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Limnological Characterization and First Data on the Occurrence of Toxigenic Cyanobacteria and Cyanotoxins in the Plankton of Some Lakes in the Permafrost Zone (Yakutia, Russia)

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Abstract—The first data on the distribution of cyanobacterial toxins have been obtained and a molecular genetic determination of cyanotoxin producers in the plankton of some lakes in the permafrost zone in Yakutia have been performed for the first time. Most of the lakes are characterized by high concentrations of nitrogen, phosphorus, and chlorophyll *a* and by an increased content of organic matter, which could be associated with a high anthropogenic load. Water blooms were visually observed in three of the six studied lakes during the summer period. The abundance and biomass of cyanobacteria in the lakes varied within 13.7–676.3 million cells/L and 0.6–4.8 mg/L, respectively. Eight species of potentially toxigenic cyanobacteria were found in phytoplankton using light microscopy. Regions of the *mcyE* gene involved in the biosynthesis of microcystin were amplified using the polymerase chain reaction method in environmental DNA isolated from planktonic samples. Cyanobacteria capable of producing neurotoxic anatoxin-a, saxitoxins, and hepatotoxic nodularins were absent in the lakes during the study period. The use of genus-specific primers to the *mcyE* gene enables us to find that the main producers of microcystins were represented by species of the genus *Microcystis* in most of the lakes and by species of *Dolichospermum* only in one of the lakes. Up to eight structural variants of microcystins, in general, arginine-containing isoforms MC-LR, MC-RR, MC-YR, MC-LY, MC-HIlR, [Asp^3]MC-LR, [Asp^3]MC-RR, and [Asp^3]MC-YR, were identified in lake plankton using liquid chromatography–mass spectrometry. The maximal concentration of microcystins in plankton (intracellular fraction 803 ng/L) was recorded in a sample from Ytyk-Kyuyol Lake. The calculated content of microcystins per unit biomass of producing cyanobacteria (toxin quota) was low (0.005–0.069 μg/mg). In order to assess the potential hazard of toxigenic species of cyanobacteria to human health, the distribution of cyanotoxins and their producers should be further studied in water bodies of the region.

Keywords: toxigenic cyanobacteria, cyanotoxins, microcystins, plankton, lakes, permafrost zone
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INTRODUCTION

Cyanobacterial harmful algae blooms (cyanoHAB) of water widespread all over the world result in serious negative environmental and economic consequences (Sivonen and Jones, 1999; Chorus and Welker, 2021). This phenomenon consists of the mass reproduction of cyanobacteria in a reservoir, which causes a visible change in the water color (Huisman et al., 2018). It is known that cyanobacteria produce highly toxic metabolites—cyanotoxins—that can cause adverse effects on the health of humans and domestic animals, as well as on terrestrial and aquatic organisms, and even their death. Cyanobacteria are able to synthesize hepatotoxic nodularins (NOD) and neurotoxins: anatoxin-a (AN-a) and saxitoxins (SXT) (van Apeldoorn et al., 2007). Hepatotoxic microcystins (MCs) are the most common among cyanobacterial toxins in freshwater ecosystems; they are represented by more than 250 structure variants (Meriluoto et al., 2017) and are characterized by different toxicity (Rinehart et al.,

1994). Microcystins are assigned to the class of cyclic oligopeptides. Their general structure contains a cycle of seven amino acids, five of which, including the characteristic Adda acid (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), are constant in compounds of this class, and two L-amino acids at positions 2 and 4 are variable. Conventional abbreviations of variable amino acids are indicated as a suffix in the name of MCs. The structure of the most toxic variant of MC-LR (molecular weight is 994) includes leucine (Leu = L) at position 2 and arginine (Arg = R) at position 4 (Botana, 2008).

Although cyanobacteria colonize a wide range of habitats and occur under very severe temperature conditions (Paerl and Paul, 2012; Huisman et al., 2018), almost all current knowledge about the distribution of cyanotoxins is limited to fresh waters of temperate and tropical latitudes due to the frequent mass reproduction of cyanobacteria in these reservoirs (Svirčev et al., 2019). However, recent works reflect the presence of cyanotoxins in reservoirs of the northern and polar regions (Kleinteich et al., 2013, 2014; Denisov et al., 2021; Smirnova et al., 2021).

