Cyanobacteria in freshwater Lake Dikoye (Pribaikalsky district, Buryatia, Siberia) under intensive eutrophication

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Abstract. We studied freshwater Lake Dikoye located in the coastal zone of Lake Baikal. Negative changes associated with cyanobacterial bloom were observed in the lake. Phototrophs were represented by cyanobacteria, green algae, and diatoms. In the microbial community, Cyanobacteria were the dominant phylum and accounted for up to 48% of the total diversity. Cyanobacteria were represented by 7 genera and 9 species. *Microcystis aeruginosa*, a potentially toxic species, was dominant among cyanobacteria. According to chlorophyll \(a\) content, the lake should be assigned to eutrophic ones. The bacterial eutrophication index for the lake studied varied from 1.17 (middle eutrophic) to 28.2 (hypereutrophic) during cyanobacterial bloom.

1. Introduction
Lake Dikoye is located 138 km from the city of Ulan-Ude (capital of the Republic of Buryatia) and within walking distance (200 m) from Lake Baikal. Dikoye is at an altitude of 459 m a.s.l.; its depth is 2.2 m, and an area reaches 4 km\(^2\). Previously, the reservoir was described as a picturesque shallow lake with a sandy bottom. In summer, the lake was a place of intensive recreation for local residents. As a result of natural and anthropogenic changes in the lake, the processes of eutrophication have intensified. The area and depth of the reservoir decreased significantly. Intensive algae and cyanobacterial growth in the water column, and formation of microbial mats at the bottom resulted in a deterioration of water quality, a change in its consistency up to gelatinous, and accumulation of toxins.

Lake Dikoye is an acceptable model for studying the microbial community of shallow lakes. This work aimed to assess the trophic status of Lake Dikoye and to identify cyanobacteria, which are a key component of water blooms that lead to eutrophication and intoxication.

2. Objects and methods
A comprehensive physicochemical and microbiological study of Lake Dikoye was carried out in the summer-autumn period 2015-2017. Sampling was carried out from the eastern side of the lake (52\(^°\)47.361\(^’\) N, 107\(^°\)59.700\(^’\) W). The temperature, \(pH\), and total dissolved solids (TDS) in water were measured directly at the sampling site with a Prima sensor thermometer (Portugal), potentiometric portable \(pH\) meter (pHer2, Portugal), and a portable MASTER-PM refractometer (Atago, Japan). The concentration of carbonates and hydrocarbonates was determined by the titrimetric method [1]. Chlorophyll \(a\) was measured by standard methods [2].

Total DNA was isolated using the commercial kits DNK-sorb (AmpliSens, Russia) and Bacterial Genomic DNA kit (Axygen, United States). Sequencing analysis of the V3–V4 variable regions of
the 16S rRNA gene was performed on a MiSeq sequencer (Illumina, United States) in the SB RAS’ Genomics Core Facility (Novosibirsk, Russia) using a protocol described by Fadrosh et al. [3]. Bioinformatic treatment included paired reads overlapping, quality and length filtration, assessment of identical sequences, singleton removal, chimera deletion, and isolation of operational taxonomic units (OTUs) using the UPARSE clustering algorithm. Taxonomic identification of the OTU sequences was determined using the 16S RDP pipeline (https://rdp.cme.msu.edu).

Morphological identification of cyanobacteria was carried out using identification manuals – Elenkin and Gollerbach et al. [4, 5], Komárek and Anagnostidis [6, 7]. The representatives of Bacillariophyta were identified according to the manual by Zabelina et al. [8]; for identification of Chlorophyta species, the manuals by Dedusenko-Shchegoleva et al. [9], Moshkova and Gollerbach [10], and Palamar'-Mordvintseva [11] were used. Microscopic examination of the samples was carried out using an Axiosstar Plus microscope (Carl Zeiss, Germany). Bacterial eutrophic index (BEI) was determined as a biotic complex assessment index based on the function of temperature and abundance of Cyanobacteria and Actinobacteria [12].

