

## Participation of Planktonic and Benthic Bacteria in the Polyphosphate Accumulating Process in Mesocosms Contaminated with Phosphates and Nitrates

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**Abstract:** Participation of planktonic and benthic bacteria in accumulation of polyphosphates in conditions of nitrates and phosphates overload was investigated in model mesocosms with natural water containing hydrobionts and curtains of *Typha angustifolia*. Experiments were performed in seasonal dynamics. Dynamic of number of bacterioplankton and benthic bacteria was studied in ecosystems of water and ground. It was detected that structure and number of bacterioplankton and benthic bacteria depended on the presence of higher aquatic plants in model biotopes. In overgrown biotopes overload on nitrates and phosphates resulted in development of planktonic and benthic bacteria. Reduction of trophic status of hydro ecosystem on bacterioplankton and low concentrations of biogens were favorable conditions for accumulation of polyphosphates by bacteria.

**Key words:** Bacterioplankton • Nitrates • Phosphates • Benthic Bacteria • Auto Purification

### INTRODUCTION

Intensive pollution of surface waters and sediments results in reduction of their ability for auto purification. Bacterioplankton is one of the most important groups of hydrobionts determining elimination of nitrates and phosphates from polluted aquatic ecosystems [1-6]. It is known that bacterial activity is higher in eutrophic systems in comparison with oligotrophic ones. Nevertheless, it is detected that bacterioplankton influences actively intra reservoir processes in oligotrophic systems. Therefore, in oligo- and mesotrophic lakes the significant proportions of phosphorus are consumed by bacterioplankton [6-10]. Bacteria are able to consume substances at low concentrations. In eutrophic systems where the rate of introduction of inorganic substances is very high, phytoplankton may fix introducing phosphorus for cell growth [8, 11, 12]. Some bacteria may preserve phosphorus as inorganic polyphosphate granules

(volutin) [2, 5, 13]. Inorganic polyphosphates are detected in a variety of microbes [1, 14-18]. Accumulation of these polymers is one of the ways to survive in severe environment. Bacteria may grow even in conditions of biogenic element deficiency in ecosystems of various types [19-21]. Intracellular polyphosphates influence some bacterial processes like motility, biofilm formation, competence and virulence [14, 16].

Aquatic macrophytes play an important role in consumption and accumulation of biogenic elements [22, 23]. At high concentrations of nitrogen and phosphorus, plants have more high concentrations of these elements in plant tissues [24-26]. It is known that interaction between macrophytes and microorganisms have a vital importance in the process of turnover of biogenic elements in reservoirs [27, 28]. Therefore, it was of special interest to investigate in this research the role of bacterioplankton in accumulation of polyphosphates and adaptation of hydro ecosystems with aquatic macrophytes toward overload of nitrates and phosphates.

## MATERIALS AND METHODS

Investigations were performed in experimental tanks with natural ground (10 L), water (30 L), organisms of sediment and planktonic communities and curtains of *Typha* plants taken from Sredniy Kaban Lake (Kazan, Russia). This volume of water satisfied with requirements for experimental justification [29, 30]. There were two types of biotopes – overgrown with *Typha* plants and without them. Investigations were performed over 2 years (2009-2010). In 2009 nitrates and phosphates were introduced at 100 и 20 mg/L ( $N_{100}P_{20}$ ) and 400 and 20 mg/L ( $N_{400}P_{20}$ ). Then, water samples were taken since June till October with the aim of detection of total bacterioplankton number and the number of polyphosphate-accumulating bacteria. In 2010, nitrates and phosphates were introduced at concentrations of 600 and 30 mg/L ( $N_{600}P_{30}$ ). Since May till September samples of water and ground were taken to study a number of bacteria. Bacterioplankton number and the amount of polyphosphate-accumulating bacteria were assessed using a direct calculation on membrane filters with MBI-4 microscope. For this purpose, water samples were placed to sterile flasks with 2% formalin. Ground samples were taken from depths of 0.5-1 cm. Then, 1 g of the sample was placed to sterile tube with 4 mL of distilled water and 2% formalin. To separate the adsorbed bacteria, standard procedures were used [31-33]. To intensify the separation, ultrasound device UZV-1,3TTC was used (35 kHz, «Sapphire», Russia) for 6 min (2 min with 30 s interruption). Then, samples were washed with 5 mL of distilled water and the necessary volume was sampled for the following dilutions in distilled water. All samples were filtered through Vladipor filters (Russia) (pore size of 0.2 mcm). Then, the filters were stained with toluidine blue to obtain metachromatic color of volutin granules. Over 500 cells in 10 visual fields were assessed at each filter to check the number of bacterioplankton and bacteria. Considering the number of bacterioplankton, waters in model mesocosms

