Taxonomic composition of the bacteriobenthos of the littoral and pelagic zones of the Lake Kandry-Kul in June and September 2010 (Natural Park "Kandry-Kul", Republic of Bashkortostan, Russia)

N G Sherysheva1,2, T A Rakitina1 and L P Povetkina1
1 Samara Federal Research Scientific Center RAS, Institute of Ecology of Volga River Basin RAS, Togliatti, Russia
2 Togliatti State University, Togliatti, Russia

E-mail: sapfir-sherry@yandex.ru

Abstract. The taxonomic composition of the bacteriobenthos of the littoral and pelagic zones of the Lake Kandry-Kul' was analyzed by gas chromatography-mass spectrometry (microbial markers). The studied communities comprise 39 taxa of the Bacteria domain. Two dominant taxa were a characteristic of bacteriobenthos in the littoral zone in June, one dominant taxon, in September. The taxonomic structure of bacteriobenthos of the pelagic zone was characterized by two dominant taxa both in June and September. There were common dominants in the littoral and pelagic biotopes, namely, representatives of the genera Butyrivibrio, Spirochaeta, and of the group of iron-reducing bacteria (FeRB). The species Clostridium difficile, Geothrix fermentans, and the genus Thiobacillus were specific to the littoral bacteriobenthos community. The bacterial community of the pelagic zone was characterized by a change of dominant taxa in autumn. The increase of bacteriobenthos biomass and its particular taxa was significantly affected by the water temperature, redox potential, and the content of humic substances.

1. Introduction
The Lake Kandry-Kul' is the largest water body in the Republic of Bashkortostan, representing a unique water ecosystem. In 1996, the Natural Park "Kandry-Kul" was established especially for the lake protection [1]. Bottom sediments of aquatic ecosystems serve as a habitat for microorganisms that play an important role in organic matter destruction. Chemical components accumulated by the bottom sediments are transformed due to the vital activity of microorganisms. The bacterial community of bottom sediments (i.e. bacteriobenthos) comprises a number of bacteria species. The taxonomic structure of the bacteriobenthos is formed depending on local environment and is affected by various factors. The redox potential, availability of organic matter and its qualitative composition, environmental pH, and concentration of biogenic elements may be named as limiting factors [2].

In 2010, an expedition was performed by the Institute of the Volga River Basin of the Russian Academy of Sciences. The comprehensive studies of the ecosystem of the Lake Kandry-Kul' were carried out in order to identify the trends and patterns of spatiotemporal variability of abiotic and biotic parameters of the water column and bottom sediments of the Lake Kandry-Kul'. As a result of
these studies, the data on the structure and functioning of biotic communities of bacteria, algae, ciliates and zooplankton were obtained, which were then summarized as the monograph [3]. However, the information about the species composition of bacteriobenthos of the Lake Kandry-Kul' is still missing in the literature.

The study aims to analyze the taxonomic structure of the bacterial communities of littoral and pelagic zones of the Lake Kandry-Kul' in summer and autumn. Certain abiotic factors significant for bacteria have been considered (pH, redox potential, granulometric composition of bottom sediments, the content of humic substances, carbonates, and total iron content in silts), since these factors affect greatly the taxonomic structure of bacterial community.

The method of gas chromatography–mass spectrometry (GC-MS) of microbial markers was applied; this made it possible to study the qualitative and quantitative composition of natural communities of microorganisms to a certain extent.

2. Materials and Methods
The study of the bacterial communities of the littoral and pelagic zones was conducted in June and September 2010 at two stations of the Lake Kandry-Kul' (figure 1). Bottom sediments were sampled from the surface layer (0—5 cm) by a 100-cm³ sinker.

![Figure 1. Sampling site map in the Lake Kandry-Kul' in 2010: 1 – station in the littoral zone (2.5-m depth), 2 – station in the pelagic zone (14.6-m depth). Bottom sediments: I – grey and black nitidous silts; II – grey silts; III – aleurite and sandy silts; IV – sandy silts, silted sands, macrophytic silts, local areas of black silts; V – sand; VI – unexplored area around the protected Utrau Island.](image)

The temperature, pH, and redox potential (Eh) were measured in the silt during sampling using the ELWRO N5123 pH-meter. The grain-size composition [4, 5], the content of carbonates [6], humic substances [7], and total iron [8] were determined in the laboratory.