Reservoirs under conditions of the permafrost zone are characterized by a short ice-free season (*Resursy...,* 1970), which shortens the vegetation period of phytoplankton and toxigenic cyanobacteria (Magnuson et al., 2000). For example, on lakes within the boundaries of the city of Yakutsk and its environs in the northeast of Russia, the ice-free season lasts only 120–125 days (Arzhakova et al., 2007). However, global climate changes in recent 10-year periods caused shifts in ice formation in northern reservoirs. The increase in the ice-free season on various water bodies of Asian Russia averaged from 4.63 to 11 days per 10-year period from 1980 to 2014 (Vuglinsky and Valatin, 2018). The forecast for the near future confirms the preservation of this trend (Field et al., 2014). A number of authors have already mentioned the massive development of algae in the plankton of Fennoscandia reservoirs, explaining this phenomenon by positive temperature anomalies in recent 10-year periods (Denisov and Kashulin, 2016). Annual local mass development of cyanobacteria, resulting in water blooming, has been observed in reservoirs of the Kola Peninsula since 2000. In addition to the risk of increased mass development of phytoplankton, changes in climatic parameters may affect the structure of algal communities of reservoirs in the permafrost zone (Winder and Sommer, 2012). Both global climatic changes and the increasing anthropogenic load may cause changes in the composition of phytoplankton communities in lakes of the permafrost zone, an increase in the biomass of cyanobacteria, and a greater risk of accumulation of toxic metabolites in water and hydrobionts.

Until recently, studies of the spread of toxic cyanometabolites in the circumpolar and polar regions included only benthic cyanobacteria and species, forming so-called mats and biofilms (Hitzfeld et al., 2000; Jungblut et al., 2006; Wood et al., 2008; Kleinteich et al., 2012, 2013; Chrapusta et al., 2015). The first work devoted to the determination of MC concentrations in the plankton of some polar lakes was published recently (Trout-Haney et al., 2016). This study showed that water samples taken from 18 Greenland lakes contained measurable amounts of hepatotoxic cyanotoxin MCs. However, a nonselective method of enzyme immunoassay (the ELISA method) was used to determine the MC concentrations, so it was unclear which of them were found in plankton of the studied lakes. In addition, it was colonies of benthic cyanobacteria *Nostoc pruniforme* and not planktonic cyanobacteria that were probably the source of MCs in water (Trout-Haney et al., 2021). The presence of arginine-containing MC (MC-LR, MC-RR) and their demethylated forms in plankton was first detected in polar Lake Imandra (northwest of Russia) by high-performance liquid chromatography–mass spectrometry (Denisov et al., 2021). However, it was not possible to reliably identify MC producers in the lake plankton (Denisov et al., 2021). The relative contribution of planktonic and benthic cyanobacterial communities to the production of MCs in northern circumpolar and polar freshwater bodies remains unknown.

Yakutsk is the largest city in the world located in the permafrost zone. Its population is rapidly increasing and has grown by almost 40% over the past 15-years (to 330000 inhabitants). There are a lot of lakes in the city and its surroundings, which are an important component of the urban environment. With the development of agriculture and an increase in the municipal and household load on the studied reservoirs, the input of nutrients and organic matter to them from adjacent areas increases, which can stimulate toxic cyanobacterial water blooms. We have not found published data on the possible spread of cyanotoxins in reservoirs of Yakutia characterized by a severe arctic climate and the presence of permafrost. No studies on the molecular genetic diagnostics of producers of MCs among planktonic cyanobacteria living in the reservoirs of this region have been performed.

The purpose of this study was to obtain the first data on the occurrence, concentrations, and structural variants of intracellular cyanotoxins, as well as on the species composition of potentially toxic cyanobacteria in lakes on permafrost in Yakutia. Special attention was paid to answering the following questions: (1) is water blooming with the participation of planktonic toxigenic cyanobacteria possible in reservoirs located in severe climatic conditions of the permafrost zone in Yakutia? (2) which cyanobacteria are the main producers of toxins in the region? and (3) do the studied reservoirs contain cyanotoxins in concentrations dangerous for humans?