were assessed according to nomenclature of ROSHYDROMET [34]. Paired t-tests were used for statistical analysis;  $p < 0.05$  was considered to indicate significance and data were presented as mean  $\pm$  SD.

## RESULTS

It was detected that the combined introduction of nitrates and phosphates in concentrations 100 and 20 ( $N_{100}P_{20}$ ) and 400 mg/L and 20 mg/L ( $N_{400}P_{20}$ ) to model biotopes resulted in increase of bacterioplankton number. In open biotopes, by the end of the observation period (October) the water quality was characterized as slightly polluted (Fig. 1 and Table 1). In overgrown biotope, two months later after the introduction of nitrates and phosphates ( $N_{100}P_{20}$ ) there was a process of bacterial auto purification and water quality was stated as pure (Fig 1, Table 1). After introduction of the pollutants at concentrations of  $N_{400}P_{20}$  the water quality both in open and overgrown biotopes was characterized as slightly polluted (Fig. 1 and Table 1).

In all biotopes by the end of the experiment, there was increased number of bacteria that may be connected with a death of higher aquatic plants or/and phytoplankton. It should be noted that there was a high positive correlation between the number of bacterioplankton and BCO<sub>2</sub>/5 days (biological O<sub>2</sub> consumption over 5 d) – with correlation coefficient of 0.7.

The most prominent number of polyphosphate-accumulating bacteria ( $2.2 \times 10^6$  cells/mL) was detected in overgrown biotope at  $N_{400}P_{20}$ . It is very likely that this development of polyphosphate-accumulating bacteria may also be connected with a high number of bacterioplankton characteristic to this type of biotope ( $6.3 \times 10^6$  cells/mL) (Fig. 1 and 2). The correlation analysis showed that in all biotopes the number of polyphosphate-accumulating bacteria was positively correlated with the total number of bacterioplankton (coefficient 0.9).

Table 1: Classification of water quality according to RosHydroMet index [34]

Class of water quality	Level of water pollution	Total amount of bacteria, $10^6$ cell/mL	Number of saprophytic bacteria, $10^3$ cell/mL	Total number of bacteria/ Number of saprophytic bacteria
1	Very pure	< 0.5	< 0.5	> $10^3$
2	Pure	0.5-1.0	0.5-5.0	> $10^3$
3	Slightly polluted	1.1-3.0	5.1-10.0	$10^3$ - $10^2$
4	Polluted	3.1-5.0	10.1-50.0	< $10^2$
5	Dirty	5.1-10.0	50.1-100.0	< $10^2$
6	Very dirty	> 10.0	> 100	< $10^2$

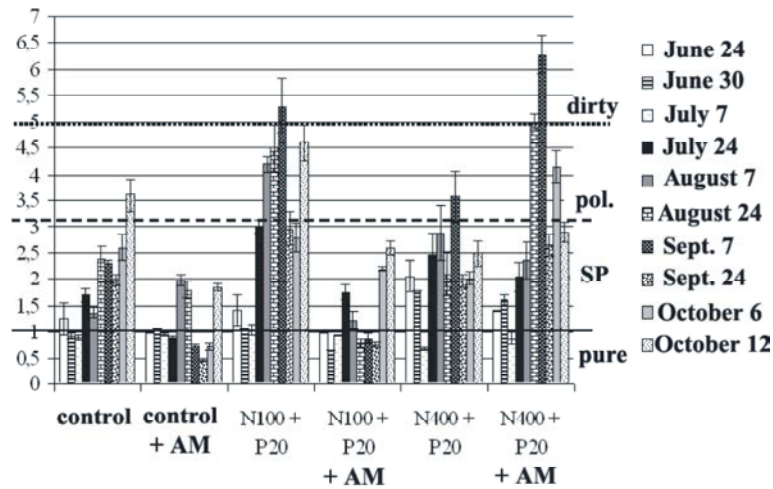


Fig. 1: Total number of bacterioplankton (axis Y), cell/mL x 10<sup>6</sup>.

