Ecological trends of spatial distribution and of size-morphological structure of bacteriobenthos in the Kuibyshev Reservoir, the Volga River, Russia

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Abstract. The abundance and biomass of bacteriobenthos, as well as morphological and size structure of bacteriobenthos community have been studied in 2016 in the Kuibyshev Reservoir. Three areas of the reservoir, differing by the features of the spatial distribution of bacteriobenthos, were defined: the downstream area of lake-like reaches, the mixed area of the Volga and Kama rivers confluence, and the upstream area of variable backwater. Trends in the increase in the number and biomass of bacteria along the longitudinal axis from the upstream reaches to the dam were revealed in the downstream area of lake-like reaches and in the upstream area of variable backwater. The mixed area of the Volga and Kama rivers confluence was characterized by a patchy distribution of bacteriobenthos. The abundance of bacteria in the bottom sediments was 3.6-10.5×10^9 cells mL^{-1}, biomass, 263-971 μg mL^{-1}. There was an increase in the abundance and biomass of bacteria in the area of the Volga and Kama rivers confluence. In the morphological structure of bacteriobenthos, the main morphological group were bacilli, followed in importance by coccobacilli. The predominant size classes of bacteria were 0.2-0.5 μm, 0.5-1.0 μm, and 1.0-2.0 μm. A number of reliable correlations were obtained between the characteristics of bacteriobenthos and the grain-size composition of sediments, organic matter content, and humus content. The share of aleurite fraction, organic matter content, and humus content affect the trends in the spatial distribution of quantitative indicators of bacteria. Main morphological and size groups of bacteria were significantly related to certain size fractions of the mechanical composition of bottom sediments, which affects the spatial distribution of these groups along the bottom of the reservoir.

1. Introduction
The Kuibyshev Reservoir is of particular interest for the study of bacteriobenthos in regard to the peculiarities of the morphometry and hydrological regime, significant size, physico-chemical diversity and heterogeneity of bottom sediments, and the functional importance of the bacterial community in the processes of the natural cycles of organic and biogenic matter. The Volzhsky Reach and the Kamsky Reach are two areas of variable backwater in the upstream reaches of the reservoir [1]. The fluctuating margin is a characteristic feature of these areas, the instability of their borders keeps throughout the year, pronounced especially at high water period. The reservoir is characterized by well-expressed alternation of lake-like extensions (reaches) and sharp narrowings. The Volga-Kama Reach and the Tetyushinsky Reach form the upstream part of the reservoir proper. In this area, the waters coming from the Volzhsky...
Reach and Kamsky Reach are merging and mixing. The middle part of the reservoir includes the Ulyanovsk Reach, the downstream part includes the Novodevichensky and Priplotinny Reaches [1].

Regular microbiological studies of bottom sediments of the Kuibyshev Reservoir were carried out from the very beginning of its establishing at the operation mode (in 1957) and lasted until 1997. The regularities of development, structure and seasonal dynamics of the bacteriobenthos community, its role in the functioning of the reservoir ecosystems, bacterial reduction of sulphates, the intensity of microbiological destruction processes, and the methane cycle were studied [2-6]. A.V. Ivatin noted that there was an increase in the abundance of bacteria along the longitudinal axis of the reservoir [5]. Water temperature, content and qualitative composition of labile organic matter, and redox potential were named as the most important factors for the development of bacteria in bottom sediments [6].

The studies of bacteriobenthos were renewed in 2010 during the complex expeditions of the Institute of the Ecology of the Volga Basin of Russian Academy of Sciences. The total abundance and biomass of bacteriobenthos were determined, an increase in the bacteria abundance in the Volga-Kama Reach (the contact zone of the Volga and Kama rivers’ water masses) was reported, and positive correlations of the total abundance of bacteria with the content of organic matter and aleurite-pelite fraction of bottom sediments were found [7]. In 2015, the studies were aimed to determine the abundance of aerobic (saprophytes) and anaerobic (butyric-acid and sulphate-reducing) groups of bacteriobenthos; as a result, the functional relationship of sulphate-reducing, butyric-acid, and saprophytic bacteria with plant pigments was shown [8]. However, the regularities of the distribution of bacteriobenthos in the bottom sediments of the reservoir remain poorly understood. The study aims to analyze the influence of the grain-size composition, organic matter content, and humus content on the spatial distribution of bacteriobenthos (its abundance, biomass, and size-morphological structure of community) in the Kuibyshev Reservoir.