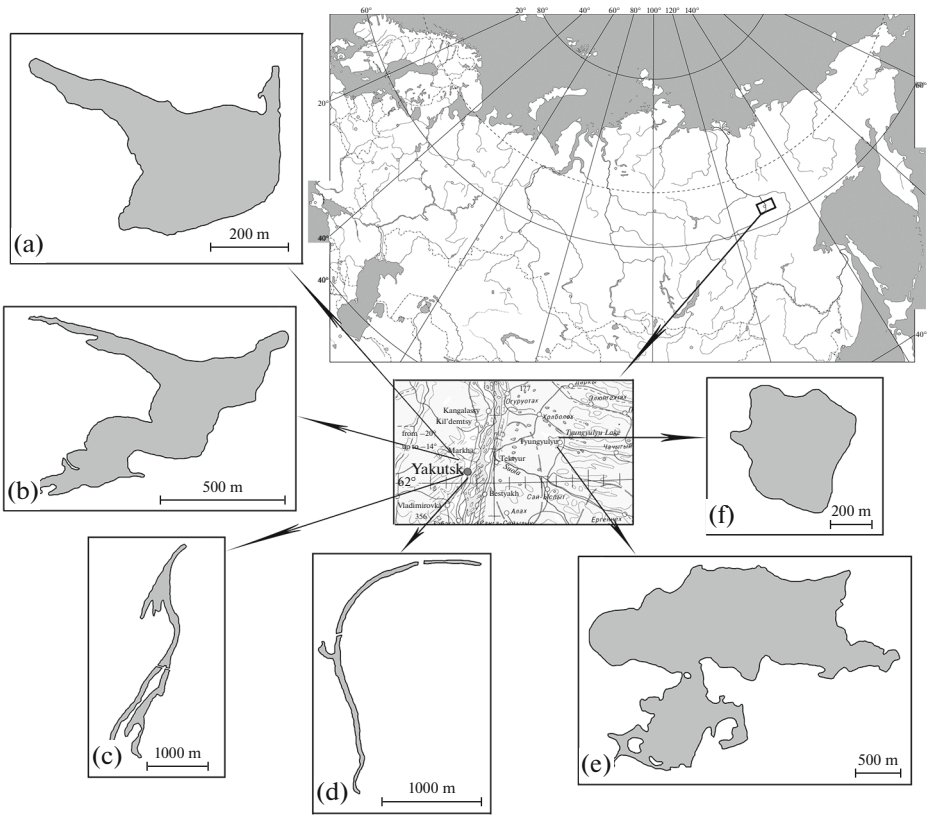

Fig. 1. Schematic map of the location of the survey area and lakes of Yakutsk and its environs: (a) Leontiyevskoye Lake, (b) Dachnoye Lake, (c) Ytyk-Kyuyol Lake, (d) Log Lake, (e) Nal-Tyungyulyu Lake, and (f) Tyungyulyu Lake.

MATERIALS AND METHODS

Description of the Research Region

The research area is located on the 62nd parallel north in the middle reaches of the Lena River in the zone of continuous permafrost. A great effect on climatic conditions of the region is exerted by the Siberian anticyclone formed in the center of Asia in winter, a great spur of which occupies the entire Eastern Siberia. Frequent invasions of air masses from the Arctic Ocean with very low water vapor content in summer strongly affect the weather conditions. The climate is sharply continental with long severe winter and short hot summer. There is the pole of cold in the region (the settlement of Oimyakon), where the lowest temperatures for the Northern Hemisphere are recorded. According to the data received on www.worldclim.org, the mean annual air temperature for the sampling area ranges from −2.4 to −8.7°C, and the maximal temperature in summer is from 22.2 to 25.0°C.

We performed the research on six lakes of different types (Fig. 1, Table 1). Some of them are located on the terrace above the floodplain of the Lena River and are assigned to oxbow cutoff lakes, represented by channels separated from the river. Other lakes are cryogenic: their basins were formed as a result of thawing of underground ice permafrost. One of the studied reservoirs is an artificial damming lake (Table 1).

Water Sampling

The samples were taken from the surface water layer (0–0.3 m) in the summer low-water period in August 1–5, 2021. Samples for qualitative and quantitative analyses of phytoplankton were taken by an Apstein network (Sefar Nitex material with a mesh size of 15 μm). The initial volume of the sample for quantitative analysis was 20 L. The volume of the condensed phytoplankton sample was 15 mL and was fixed by three drops of 40% formalin solution. Phytoplankton for the detection of intracellular fraction of toxins and for the molecular genetic analysis was sampled by water scooping, and cells were precipitated on Sartorius cellulose nitrate filters (with a pore size of 0.8 μm) under excessive pressure. Filters with biomass were immediately frozen at −20°C. Lake water for hydrochemical analysis was taken by scooping and transported to the laboratory for immediate analysis.