P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes. SP - slightly polluted, pol. - polluted. Water quality was presented according to RosHydroMet.

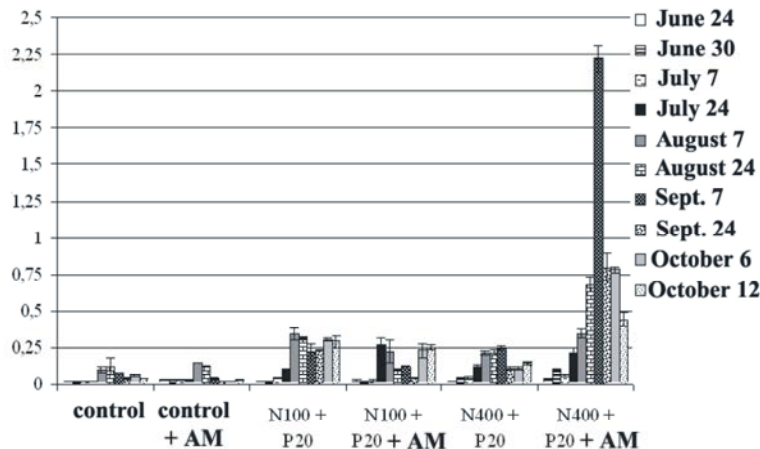


Fig. 2: Total number of polyphosphate-accumulating bacteria (axis Y), cell/mL x 10<sup>6</sup>.

P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

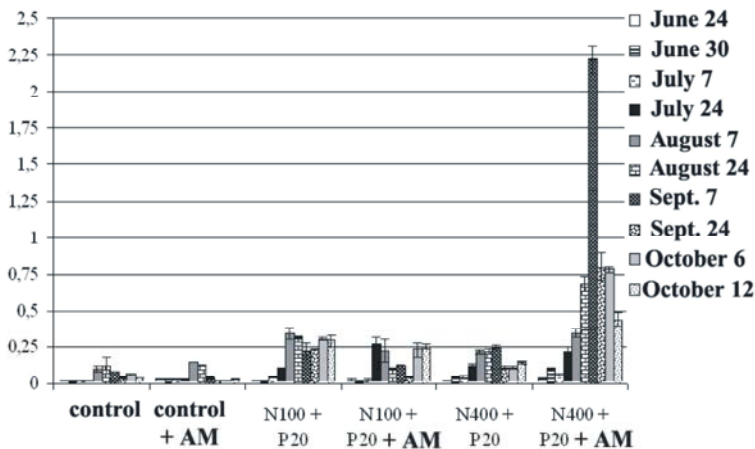


Fig. 3: Proportion (%) of polyphosphate-accumulating bacteria (axis Y) of total number of bacterioplankton.

P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

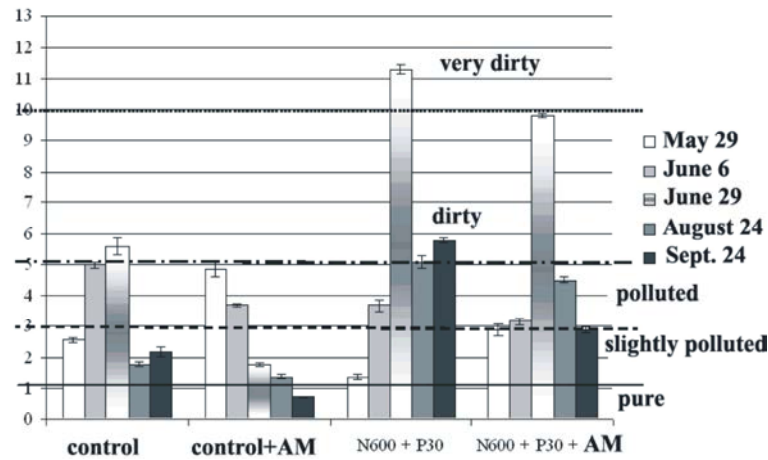


Fig. 4: Total number of bacterioplankton (axis Y), cell/mL x 10<sup>6</sup>.