2. Materials and Methods

The bottom sediments were sampled in July 2016 during the complex expedition of the Institute of Ecology of the Volga Basin of Russian Academy of Sciences. In total, 21 samples were obtained from the 0-5-cm surface layer of bottom sediments using a lot with a capture area of 100 cm$^3$ (figure 1).

![Figure 1. Schematic map of sampling stations in the Kuibyshev Reservoir in July 2016](image)
carried out by a combined method: wet sieving and further gravity precipitation. Several size fractions of grain-size composition were distinguished: coarse sand (> 1 mm), medium-size and fine sand (0.1-
0.1 mm), aleurite (0.1-0.01 mm), and pelite (< 0.01 mm) [13]. The sediment types were identified by standard approach [14].

The abundance and biomass of bacteria were determined under fluorescence microscopy using the fluorescein isothiocyanate (FITC) dye [15]. Sediment samples were fixed with a 25% glutaraldehyde solution to a final concentration of 2.5%. A fixed and suspended suspension was filtered through nuclear filters with a 0.2-μm pore diameter (Joint Institute for Nuclear Research, Russian Academy of Sciences, Dubna, Russia). Cells were counted under a Leica DM5500 B microscope at ×1000 magnification for 20 visual fields per slide. The morphology of bacteria was assessed by the length to width ratio of cell: cocci (< 1.2), coccobacilli (1.2-1.8), bacilli (1.8-10), filaments (> 10) [16]. The size classes were: < 0.01 μm, 0.1-0.2 μm, 0.2-1.0 μm, 1-2 μm, 2-5 μm, 5-10 μm, > 10 μm. Statistical data processing was performed in the Excel 2010 program; the significance level was set as \( p \leq 0.05 \). Mean values are presented with confidence intervals \( (M \pm CI) \). Correlation analysis was used to determine the relationships between the quantitative characteristics of bacteriobenthos and abiotic factors. The main variables were the granulometric composition, the content of organic and humus matter, the abundance and the biomass of bacteria.

3. Results and Discussion

In 2016, the bottom sediments of the reservoir were represented mainly by grey aleurite silts (table 1). Along the channel sections located nearby Mordovo, Ulyanovsk, and Soroch’i Gory, finely dispersed grey silts with an olive tint were formed. In the left-bank floodplain, nearby Volostnikovka village and in the Syukeevsky Channel, there were sandy silts, in the estuaries of the Ilet’ and Tsivil’ rivers, silty sands.

| No. | Stations               | Bottom sediments          | Particle content (%) | CI (%) | Hs (%) |
|-----|------------------------|---------------------------|----------------------|--------|--------|
| 2   | Yablonevy ravine (riverbed) | Aleurite silt             | 5.53                 | 11.38  | 2.6    |
| 4   | Akhtushi (riverbed)     | Aleurite silt             | 11.24                | 10.89  | 4.3    |
| 6   | Mordovo (riverbed)      | Fine silt                 | 4.92                 | 10.18  | 2.5    |
| 8   | Sengleevsky (riverbed)  | Aleurite silt             | 7.00                 | 9.17   | 1.9    |
| 10  | Ulyanovsk (riverbed)    | Fine silt                 | 3.38                 | 11.61  | 1.8    |
| 11  | Ulyanovsk-Zavolzhye (left-bank floodplain) | Sandy silt | 34.48             | 5.67   | 1.6    |
| 12  | Volostnikovka (riverbed) | Aleurite silt             | 8.08                 | 10.53  | 2.3    |
| 13  | Volostnikovka (left-bank floodplain) | Medium-size and fine sands | 90.40          | 1.28   | 1.7    |
| 14  | Syukeevsky Vzvo (riverbed) | Sandy silt | 47.10             | 11.66  | 2.1    |
| 16  | Atabaevo (riverbed)     | Aleurite silt             | 5.45                 | 9.76   | 2.6    |
| 18  | Imenkovo (riverbed)     | Aleurite silt             | 3.32                 | 9.08   | 2.1    |
| 19  | Imenkovo (left-bank floodplain) | Aleurite silt | 1.77             | 7.97   | 2.1    |
| 20  | Krasnovidovo (riverbed) | Aleurite silt             | 5.36                 | 13.04  | 2.2    |
| 21  | Sviyaga River estuary    | Aleurite silt             | 3.40                 | 10.72  | 1.8    |
| 23  | Ilet’ River estuary      | Silt sand                 | 75.65                | 2.8    | 1.5    |
| 26  | Tsivil’ River estuary    | Silt sand                 | 84.75                | 2.3    | 1.5    |
| 28  | Soroch’i Gory (riverbed) | Fine silt                 | 7.56                 | 8.76   | 2.1    |