Hydrochemical Analysis

Chemical-analytical works were performed by the methods described in the guidance on the chemical

Table 1. Brief characterization of the studied lakes

Lake	Geographical coordinates, deg (N/E)	Length, m	Width, m	Depth, m	Area of water surface, thousand m^2	Use	Type, genesis
Log	62.017514 129.707035	3500	60	2.5	221.9	RP	R
Ytyk-Kyuyol	62.023662 129.618245	4400	400	3.0	790.3	RP, ACI, D	R
Dachnoye	62.124150 129.624445	1270	610	3.5	229.3	RP, D	A
Leonti-yevskoye e	62.117600 129.558634	700	500	2.5	126.7	RP, ACI	C
Nal-Tyung-yulyu	62.169853 130.658904	3500	1500	2.5	3510	L, D, P (in winter)	C
Tyungyulyu	62.202739 130.655056	600	600	1.5	237.6	L	C

Designations of type and genesis: (R) river (oxbow cutoff lake), (A) artificial damming lake, and (C) cryogenic lake. Ways in which the lakes are used: (P) potable water supply, (RP) recreational purposes, (ACI) agricultural irrigation, (D) domestic water consumption, and (L) livestock watering.

analysis of surface inner waters (Semenov, 1977). The water temperature was measured by a Chektemp electronic thermometer (Hanna Instruments, United States). The oxygen concentration was determined by the titration method with iodometric determination. We calculated the water salinity as the sum of anions and cations: we determined sulphate anion by turbidimetry, chloride ions by mercurimetry, bicarbonate ions by titration, calcium by the titration method with trilon B, potassium and sodium cations by atomic emission spectroscopy on an AAC AAnalyst400 device (Perkin Elmer, United States), water color by the photometric method on a PE-5300VI spectrophotometer (Ekroskhim, Russia), and pH by the potentiometric method on a Multitest IPL-101 device (Semiko NPP, Russia).

The following components were determined by the photometric method: total iron with the sulfosalicylic acid, ammonium ion with Nessler's reagent, nitrite ion with the Griess reagent, and nitrate ion with sodium salicylate. We also detected phosphate ions by the phosphorus–molybdenum complex formation method, total phosphorus by persulfate oxidation, and the index of chemical oxygen demand (COD) by the photometric method on a Fluorat-02 spectrofluorimeter (Lumeks, Russia).

For comparative analysis, the hydrochemical parameters measured in various scales were reduced to a conditionally general scale by normalized values of array variables characterized by the mean and standard deviation.

Treatment of Phytoplankton Samples

The qualitative and quantitative analysis of phytoplankton was performed with the use of an Olympus BH-2 light microscope (Olympus, Japan). The biomass was determined as the product of the result of counting the abundance of cells by their volume, which was estimated by the stereometric method and calculated by the data of our own measurements of cells (Makarova and Pichkily, 1970). The specific weight of algae was assumed to be equal to one. The abundance of algae cells was counted in a Nageotte chamber 0.01 cm^3 in volume. Cyanobacteria species were identified using the identification guides by J. Komárek and K. Anagnostidis (1998, 2005, 2013). The taxonomy of the species was given according to the data published on portal algabase.org.

Chlorophylls were determined by concentrating phytoplankton cells on membrane filters with the subsequent extraction of pigments with 90% acetone and their spectrophotometry (*Rukovodstvo...,* 1983).

DNA Isolation from Plankton Samples and Polymerase Chain Reaction Analysis

DNA was isolated from the sampled plankton on filters by the sorption method using a set of reagents Diatom DNA Prep 200 (Izogen Laboratory, Russia) according to the manufacturer's instructions.