P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes. SP - slightly polluted, pol. - polluted. Water quality was presented according to RosHydroMet.

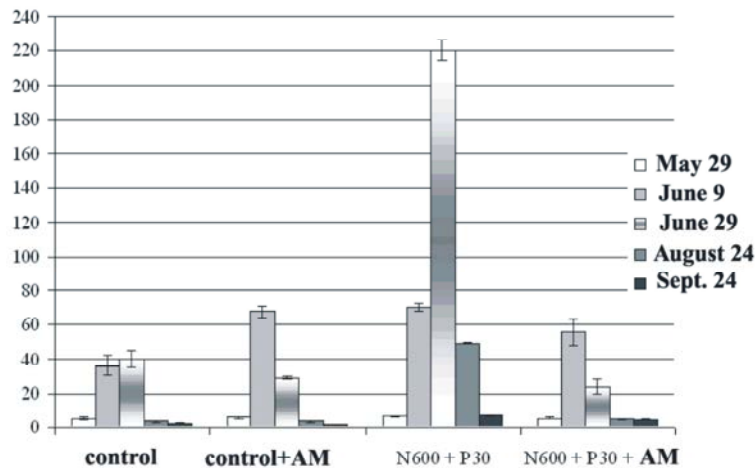


Fig. 5: Total number of bacteria from ground (axis Y), cell/mL x 10<sup>6</sup>.

P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

At concentrations  $N_{100}P_{20}$  и  $N_{400}P_{20}$  in overgrown biotopes the percent of polyphosphate-accumulating bacteria reached 18% and 35% (Fig. 3). In open biotopes, the proportion of polyphosphate-accumulating bacteria was at the control value (8-10%) (Fig. 3).

In all biotopes with *Typha* plants the concentrations of the introduced nitrates and phosphates were reduced and reached the control values. In open biotopes contaminated with nitrates and phosphates, the amount of the latter was higher than in control during the whole period of observation. In open biotopes with low concentrations of biogens ( $N_{100}P_{20}$ ) concentrations of nitrates were reduced rapidly while at  $N_{400}P_{20}$  concentration of nitrates was high and reduced only by the end of the experiment.

Due to extremely high summer temperatures in 2010, a number of bacterioplankton was high both in control and experimental variants. The most prominent number of bacterioplankton reached 9, 8-11,  $3 \times 10^6$  cells/mL (at  $N_{600}P_{30}$ ) (Fig 4). By the end of the observation period, the water quality was characterized as slightly polluted (in overgrown biotopes - 2,  $1-3 \times 10^6$  cells/mL) and dirty (in open biotopes - 5,  $8 \times 10^6$  cell/mL) (Fig. 4 and, Table 1).

In all variants, we detected increase of benthic bacteria right after the starting of the experiment (Fig. 5). Number of bacteria was 2 order higher till  $2,2 \times 10^{10}$  cell/g of wet ground in open biotope and till 3, 5-6,  $5 \times 10^9$  cell/g in other mesocosms that suggested eutrophication of the hydro ecosystems of the model biotopes.