The aleurite fraction (0.1-0.01 mm) prevailed in silts (73.73±4.59%). The fraction of coarse sand (> 1 mm) was practically absent and was found only in the left-bank floodplain of Ulyanovsk-Zavolzhye.
transect, in the estuaries of the Ilet’ and Tsivil’ rivers (0.65-6.61%). The share of medium-size and fine sand (1.0-0.1 mm) varied from 1.77% to 47.10% in sandy silts, and from 75.65% to 90.40%, in silty sands. The share of pelite fraction in fine and aleurite silts was 20.69±4.46%, in sandy silts, 5.58±1.47%.

The grey silts contained the largest amount of organic matter (7.97-13.04%), the minimum values were found in sandy sediments (1.28-2.80%). In the studies of other authors, it is shown that, there is an increase in the content of biogenic elements and organic matter with a decrease in particle sizes in the main types of bottom sediments in all studied reservoirs [13, 17]. There was also a relationship between particular physical and chemical indicators of sediments and quantitative indicators of bacteria (table 2). In particular, the content of organic matter and humus content correlated significantly with the share of aleurite and pelite fractions, and the abundance and biomass of bacteria depended greatly on the share of aleurite fraction, calcination losses, and humus content. Therefore, the growth of bacteria was significantly influenced by the share of aleurite fraction, which accumulated organic matter and humus.

Table 2. Correlation of physical and chemical characteristics of bottom sediments and quantitative indicators of bacteriobenthos of the Kuibyshev Reservoir in July 2016 (ρ ≤ 0.05)

| Variables | 1.0-0.1 | 0.1-0.01 | < 0.01 | C1 (%) | Hs (%) | N (×10⁷ cells mL⁻¹) | B (µg mL⁻¹) |
|-----------|---------|---------|--------|--------|--------|------------------|-------------|
| 1-0.1     | 1.00    | -0.96   | -0.67  | -0.83  | -0.43  | -0.56            | -0.32       |
| 0.1-0.01  | -0.96   | 1.00    | 0.44   | 0.82   | 0.49   | 0.59             | 0.37        |
| < 0.01    | -0.67   | 0.44    | 1.00   | 0.54   | 0.14   | 0.25             | 0.06        |
| C1 (%)    | -0.83   | **0.82** | **0.54** | 1.00   | 0.51   | 0.54             | 0.38        |
| Hs (%)    | -0.43   | **0.49** | 0.14   | **0.51** | 1.00   | 0.45             | 0.39        |
| N (×10⁷ cells mL⁻¹) | -0.56 | **0.59** | 0.25   | **0.54** | 0.45   | 1.00             | 0.82        |
| B (µg mL⁻¹) | -0.32 | **0.37** | 0.06   | **0.38** | **0.39** | 0.82             | 1.00        |

The total abundance of bacteriobenthos varied as 3.6-10.5×10⁶ cells mL⁻¹ of wet weight of sediment, the biomass, as 263-971 µg mL⁻¹, in the Kuibyshev Reservoir in July 2016 (figure 2). The minimum values of the abundance and biomass of bacteria were observed in sandy sediments in the areas adjacent to the estuaries of the Ilet’ and Tsivil’ rivers (stations nos. 23 and 26), the maximum values were found in the silts of the Volzhko-Kamsky Reach (station no. 16) and at downstream areas of the Priplotinny Reach (station no. 2). Comparison of our data with the results of long-term studies [2, 5-7] evidenced that the abundance of bacteria in the bottom sediments increased from 1966 to 2016. In 1966, the abundance of bacteria was 0.3-6.0×10⁶ cells mL⁻¹ [5], in 1997, 0.8-3.7×10⁶ cells mL⁻¹ [6], in 2010, it increased up to 1.9-8.6×10⁶ cells mL⁻¹ [7].