The polymerase chain reaction (PCR) was performed using primers known from published works specific to genes of the synthesis of hepatotoxins MC (*mcyE* gene) and neurotoxins SXT (*sxtA* and *sxtI* genes). To detect the genes responsible for SXT biosynthesis, pairs of primers sxtaf/sxtar for the sxtA gene (PCR product of size 600 bp) and sxtIf/sxtIr for the sxtI gene (910 bp) were used (Ballot et al., 2010; Casero et al., 2014). The search for MC-producing taxa directly in natural samples (metagenomic DNA) with a mixed composition of cyanobacteria consisted

of two stages. First, the reactions included universal primers HEPF and HEPR complementary to the ends of a fragment of the nucleotide sequence of the *mcyE* gene of 472 bp in length. These primers were developed to diagnose the presence of MC producers in the sample regardless of their taxonomy (producers of the genera *Microcystis, Planktothrix,* and *Dolichospermum*) (Jungblut and Neilan, 2006). In case of a positive result with HEPF/HEPR primers, genus-specific primers mcyE-F2/MicmcyE-R8 (the genus *Microcystis*, 250 bp) and mcyE-F2/AnamcyE-12R (the genus *Dolichospermum*, 250 bp) were used (Vaitomaa et al., 2003). They enabled us to identify MC producers from samples with a mixed composition of cyanobacteria to the level of genus. The DNA of the MC-producing strain *Microcystis aeruginosa* PCC 7806 and the SXT-producing strain *Aphanizomenon* sp. AB59 were used as a positive control.

Genes were amplified with the use of a set of reagents DreamTaq PCR Master Mix (Thermo Scientific, United States) in a CFX96 Touch thermal cycler (Bio-Rad, United States) according to the following protocol: the preliminary denaturation of DNA at a temperature of 95°C for 3 min. It was followed by 37 amplification cycles: at 95°C for 30 s, at 58°C for 30 s, and at 72°C for 1 min. In the last cycle, the elongation stage at 72°C lasted 10 min. PCR products were electrophoretically fractionated in 1.5% agarose gel and analyzed in UV light after staining by ethidium bromide using a Gel Doc XR+ gel documenting system (Bio-Rad, United States). The size of the amplified DNA fragments was determined using a FastRuler Low Range DNA Ladder marker of molecular masses (Thermo Scientific, United States).

Determination of the Content of Cyanotoxins by Liquid Chromatography–Mass Spectrometry

Cyanotoxins were extracted from cells deposited on filters by treatment with 75% aqueous methanol under the action of ultrasound. Structural variants of cyanotoxins of different toxicity were identified and quantified by the high performance liquid chromatography–tandem mass spectrometry of high resolution (HPLC-HRMS/MS).

The analysis was performed using the Prominence LC-20 HPLC system (Shimadzu, Japan) in combination with a LTQ OrbitrapXL mass spectrometer (Thermo Fisher Scientific, United States). Toxins were separated on a Thermo Hypersil Gold RPC 18 column (100 × 3 mm, 3 μm, Thermo Fisher Scientific) in a gradient elution mode (0.2 mL/min) by a mixture of water and acetonitrile containing 0.05% of formic acid. The mass spectrometric analysis of cyanotoxins was performed under conditions of the positive ion electrospray ionization. Tandem mass spectra were obtained using the mode of collision-induced dissociation (CID). The parameters of mass spectrometric analysis were the following: heater temperature 300°C, capillary temperature 300°C, sheath gas flow rate 45 Arb, aux gas flow rate 10 Arb, ISpray voltage 3.5 kV, and S-lens radio frequency (RF) 69%.

The target compounds were identified based on an accurate measurement of the mass of $[M + H]^+$ or $[M + 2H]^{2+}$ ions (resolution 30000 and accuracy within 5 ppm) (Chernova et al., 2016), data of tandem mass spectra (Chernova et al., 2016), and retention times determined with the use of commercially available standard cyanotoxin compounds (AN-a, MC, and NOD).

The quantitative analysis was performed by the method of an external standard. The calibration dependencies in the range from 1 to 500 ng/mL were constructed with the use of solutions of nine standard compounds, MC–LR, MC–RR, MC–YR (Sigma Aldrich, United States), MC–LY, MC–LA, MC–LW, MC–LF, [D-Asp^3]MC-LR and [D-Asp^3]MC-RR; AN-a fumarate (Enzo Life Sciences, Inc., United States), and two standard NOD compounds (NOD-R and [D-Asp^1]NOD-R) (Sigma-Aldrich Corp., United States).

The following reagents were used: Acetonitrile *0* (Kriokhrom, Russia), methanol (LiChrosolv hypergrade for LC–MS, Merck, Germany), formic acid (98–100%, Fluka Chemika, Buchs, Switzerland), and water purified by the Direct-Q system (Millipore, electrical conductivity 0.056 μS/cm at 25°C) (Massachusetts, United States).