3. Results and discussion

3.1. Physicochemical characteristics of Lake Dikoye

The water temperature range was 21.8°C in June, 29.7°C in July, and 9.6–15.5°C in September (table 1). In addition to seasonal temperature changes, significant fluctuations in hydrochemical parameters were observed. Thus, in the autumn samples of 2015 and 2017, the water pH varied from 8 to 10.3, the total dissolved solids – from 97 to 183 mg/l, the hydrocarbonate ion – from less than 1 to 73.2 mg/l. A more complete anionic composition was presented by Zaitseva et al. [13]. During the period of studies, we observed the negative changes associated with the intensive cyanobacterial bloom such as high alkaline water pH due to high cyanobacterial activity.

Table 1. Physicochemical characteristics of the water.

| Sampling date | $t$, °C | TDS, mg/l | pH | $E_h$, mV | CO$_3^-$, mg/l | HCO$_3^-$, mg/l |
|---------------|--------|-----------|----|-----------|----------------|-----------------|
| 2015 June     | 21.8   | 164       | 8.82 | +115      | 24             | 36.6            |
| September     | 15.5   | 97        | 10.27| +122      | 12             | 73.2            |
| 2016 July     | 29.7   | 79        | 9.57 | +140      | 36             | 48.8            |
| September     | 9.6    | 183       | 8.06 | +140      | 12             | 0               |

3.2. The microbial community diversity of Lake Dikoye

The water microbial community of Lake Dikoye in 2015 was characterized by low diversity and only 165 phylotypes were determined. Cyanobacteria were the dominant phylum and accounted for 48.2% of the total diversity (figure 1).

Proteobacteria (31.5%), Bacteroidetes (4.4%), Verrucomicrobia (3.7%) and Actinobacteria (2.1%) were co-dominants. Cyanobacteria were represented by four species: *Synechococcus* sp. (GpIIa), *Pseudanabaena* sp. (GpVI), *Microcystis* sp. (GpXI) and *Leptolyngbya* sp. (GpIV), as well as unclassified Cyanobacteria.
In 2016, the diversity of the microbial community of Lake Dikoye increased to 880 phylotypes. Proteobacteria (53.8%) dominated; Cyanobacteria (9.9%), Bacteroidetes (9.1%), Actinobacteria (3.5%) were co-dominants. Six representatives of cyanobacteria were identified: Synechococcus sp. (GpIIa), Pseudanabaena sp. (GpVI), Microcystis sp. (GpXI), Leptolyngbya sp. (GpIV), Aphanizomenon sp. (GpI), and unclassified Cyanobacteria (figure 2).

In 2017, the diversity of microbial communities was represented by 270 phylotypes. Proteobacteria (34.92%), Cyanobacteria (12.48%), Bacteroidetes (6.86%) dominated (figure 3). Cyanobacteria were represented by 3 species: Pseudanabaena sp. (GpVI), Synechococcus sp. (GpIIa), Microcystis sp. (GpXI).
Figure 3. Taxonomic diversity of the microbial community in water of Lake Dikoye in September 2017.

The species diversity of cyanobacteria, diatoms, and green algae has also been studied using microscopic methods (table 2). Cyanobacteria were represented by 7 genera and 9 species: *Microcystis aeruginosa*, *Leptolyngbya tenuis*, *L. foveolarum*, *L. laminose*, *Cyanobacterium cedrorum*, *Chroococcus minutus*, *Phormidium formosum*, *Pseudanabaena frigida*, and *Synechococcus cedrorum*. In June 2015, the greatest diversity of cyanobacteria was noted (8 species). *Microcystis aeruginosa* and *Leptolyngbya tenuis* were the dominant species. In 2016, the diversity of cyanobacteria decreased to 4 species. *Microcystis aeruginosa* was also the dominant species. In 2017, cyanobacteria were represented by 5 species. In Lake Kotokelskoye, during the “bloom” period in 2009, representatives of the genus *Microcystis* (*Aphanocapsa*) were also dominant [14]. Diatoms were represented by 4 species: *Eucocconeis dorogostaiskyi*, *Eucocconeis sp.*, *Navicula peregrine* and *Navicula* sp. Green algae were represented by one species – *Treubaria* sp.