Three areas of the bottom of the reservoir may be distinguished in regard to the spatial distribution of the abundance and biomass of bacteria (figure 2). Area I comprises the lake-like extensions with slow waters (Priplotinny, Ulyanovsky, and Novodevichensky Reaches), area II is the confluence of the Volga and Kama rivers (Volga-Kamsky Reach and Tetyushinsky Reach), area III is characterized by variable backwater and the greatest flow rates (Volzhsky Reach and Kamsky Reach). This conditional grouping of bottom sediments as the habitat for bacteriobenthos corresponds to the hydrological zoning of the reservoir [1]. In the area of river confluence, there was an increase in the content of organic matter in silts, so the highest average values of abundance (9.10±1.17 cells mL⁻¹) and biomass (841±141 µg mL⁻¹) of bacteria were noted. The increasing of the abundance of bacteriobenthos at the Volga-Kama Reach was first reported by the author in 2010 [7]. The lowest average abundance of bacteria (5.5±1.17 cells mL⁻¹) was typical for the area of variable backwater. However, there was a high biomass (583±135 µg mL⁻¹) due to the large size of bacterial cells in the estuarine areas (stations nos. 21, 23, 26). In the area of lake-like reaches (Priplotinny, Ulyanovsky, and Novodevichensky Reaches), the average abundance of bacteriobenthos was 6.1±1.51 cells mL⁻¹, the average biomass, 443±124 µg mL⁻¹.

Based on the statistical analysis of the entire data set, a number of trends in changing the studied parameters were revealed along the longitudinal axis of the reservoir, which were most pronounced in
some areas. There was an increase in the share of thealeurite fraction (0.1-0.01 mm), humus content, losses during calcination, and the abundance of bacteria from the upstream reaches of the reservoir to the Priplotinnny Reach. These tendencies were most reliably expressed in the area of lake-like extensions with a slow water flow, i.e. from Tetyushinsky Reach to the dam (figure 3, 4). The pelite fraction of silts and the bacterial biomass also had similar pattern in this area, but to a lesser extent.

**Figure 2.** Abundance and biomass of bacteria in the bottom sediments of the Kuibyshev Reservoir in July 2016: I – Priplotininny, Ulyanovsky, and Novodevichensky Reaches; II - the area of mixing of the waters of Volga-Kamsky and Tetyushinsky Reaches; III – the area of variable backwater of Volzhsky and Kamsky Reaches.

**Figure 3.** Trends in the content ofaleurite fraction (0.1-0.01 mm), organic matter (Cl), humus matter (Hs) along the longitudinal axis of the Kuibyshev Reservoir (Priplotininny, Ulyanovsky, and Novodevichensky Reaches).

No regularities in the distribution of bacteriobenthos and grain-size composition were found in the Volga-Kamsky and Tetyushinsky Reaches. Probably, this was due to a change in the hydrodynamic regime in the contact zone of the water masses; this was a result of mixing of different grain-size particles in the surface layers of bottom sediments.

The bacteriobenthos was represented by bacilli, cocci, diplococci, coccobacilli, and filamentous bacteria. Bacilli formed chains, clusters, and rosettes; cocci were found in colonies and packages. Bacilli were the main morphological group of bacteriobenthos (by abundance) along the bottom sediments (table 3). Coccobacilli contributed significantly to the total abundance of bacteria. The abundance of cocci was small, and the filamentous bacteria were found rarely.
The morphological structure of bacteriobenthos community changed along the longitudinal profile of the reservoir from the upstream reaches to the dam; in particular, the abundance of cocccobacilli increased, on the contrary, the abundance of bacillus-shaped cells decreased. According to correlation analysis, there were certain relationships, which explained the observed spatial pattern of distribution of major cell morphological types to some extent. The share of pelite fraction had a direct effect on the coccobacilli abundance (\( R = +0.51 \)) and coccobacilli abundance (\( R = +0.50 \)). The share of aleurite fraction had the same effect (cocc, \( R = +0.26 \); and cocccobacilli, \( R = +0.32 \)). Bacilli exhibited positive correlation with the share of fine and medium-size sand fraction (\( R = +0.44 \)). However, unexpectedly high negative correlation was obtained for bacilli and the share of pelite fraction (\( R = -0.62 \)). It is reasonable to assume that fine sands and fine dispersed fractions of the silts play an important role in the spatial distribution of coccus- and bacillus-shaped forms of bacteria along the bottom of the reservoir. The absence of reliable correlations between the organic matter and humus contents and the type of bacterial cells may indicate that the morphological structure of bacteriobenthos depends on a wide range of food substrates. Cell morphology is determined by physical principles, some of which are access to nutrients, attachment to surfaces, and ability to perform active movements [18]. The cell increases the cell surface to volume ratio in low-nutrient medium [18]. Therefore, bacilli prefer less organic-rich sandy sediments compared to that in fine silts; this may be considered as a manifestation of morphological plasticity that promotes their survival.