The specific content of MC in the unit biomass (B) of cyanobacteria producers (MC quota, μg MC/mg B) was calculated as the ratio of the intracellular concentration of MC to the total biomass of cyanobacteria producers identified by the PCR.

RESULTS

Chemical Composition of Waters

Water in the surface layer of the lakes was sufficiently warm during sampling, and the oxygen concentration was high (Table 2). The reaction of waters in the lakes studied was slightly alkaline, except for Dachnoye Lake, where the water reaction was neutral.

With respect to the salt content, waters of most lakes were of medium salinity, except for Tyungyulyu Lake, where the salt concentration was more than three times higher than in the other reservoirs. Ytyk-Kyuyol Lake and cryogenic lakes were distinguished by more intense water color. The COD was high in all the reservoirs and reached maximum in cryogenic lakes. The concentration of ammonium ion was high in all the lakes, with the maximum in Tyungyulyu Lake and the minimum in Dachnoye and Log lakes. The content of nitrites varied widely and reached a maximum in Tyungyulyu Lake. The amount of nitrate ions was also high and was maximal in Tyungyulyu Lake, as well as in Log and Ytyk-Kyuyol lakes. The

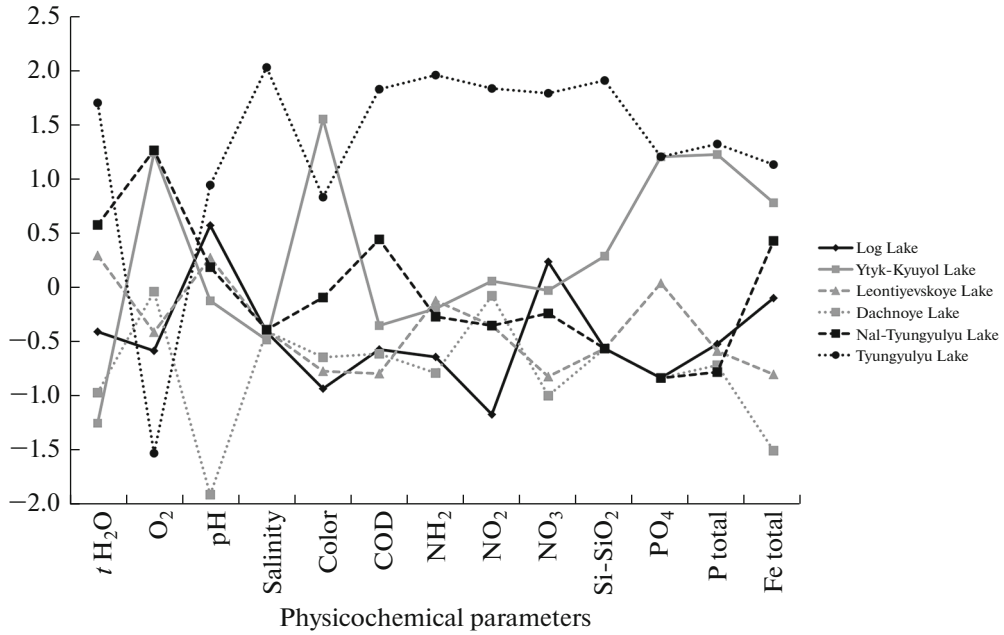

Fig. 2. Diagram of normalized physicochemical parameters of the studied lakes.

maximal concentration of phosphate ions and total phosphorus was typical for Ytyk-Kyuyol and Tyungyulyu lakes. The total iron content was high in all the lakes and reached maximal values in Tyungyulyu, Ytyk-Kyuyol, and Nal-Tyungyulyu lakes.

The diagram of normalized physicochemical parameters of waters in the studied lakes shows that Tyungyulyu Lake significantly differed from the other lakes by the maximal content of salts, nitrogen compounds, and COD (Fig. 2). This lake was also characterized by the highest pH and the total iron concentration. Tyungyulyu and the Ytyk-Kyuyol lakes were distinguished by the highest content of phosphorus compounds and the most intensive water color.

Taxonomic Composition of Cyanobacteria, Dominant Species, and Chlorophyll Concentration

We have identified 18 species of cyanobacteria of 11 genera in plankton of the lakes, and one taxon has been identified to the level of genus. The species richness of cyanobacteria was the greatest in Nal-Tyungyulyu, Leontiyevskoye, and Ytyk-Kyuyol lakes (Table 3).