A highly sensitive method of gas chromatography–mass spectrometry of microbial markers (GC-MS) was applied to analyze the taxonomic composition of bacterial community, referring to the database [9, 10]. The method allows to determine the genera/species of bacteria, which abundance exceeds \(10^4\) Cells g⁻¹ of silt. The analysis was carried out by G.A. Osipov, the author of the method, using the AT 5973 gas chromatography–mass spectrometer (Agilent Technologies), in the laboratory of the scientific group of Academician Yu. F. Isakova, Russian Academy of Medical Sciences (Moscow, Russia).

3. Results and Discussion

3.1. Bottom sediments of the lake
The littoral station was located on the southern shore of the lake, in the thickets of aquatic vegetation, at a depth of 2.5 m. The bottom sediments of this biotope were formed by marshy grey fine-aleurite
sils with whitish granular particles and the shells of the zebra mussel *Dreissena* spp. In June, the silt was soft, with some remains of aquatic vegetation and detritus. In September, the silt turned black, which indicated active microbiological destruction processes. A significant part of the silt in the littoral biotope was presented by semi-decomposed plant material in autumn.

At the deepest station in the pelagic zone (14.6 m), the bottom was covered by grey fine homogeneous silts. In June, the silts were nitidous and soft, with brown and black layers; in September, they became dark grey with olive spots. The physico-chemical characteristics of the bottom sediments of the two stations are presented in Table 1.

**Table 1.** The physico-chemical characteristics of bottom sediments of the Lake Kandry-Kul’ in June and September 2010

| Station | T (°C) | pH | Eh | C<sub>c</sub><sup>a</sup> (mg g<sup>-1</sup>) | Fe<sub>ox</sub> (mg g<sup>-1</sup>) | Hs<sup>b</sup> (%) | Particle content (%) with size (mm) |
|---------|-------|----|----|--------------------------------------|-----------------|----------------|----------------------------------|
|         |       |    |    |                                       |                 |                | > 1.0 | 1.0 - 0.1 | 0.1 - 0.01 | < 0.01 |
| Littoral | 19.8  | 7.2 | 10 | 12.9                                 | 2.1             | 3.7            | 9.4         | 16.3     | 53.3      | 21.0   |
| Pelagial | 7.5   | 7.4 | -20| 4.9                                  | 3.1             | 0.57           | 14.2        | 28.8     | 46.1      | 10.9   |
| Littoral | 17.0  | 7.7 | -60| 5.2                                  | 1.5             | 3.8            | 7.0         | 33.2     | 46.9      | 12.9   |
| Pelagial | 10.0  | 7.6 | -80| 6.0                                  | 2.8             | 1.3            | 0.7         | 28.0     | 58.6      | 12.7   |

<sup>a</sup> content of total carbonates (C<sub>c</sub> = CO<sub>2</sub> + HCO<sub>3</sub> + CO<sub>3</sub>2-).

<sup>b</sup> content of humic substances.

The silts of the littoral zone were characterized by a higher temperature and were more enriched with humic substances, in comparison with that in the pelagic zone. In contrast, the silts for the deep-water area had a higher total iron content. Fine fractions prevailed in the silts of the littoral zone in summer; in autumn, the pattern was the opposite. Thus, the content of aleurite-pelite fractions was 74.4% in the coastal silts in June and 71.4% in the deep-water silts in September.

### 3.2. Bacterial community of the bottom sediments

The bacteriobenthos abundance was determined by the method of microbial markers and was 2.72×10<sup>6</sup> Cells g<sup>-1</sup> of dry silt in the coastal area in June, and 3.96×10<sup>5</sup> Cells g<sup>-1</sup> in September. As the lake depth increased, the number of bacteria in the bottom sediments decreased down to 1.88×10<sup>5</sup> Cells g<sup>-1</sup> in June and to 1.47×10<sup>5</sup> Cells g<sup>-1</sup> in September (figures 2, 3).