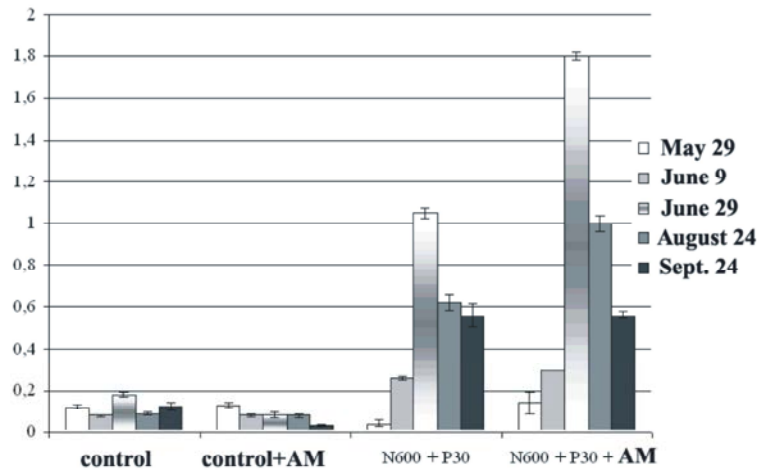


Fig. 6: Total number of polyphosphate-accumulating bacteria (axis Y), cell/mL x 10<sup>6</sup>.  
P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

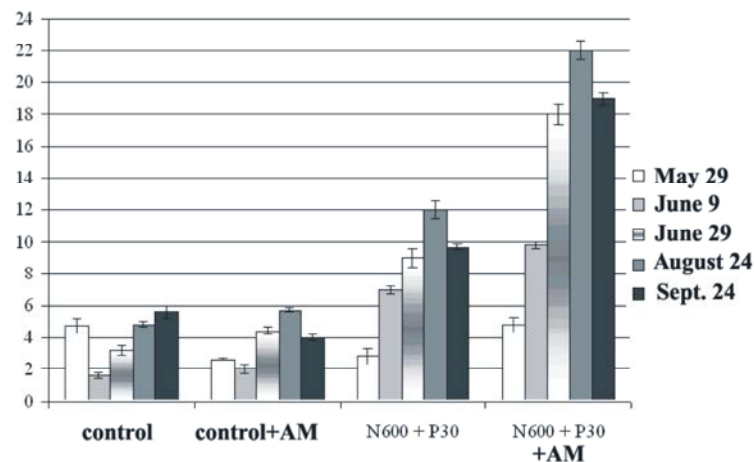


Fig. 7: Percent of polyphosphate-accumulating bacteria (axis Y) of total number of bacterioplankton.  
P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

The total amount of benthic bacteria reduced till  $1.2-7.4 \times 10^8$  cell/g by the end of the experiment (Fig. 5).

In all mesocosms, polyphosphate-accumulating bacteria grew actively. These bacteria reached  $1.05-1.8 \times 10^6$  cell/mL in comparison with control variants (0., 08-0.,  $18 \times 10^6$  cell/mL) and became reduced till  $0.56 \times 10^6$  cell/mL by the end of the experiment (Fig. 6). The percent of polyphosphate-accumulating bacteria reached maximal values (12% and 22% for open and overgrown biotope, respectively) by the end of the experiment (Fig. 7).

In biotopes with combined pollution (nitrates+phosphates) activation of the growth of polyphosphate-accumulating bacteria was similar to those in the ground (Fig. 8). Total number of bacteria was increased in the ground at eutrophication but the proportion of polyphosphate-accumulating bacteria was

about 1-4% (Fig. 8 and 9). When the total number of bacteria was reducing, the proportion of polyphosphate-accumulating bacteria was about 14 and 31% in open and overgrown biotopes, respectively (Fig. 9). In other words, in the ground, in parallel with a growth of bacterial number, there was an increase in number of polyphosphate-accumulating bacteria but their proportion was reducing. Then, simultaneously with the process of bacterial auto purification of a ground, the number of polyphosphate-accumulating bacteria was reducing but their percent was increasing (Fig. 9).

Concentration of mineral phosphorus was low in all biotopes. In variants with low biogen concentrations, the amount of phosphorus in overgrown biotope was significantly lower than in open biotope. The amount of nitrates was high in all biotopes and reduced only by the end of the experiment.

