUSSION

It is well known that bacterioplankton is responsible for phosphorus utilization in limnetic waters [15, 16] However, there is no a bulk of data on factors influencing consumption of phosphorus by bacteria; the role of planktonic community and biogeochemical cycles are not very clear yet.

The performed studies for the first time describe the role of bacterioplankton in the processes of autopurification of waters. It means that in the open and overgrown biotopes the compound is assimilated by bacteria and it may accumulate in the form of polyphosphates. It has been already found that microorganisms have developed diverse mechanisms of resistance that allow them to colonize contaminated environments. However, the mechanisms of microbial regulation and coordination in response to stress remain poorly understood. Cellular polyphosphates have been proposed to serve as reservoirs for phosphate and as chelators of metal ions and in gene regulation [17]. Polyphosphates have also been linked to a variety of microbial physiological processes for example biofilm development [18] It was found that addition of inorganic phosphorus resulted in increasing of bacterioplankton number, especially in the open biotopes. At that, only in biotopes with higher water plants, there was a process of autopurification in the second part of the vegetation period (decreasing a bacterial number).

It is known that in small lakes the growth of bactrioplankton may be limited by availability of nitrogen, phosphorus and/or dissolved organic carbon and macrophytes may directly or indirectly influence biomass and production of bacterioplankton through consumption and extraction of the biogenic elements [19, 20].

Macrophytes may negatively influence bacterioplankton through various mechanisms. Firstly, the negative effects of macrophytes may be mediated by its active consumption of phosphorus and decreasing this element in the water environment [20]. Moreover, it was found that in the presence of macrophytes, there is increasing number and activity of zooplankton [21]. Secondly, macrophytes act through inhibition of phytoplankton as a source of organic carbon for bacterioplankton. It is known that the classical viewpoint [22, 23] that phosphorus determinates the development of bacterioplankton becomes less popular. It was detected that increasing a density of macrophytes may result in decreasing phytoplankton biomass [24]. In other words, macrophytes may reduce availability of nutrient compounds for bacterioplankton.

Also, we detected that polyphosphate-accumulating bacteria have their own input into consumption of inorganic phosphorus in biotopes. However, the reasons for increasing of a number of the bacteria are different in the open and overgrown biotopes. This may be explained by the different content and amount of extra-cellular excreta of macrophytes that may influence a number of the bacteria.

While phosphorus concentration is increasing, there is enhancement of its consumption by phytoplankton [25-27]. The function of the bacteria is probably consisted in the accumulation of the biogenic compounds from the environment. Phosphorus accumulated by bacterial cells is included into plankton that, in turn, excretes the dissolved forms of the element needed at synthesis of organic compounds by phytoplankton. This is especially characteristic to oligotrophic systems where the role of bacterioplankton is consisted in the preservation of phosphorus in the photic zone of the reservoir that makes the element available for planktonic community of the hydroecosystem [28, 29].

Thus, owing to macrophytes, a number and a structure of bacterioplankton is changed. In the biotope with higher water plants, there are conditions favorable for growth of polyphosphate-accumulating bacteria. This may be connected with the presence of chemicals excreted by the plants that may be useful to bacteria as food substrate. Anyway, these bacteria accumulate polyphosphates and start playing a significant role in the preservation of this element within the planktonic community of the ecosystem.

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