According to the correlation analysis, the temperature and content of humic substances in silts are the factors influencing the quantitative distribution of the bacteriobenthos in the littoral and pelagic zones. A significant correlation (ρ < 0.05) was found between the bacteria abundance in both biotopes with the content of humic substances (R = +0.85) and with temperature (R = +0.72). The maximum abundance of the coastal bacteriobenthos in September was preconditioned by the development of a group of anaerobic bacteria, which, in turn, was caused by the decrease in the redox potential in the bottom sediments in autumn (table 1) as a result of the decomposition of dying aquatic vegetation. This is confirmed by significant inverse correlations of the redox potential (Eh) with the anaerobic bacteria abundance, i.e. iron-reducing bacteria (R = -0.82), genus *Butyrivibrio* (R = -0.80), genus *Desulfitomaculum* (R = -0.68), and genus *Acetobacterium* (R = -0.91).
Figure 2. Taxonomic composition and bacteria abundance in the silts of the littoral and pelagic zones of the Lake Kandry-Kul’ in June 2010: ■ — littoral zone, ■ — pelagic zone.

Figure 3. Taxonomic composition and bacteria abundance in the silts of the littoral and pelagic zones of the Lake Kandry-Kul’ in September 2010: ■ — littoral zone, ■ — pelagic zone.

Thirty-nine taxa of genera/species rank belonging to the Bacteria domain were identified in the bottom bacterial community of the Lake Kandry-Kul’ by the GC-MS method. The representatives of the family Enterobacteriaceae, a group of iron-reducing (FeRB Lovley, FeRed KM-2), Cyanobacteria (Anabaena, Aphanizomenon, Microcystis) and two unidentified strains of bacteria Zor-1/ZOR-2 Boldareva, ZOS-R Osipov were found. A significant similarity (86%) of the taxonomic composition of the coastal and deep-sea bacteriobenthic communities was observed (figures 2, 3). The coastal bacterial community comprised 39 taxa, the deep-water community, 35 taxa. Clostridium difficile, Geothrix fermentas, and Thiobacillus spp. were absent in the silts of the pelagic zone. Consequently, these organisms are specific to the littoral bacteriobenthos community.
The representatives of the genera *Clostridium* (27.8×10^6 Cells g⁻¹) and *Spirochaeta* (13.5×10^6 Cells g⁻¹) were the dominant taxa by biomass in littoral bacteriobenthic community. In the pelagic zone, these were the representatives of the family *Enterobacteriaceae*, genera *Butyrivibrio*, *Spirochaeta*, *Heliobacterium*, as well as *Nocardia carnea*, *Clostridium* sp., and iron-reducing strains FeRed (Turova), ranging as 3.14—7.50×10^6 Cells g⁻¹. The qualitative composition of bacterial communities did not change significantly from June to September. By autumn, the representatives of genera *Aeromonas* and *Burgholderia* disappeared or sharply reduced their abundance down to the values of less than 10^6 Cells g⁻¹ of silt). The species *Clostridium difficile* and the genus *Thiobacillus* were not found in the coastal community in autumn. In the community of the pelagic zone, *Eubacterium moniliforme* and *Actinomadura roseola* appeared in autumn.

### 3.3. Dominance structure of bacteriobenthos communities

The share of certain species in the bacterial community is another important indicator of the species structure, in addition to the indicators of the qualitative composition and bacteria abundance. Here, we studied the dominance structure in terms of abundance, ranging the bacteria taxa into four groups: insignificant or minor, secondary, sub-dominant, and dominant (table 2). The degree of dominance was set taking into account the quantitative characteristics of the community structure: minor species comprised less than 1%; secondary species, 1—5%; subdominants, 5—10%; dominants, more than 10% [11].

**Table 2. Dominance structure of the bacteriobenthos communities in the Lake Kandry-Kul' in 2010**

| Biotope  | Month | Dominants, > 10% | Sub-dominants, 5–10% | Secondary, 1–5% | Minor, < 1% |
|----------|-------|------------------|----------------------|-----------------|------------|
| Littoral | June  | 27               | 12                   | 54              | 7          |
| zone     | September | 22           | 25                   | 46              | 8          |
| Pelagial | June  | 34               | 7                    | 50              | 8          |
| zone     | September | 41           | 13                   | 35              | 11         |

The species of two genera, *Butyrivibrio* and *Spirochaeta*, dominated in the bacteriobenthos of the littoral and pelagic zones in June. Their contribution to the total abundance of bacterial communities was 27% and 34%, respectively (table 2). In September, *Butyrivibrio* became the absolute dominant in the community of the littoral zone (34%). The representatives of this genus dominated in the bottom sediments of the pelagic zone as well, but the second dominant (*Spirochaeta*) was replaced by the iron-reducing bacteria, FeRB Lovley (41%). The active development of the iron-reducing bacteria (FeRB Lovley) in the deep-water zone in September might be due to a higher content of total iron in the silt compared to that in the littoral zone, as well as due to the decrease in the redox potential in autumn (table 1). Consequently, the anaerobic conditions were formed in the silts, favourable for the development of iron-reducing microorganisms.