Small and medium-size bacteria of the size classes 0.2-0.5 \( \mu m \), 0.5-1.0 \( \mu m \), and 1-2 \( \mu m \) dominated in the bacterial community (table 3). Very small and large cells with linear sizes of 0.1-0.2 \( \mu m \) and > 5 \( \mu m \) were found in minor quantities. It should be noted that there were no bacteria belonging to the 0.1-0.2-\( \mu m \) group in the upstream reaches of the reservoir, in the Volga-Kamysky and Tetyushinsky Reaches. Bacteria of the size class of 0.2-0.5 \( \mu m \) correlated positively with the share of pelite fraction (\( R = +0.31 \)) and with calcination losses (\( R = +0.48 \)); this explained the confinement of small-cell bacteria to the downstream area of lake-like reaches. Bacteria of the size class of 1-2 \( \mu m \) showed a positive correlation

\[ y = -0.803x + 9.7796 \]
\[ R^2 = 0.817 \]

\[ y = -49.409x + 665.34 \]
\[ R^2 = 0.4584 \]

\[ y = -61.781x + 767.92 \]
\[ R^2 = 0.4036 \]

\[ y = 0.2x + 8.6 \]
\[ R^2 = 0.047 \]

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with the share of the fractions of fine and medium-size sand \( R = +0.49 \), they had the greatest abundance in the upstream area of the variable backwater.

4. Conclusion

Three areas were identified in regard to the distribution of bacteriobenthos in the Kuibyshev Reservoir, which differed by the hydrodynamic regime: (1) the downstream area of lake-like extensions from the dam to the Tetyushinsky Reach, (2) the mixed area of the Volga and Kama rivers’ confluence (Tetyushinsky and Volga-Kamsky Reaches), and (3) the upstream area of variable backwater (Volzhsky and Kamsky Reaches). The share of aleurite fraction in bottom sediments correlates positively with organic matter content, humus content, and the abundance of bacteria; this preconditions the trends in the spatial distribution and structural organization of bacteriobenthos. In the area of variable backwater, especially, in the downstream lake-like reaches, abundance and biomass of bacteriobenthos increase along the longitudinal axis, from the upstream reaches of the reservoir to the dam. In the area of joining the Volga-Kamsky and Tetyushinsky Reaches, bacteriobenthos is characterized by a patchy distribution.

In July 2016, abundance of bacteria in the Kuibyshev Reservoir was \( 3.6-10.5 \times 10^9 \) cells mL\(^{-1}\), biomass, 263–971 \( \mu \)g mL\(^{-1}\). The effect of increasing the abundance and biomass of bacteria in the area of the Volga and Kama rivers’ confluence (the Volga-Kamsky and Tetyushinsky Reaches), first discovered by author in 2010, was confirmed. The main morphological groups of bacteriobenthos were bacilli and cocccobacilli, the main size groups, small and medium-size cells (0.2–0.5 \( \mu \)m, 0.5–1.0 \( \mu \)m, and 1–2 \( \mu \)m). There were opposite trends in the distribution of cocccobacilli and bacilli along the longitudinal axis of the reservoir from the upstream sections to the dam, preconditoned by the share of fine and sandy fractions of bottom sediments. In particular, there was an increase in the share of cocci and, on the contrary, a decrease in the share of bacilli. Very small cells with a linear size of 0.1–0.2 \( \mu \)m were common only in the downstream part of the reservoir in the area of lake-like extensions, up to the Tetyushinsky Reach. The main morphological and size groups of bacteria correlated significantly with the certain fractions of bottom sediments, which preconditoned spatial distribution of the bacterial groups of certain cell size and morphology along the bottom of the Kuibyshev Reservoir.

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