Table 2. Taxonomic composition of phytoplankton of Lake Dikoye.

| Year | Cyanobacteria | Diatoms | Green algae |
|------|---------------|---------|-------------|
| 2015 | *Microcystis aeruginosa*<sup>*</sup>  
*Leptolyngbya tenuis*<sup>*</sup>  
*L. foveolarum*  
*L. laminose*  
*Cyanobacterium cedrorum*  
*Chroococcus minutus*  
*Phormidium formosum*  
*Pseudanabaena frigida* | *Eucocconeis dorogostaiskyi*<sup>*</sup>  
*Eucocconeis sp.*  
*Navicula peregrine*  
*Navicula* sp. | *Treubaria* sp. |
| 2016 | *M. aeruginosa*<sup>*</sup>  
*Ph. formosum*  
*L. tenuis*  
*L. foveolarum* | *Eucocconeis sp.*  
*Navicula sp.* | *Treubaria* sp. |
| 2017 | *M. aeruginosa*<sup>*</sup>  
*Ps. frigida*  
*L. foveolarum*  
*Ch. minutus*  
*Synechococcus cedrorum* | *Eucocconeis ssp.*  
*Navicula sp.* | *Treubaria* sp. |

*<sup>*</sup> dominant species
Thus, the composition of the microbial community in water of Lake Dikoye was unstable for three years. The largest number of taxa was recorded in 2016. Proteobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobia were the main dominant phyla, as in Lake Kotokelskoye (unpublished data). There is a change in dominants in different years. In total, 8 species of cyanobacteria were identified. The maximum diversity of cyanobacteria was revealed in 2015. A colony-forming cyanobacteria Microcystis aeruginosa was the dominant species for all three years. Also, Microcystis aeruginosa is the key component of harmful algal blooms in freshwater systems worldwide [15, 16]. During the 2014 cyanobacterial algal bloom, Microcystis was the dominant large-colony forming cyanobacterial genus in the algal bloom in western Lake Erie [17]. In addition to cyanobacteria, diatoms were found, represented by four species, and green algae, represented by one species.

3.3. The trophic status of Lake Dikoye

The trophic status of the lake was estimated by the concentration of chlorophyll \( a \). In June 2015 Lake Dikoye was assigned to the mesotrophic type: chlorophyll \( a \) concentration was 18 mg/l. In July 2016 the chlorophyll \( a \) increased by an order of magnitude and reached a maximum value of 22.19 mg/l, which made it possible to classify the lake as a eutrophic reservoir. However, in September 2017, the content of chlorophyll \( a \) decreased to 1.265 mg/l, which indicates the death of phototrophic microorganisms. The change in the chlorophyll \( a \) concentration directly depends on the number of phototrophic species, in particular cyanobacteria. According to the data of 2009, freshwater Lake Kotokelskoye, located 11 km from Lake Dikoye, was also classified as a eutrophic water body in terms of chlorophyll \( a \) [14].

A modern method for assessing the state of freshwater ecosystems is the recently proposed bacterial eutrophic index. The bacterial eutrophic index (BEI) represents a biotic complex assessment index based on the function of temperature and abundance of Cyanobacteria and Actinobacteria [12]. The values of the bacterial eutrophic index for the studied lake varied from 1.17 (middle eutrophic) in summer 2016 to 4.76 (hypereutrophic) in September 2017, and 28.24 (hypereutrophic) in summer 2015. The highest values were found during cyanobacterial bloom. These data on assessment of trophic status based on both chlorophyll \( a \) content and bacterial eutrophic index confirm the intensive eutrophication of this lake.

4. Conclusion

For the first time, hydrochemical and microbiological studies of freshwater lake Dikoye, located in the coastal strip of Lake Baikal, were carried out. Phototrophs in the microbial community were represented by cyanobacteria, diatoms, and green algae. Cyanobacteria along with Proteobacteria, Actinobacteria, and Bacteroidetes were the main dominant phyla in the lake water microbial community. The maximum diversity of cyanobacteria in Lake Dikoye was noted in 2015 – 9 species. Microcystis aeruginosa, a potentially toxic species, was dominant. In terms of chlorophyll \( a \) content, the lake has corresponded to the eutrophic type of water bodies. The bacterial eutrophication index for the lake studied varied from 1.17 (middle eutrophic) to 28.2 (hypereutrophic) during cyanobacterial bloom.

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