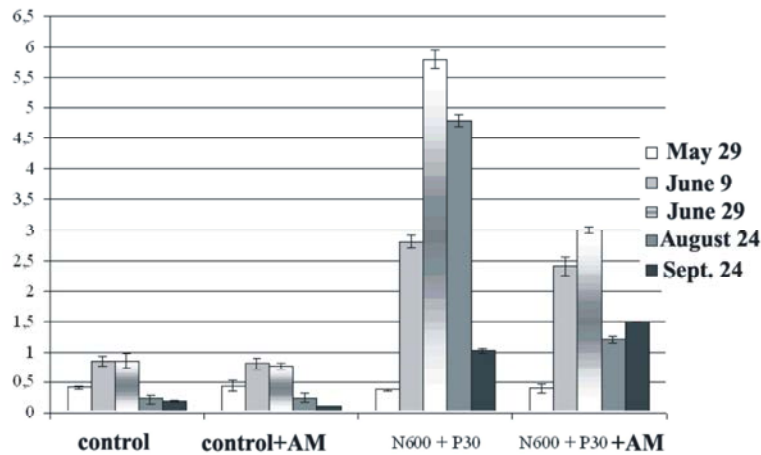


Fig. 8: Total number of polyphosphate-accumulating bacteria in ground (axis Y), cell/mL x 10<sup>8</sup>. P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

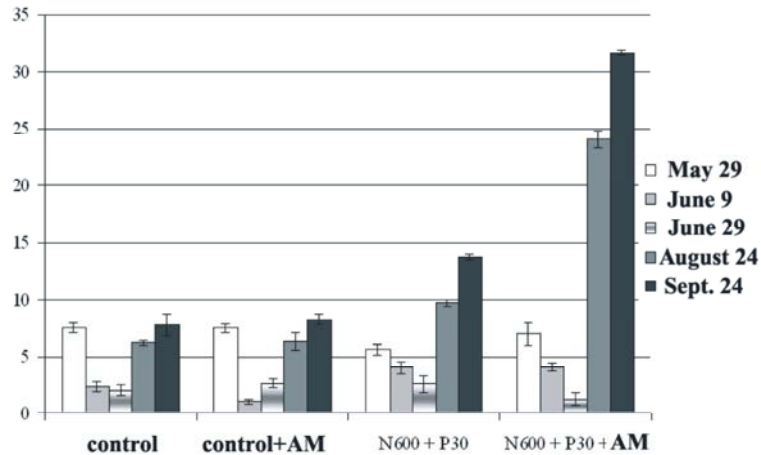


Fig. 9: Percent of polyphosphate-accumulating bacteria (axis Y) of total number of bacteria from ground. P - mineral phosphorus, N - nitrate nitrogen, AM - aquatic macrophytes.

## DISCUSSION

According to modern studies, microorganisms are important and dominating components of the planktonic trophic networks in freshwater hydroecosystems [35-37]. It is known that heterotrophic bacteria are responsible for phosphate accumulation but little is known about factors that may mediate the effect of planktonic communities and biogeochemical cycles on this process [2, 6, 8, 9]. Our results showed changes in the bacterioplankton and trophic status of hydro ecosystem. It should be noted that eutrophication process positively depends on the concentration of biogens. It is likely that in conditions of successive organics there was a prominent ability of heterotrophic bacteria to utilize easy-to-degrade organic substrate. In conditions of bacterial auto purification, the function of volutin-accumulated bacteria was in storage

of phosphorus inside the planktonic community of ecosystem [38].

It should be noted that activation of bacterial growth in overgrown model biotopes occurred at low concentrations of biogens that is in agreement of our previous investigations [39]. Owing to aquatic macrophytes, we detected changes both in number and structure of bacterioplankton and microbial community of the ground. Only in overgrown biotopes we detected favorable conditions (low biogen concentrations and reduction of the trophic status of hydro ecosystem) for accumulation of polyphosphates by bacteria.

At the present study, little is known on key mechanisms mediating dynamics of phosphorus in water and sediments. By this reason, investigation of dynamics of number of polyphosphate-accumulating bacteria seems perspective in researching auto purification processes,

detection of water quality as well as in utilization of this bacterial group for indication of pollution of hydroecosystems with mineral phosphorus.

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