Water blooming was visually detected in Ytyk-Kyuyol, Dachnoye, and Leontiyevskoye lakes in the

Table 2. Physicochemical parameters of water in the studied lakes

Parameter	Lakes					
	1	2	3	4	5	6
Water temperature, °C	22.6	22.0	23.1	22.2	23.3	24.1
Oxygen (O_2), mg/L	9.5	12.9	9.8	10.5	12.9	7.7
pH, units	7.30	9.06	8.74	9.30	8.99	9.60
Salinity, mg/L	489.6	493.2	448.6	486.3	497.2	1725.5
Water color, deg	32	29	87	25	46	69
COD, mg/L	42.15	38.60	47.17	42.93	62.58	89.40
NH_4, mg/L	0.33	0.42	0.41	0.35	0.40	0.70
NO_2, mg/L	0.10	0.08	0.11	0.02	0.08	0.24
NO_3, mg/L	0.68	0.72	0.90	0.96	0.85	1.31
PO_4, mg/L	0.08	0.11	0.15	0.08	0.08	0.15
P total, mg/L	0.16	0.18	0.46	0.19	0.15	0.48
Fe total, mg/L	0.32	0.40	0.58	0.48	0.54	0.62
N/P	7.0	3.1	6.8	6.9	8.9	4.7

Numbers designate the lakes: (1) Dachnoye, (2) Leontiyevskoye, (3) Ytyk-Kyuyol, (4) Log, (5) Nal-Tyungyulyu, and (6) Tyungyulyu.

Table 3. Species composition and abundance (million cells/L) (numerator) and biomass (mg/L) (denominator) of cyanobacteria in the studied lakes

Species	Lakes					
	1	2	3	4	5	6
Anabaena aequalis O. Borge	–	–	0.36 0.04	–	–	–
A. contorta H. Bachmann	–	–	–	–	0.66 0.07	–
Anathece clathrata (West & G.S. West) Komárek, Kastovsky & Jezberová	–	21.28 0.03	31.01 0.04	–	50.36 0.07	–
Aphanizomenon flos-aquae Ralfs ex Bornet & Flahault*	22.86 1.6	4.62 0.30	6.52 0.60	2.35 0.20	6.74 0.60	–
Aphanocapsa incerta (Lemmermann) G.Cronberg & Komárek*	52.63 0.10	18.18 0.04	–	–	19.05 0.04	–
A. planctonica (G.M. Smith) Komárek & Anagnostidis	–	–	4.37 0.04	–	–	–
Dolichospermum spiroides (Klebhan) Wacklin, L.Hoffmann & Komárek*	–	0.29 0.07	0.65 0.15	–	–	–
D. viguieri (Denis & Frémy) Wacklin, L. Hoffmann & Komárek*	–	–	2.00 0.30	1.11 0.20	–	–
Gomphosphaeria aponina Kützing	–	–	–	–	–	0.94 0.10
Merismopedia glauca (Ehrenberg) Kützing	–	0.50 0.03	–	–	–	1.59 0.10
M. minima G. Beck in G. Beck & Zahlbruckner	–	–	198.7 0.03	662.3 0.01	–	–
M. tranquilla (Ehrenberg) Trevisan	–	–	–	–	4.19 0.06	-
Microcrocis irregularis (Lagerheim) Geitler	–	–	–	–	3.40 0.04	–
M. aeruginosa (Kützing) Kützing*	3.08 0.20	46.67 3.50	-	1.43 0.10	2.78 0.20	–
M. flos-aquae (Wittrock) Kirchner*	–	–	79.65 3.20	–	–	–
M. pulverea (H.C. Wood) Forti	10.0 0.10	9.09 0.10	13.33 0.20	–	4.29 0.06	–
Oscillatoria sp.	0.84 0.10	0.25 0.03	–	0.84 0.10	0.34 0.04	1.69 0.20
Snowella lacustris (Chodat) Komárek & Hindák*	–	–	–	8.33 0.10	3.33 0.06	6.25 0.10
Woronichinia naegeliana (Unger) Elenkin*	3.13 0.10	–	6.06 0.20	–	1.76 0.06	3.23 0.10
Total abundance/biomass of cyanobacteria	92.53 2.20	100.9 4.10	342.6 4.80	676.3 0.80	96.89 1.30	13.70 0.60

Numbers of lakes are the same as in Table 2; * potentially toxic species. A dash signifies no species is detected.