In summer, there were two dominant taxa in the bacterial communities: in shallow waters, these were the representatives of the genera *Butyrivibrio* and *Spirochaeta*, in the deep-water zone, the species of the genus *Butyrivibrio* and the iron-reducing bacteria FeRB Lovley. It should be noted that *Butyrivibrio* had a stable leading position throughout the summer and autumn period, becoming the only dominant in September in the coastal bacterial community (figures 2, 3). The contribution of *Butyrivibrio* to the total bacteria abundance was the highest both in June (15—24%) and in September (22—26%). It is known that *Butyrivibrio* is a strict anaerobe characterized by a fermentation-type metabolism; it is able to decompose cellulose, starch, and other polysaccharides, so this microbe prefers to inhabit the thickets of aquatic vegetation. In the coastal area, there are favourable conditions, especially in autumn, due to the decrease of redox potential as a result of intensive drying and decomposing of macrophytes.

In the littoral community, *Nocardia carnea* and iron-reducing bacteria (FeRB Lovley) were the sub-dominants in summer. In September, *Desulfotomaculum* and *Spirochaeta* developed, so *N. carnea*
held the secondary rank. In the pelagic zone, one genus *Rhodococcus* was the sub-dominant in summer, but in September, there were two sub-dominants, genera *Eubacterium* and *Spirochaeta*.

The taxonomic diversity of bacterial communities in both biotopes was based on secondary species (table 2). In summer, their share in the bacteria abundance was 54% in the shallow waters and 50% in the deep-water zone. In autumn, the abundance of secondary species has decreased due to the development of dominant and sub-dominant organisms to 46% and 35%, respectively. Minor species made up 7-11% in the bacteriobenthos communities.

The composition of bacterial communities, studied by different authors, demonstrates large spatial variations of taxa in bottom sediments of freshwater lakes of various types. Thus, quantitative PCR results revealed 25 types and 68 orders of bacteria in sedimentary bacterial communities of Lake Poyang (China), including the most common taxa in freshwater bottom sediments: *Burkholderiales*, *Myxococcales*, *Sphingobacteriales* (Bacteroidetes), *Gallionellales*, *Nitrospirales*, *Xanthomonadales*, *Desulfuromonadales* [12]. In the large ecosystem of Lake Erie (Great Lakes, USA), a wide variety of bacterial taxa with a wide range of metabolism have been identified – genera *Azospirillum*, *Nitrospira*, *Steroidobacter*, *Acidovorax*, *Geobacter*, *Desulfovomonas*, *Desulfovibrio*, *Caldithrix*, *Methylphilus*, *Anaeromyxobacter*, *Geobacter*, *Thiobacillus* [13]. *Anaeromyxobacter* and *Desulfovomonas* were the most dominant taxa among the anaerobes. Typical for the entire lake are *Acidobacteria*, *Bacteroidetes* and *Nitrospira*. Using the method of microbial markers, 42 taxa of species ranks from the domain Bacteria were identified in the bottom sediments of the Selenga River (Buryatia, Russia) [14]. The leading role in the formation of the qualitative composition of the community is played by representatives of the phyla *Firmicutes*, *Proteobacteria* and, to a lesser extent – *Actinobacteria* and *Cyanobacteria*. The dominant Proteobacteria are microorganisms of the genus *Aeromonas*. Thus, the taxonomic structure of bacteriobenthos of Lake Kandy-Kul', in general, corresponds to that of bacterial communities in sediments of other large freshwater lakes.

4. Conclusion

The taxonomic structure of bacteriobenthos of the bottom sediments of the littoral and pelagic zones of the Lake Kandy-Kul' in summer and autumn was described using the method of mass spectrometry of microbial markers and traditional hydrochemical methods. The bacteriobenthos was represented by 39 taxa of the genera/species rank, belonging to the family Enterobacteriaceae, to cyanobacteria of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, and to the group of iron-reducing bacteria (FeRB Lovley, FeRed KM-2).

Analysis of environmental factors made it possible to conclude that the quantitative development of bacteria was significantly affected by the temperature regime and the content of humic substances; in case of dominant anaerobic bacteria, by redox potential.

The taxonomic composition of the bacteriobenthic communities of the littoral and pelagic zones was characterized by high similarity. In the coastal biotopes, specific organisms developed, namely, *Clostridium difficile*, *Geothrix fermentans*, and the representatives of the genus *Thiobacillus*. During summer and autumn, certain bacteria species disappeared from the bacteriobenthic communities, replaced by new ones. The domnants were the representatives of the genera *Butyrivibrio* and *Spirochaeta*, and iron-reducing bacteria (FeRB Lovley, FeRed KM-2), subdominants, *Nocardia carnea*, genera *Desulfotomaculum*, *Spirochaeta*, *Rhodococcus*, and *Eubacterium*. The representatives of genus *Butyrivibrio* were the absolute leaders in terms of abundance both in summer and autumn. The community of the pelagic zone was characterized by a change of dominant taxa in autumn.

The taxonomic structure of the bacteriobenthic community of the pelagic zone was characterized by a presence of two dominant taxa. The taxonomic structure of the littoral bacteriobenthic community was characterized by two dominant taxa in June, but only one taxon, in September.

Acknowledgements

The authors express their gratitude to the administration and staff of the Natural Park "Kandy-Kul" for their assistance in organizing and conducting field work. The study was carried out with the
financial support of the Natural Park "Kandry-Kul" (contracts nos. 01/12 of December 1, 2011 and 08/12 of December 12, 2011).

References
[1] Register of specially protected natural areas of the Republic of Bashkortostan 2010 (Ufa: Gilem)
[2] Dzyuban A N 2010 Destruction of organic matter and methane cycle in bottom sediments of inland water bodies (Yaroslavl: Printhaus)
[3] Zharkov V V, Gorbunov M Yu, Umanskaya M V, Tarasova N G, Bykova S V, Sherysheva N G, Mukhortova O V, Sabitova R Z and Krasnova E S 2018 The current state of the ecosystem of Lake Kandy-Kul ed M V Umanskaya (Togliatti: Anna)
[4] Butorin N V, Ziminova N A and Kudrin V P 1975 Bottom sediments of the Upper Volga reservoirs (L: Publishing house Science)
[5] Kuzyakmetov G G, Miftakhova A M, Kireeva N A and Novoselova E I 2004 Practicum on soil science (Textbook. Ufa: RIO BashGU)
[6] Romanenko V I, Rybakova I V, Sokolova E A and Lajos Veres 1990 A variant of the diffusive method for determining free carbon dioxide, carbonates, and sulfides in water and bottom sediments in a closed vessel Hydrobiol. Journal 26 (5) 64-69
[7] Koleshko O I 1981 Ecology of soil microorganisms: Lab. workshop (Minsk: Higher. School)
[8] Lovely D R and Phillips E J P 1986 Mineralization of organic matter with the reduction of ferric iron in anaerobic sediments Appl. Environment Microbiology 51 683-689
[9] Verkhovtseva N V and Osipov G A 2008 Method of gas chromatography-mass spectrometry in the study of microbial communities of agrocenosis soils Problems of Agrochemistry and ecology 1 51-54
[10] Sherysheva N G, Osipov G A and Khalko V V 2015 Studying composition of bacteriobenthic communities in the sediments of water ecosystems by fatty acid markers J. Inland Water Biology 8 (3) 242-249
[11] Bakanov A I 2006 Quantitative assessment of dominance in ecological communities Ecological monitoring. Methods of biological and physic-chemical monitoring (VI) ed D B Gelashvili. (N. Novgorod: Publishing house of NNSU) pp 61-116
[12] Kou W, Zhang J, Lu X, Ma Y, Mou X and Wu L 2016 Identification of bacterial communities in sediments of Poyang Lake, the largest freshwater lake in China SpringerPlus 5 (1) 401 https://doi.org/10.1186/s40064-016-2026-7
[13] Bouzat J L, Hoostal M J and Loof T 2013 Spatial patterns of bacterial community composition within Lake Erie sediments J Great Lakes Res 39 344-351
[14] Pintaeva N Ts, Bazarsadueva S V and Radnaeva L D 2013 Chromato-mass spectrometric determination of the structure of the microbial community of bottom sediments of the river Selendi. School-seminar “Problems of sustainable development of the region” (Ulan-Ude: BNC SB RAS publishing house) pp 213-216