Table 4. Concentration of chlorophylls (μg/L) of phytoplankton in the studied lakes

Lake	chl *a*	chl *b*	chl *c*
Dachnoye	20.15	4.86	15.54
Ytyk-Kyuyol	26.14	1.81	15.67
Leontiyevskoye	10.65	0.46	5.34
Log	18.39	1.35	7.46
Nal-Tyungyulyu	33.05	10.04	47.40
Tyungyulyu	5.35	0.47	2.78

form of clusters of cyanobacteria in the surface water layer. The total abundance and biomass of cyanobacteria in the studied lakes varied within 13.7–676.3 million cells/L and 0.6–4.8 mg/L, respectively (Table 3). Species *Microcystis flos-aquae, M. aeruginosa,* and *Aphanizomenon flos-aquae* were the most abundant. The share of cyanobacteria in the total phytoplankton biomass ranged from 60 to 98%.

The results of pigment analysis showed that the highest chlorophyll *a* concentration was typical for Nal-Tyungyulyu Lake, and its content was also high in Ytyk-Kyuyol and Dachnoye lakes (Table 4). The content of chlorophyll *a* was the lowest in plankton of Tyungyulyu Lake. The content of chlorophylls *b* and *c* in most lakes was lower when compared to the chlorophyll *a* content, except for Nal-Tyungyulyu Lake, where the chlorophyll *c* concentration was the highest.

Cyanotoxins and Their Biosynthesis Genes

Fragments of the gene mcyE of MC biosynthesis of about 470 bp in size were amplified using HEP primers universal to hepatotoxic cyanobacteria. The *mcyE* gene was detected in environmental DNA isolated from the plankton of Dachnoye, Leontiyevskoye, Ytyk-Kyuyol, Log, and Nal-Tyungyulyu lakes. PCR products of the expected size (~250 bp) were obtained from the same DNA samples using mcyE-F2/MicmcyE-R8 primers specific for MC-producing *Microcystis* (Table 5).

The result of the PCR with *Dolichospermum*-specific primers mcyE-F2/AnamcyE-12R was positive only for Ytyk-Kyuyol Lake. The genes involved in SXT biosynthesis were not found in the metagenomic DNA isolated from plankton of the surveyed lakes (Table 5).

We detected MCs by liquid chromatography–mass spectrometry in five of the six surveyed lakes; they were not revealed in the sample from Tyungyulyu Lake.

Table 5. Results of an analysis of plankton samples for the presence of cyanotoxins and genes of their biosynthesis by liquid chromatography–mass spectrometry and PCR

Lakes	Total concentration of cyanotoxins, ng/L (identified structural variants)				MC quota, μg/mg	Cyanotoxin-producing genes			
	MC	NOD	SXT	AN-a		*mcyE* (MC producers)	*mcyE* (MC producers of the genus *Microcystis*)	*mcyE* (MC producers of the genus *Dolichospermum*)	*sxtA*, *sxtI* (SXT producers)
1	11 (MC-LR, MC-RR, MC-YR)	ND	ND	ND	0.055	+	+	–	–
2	7 (MC-LR, MC-YR)	ND	ND	ND	0.002	+	+	–	–
3	803 (MC-LR, MC-RR, MC-YR, MC-LY, MC-HIIR, [D-Asp^3]MC-LR, [D-Asp^3]MC-RR, [D-Asp^3]MC-YR)	ND	ND	ND	0.220	+	+	+	–
4	3 (MC-RR)	ND	ND	ND	0.030	+	+	–	–
5	12 (MC-LR, MC-RR, MC-YR, [D-Asp^3]MC-RR)	ND	ND	ND	0.060	+	+	–	–
6	ND	ND	ND	ND	ND	–	–	–	–

Numbers of lakes are the same as in Table 2; (MC) microcystins, (NOD) nodularins, (SXT) saxitoxins, (AN-a) anatoxin-a, (ND) concentrations below the detection limit (<0.001 μg/L), (+) the presence of MC-producing gene *mcyE* in metagenomic DNA is confirmed by PCR, and a dash signifies that specific regions of genes of biosynthesis of cyanotoxins in metagenomic DNA have not been detected.

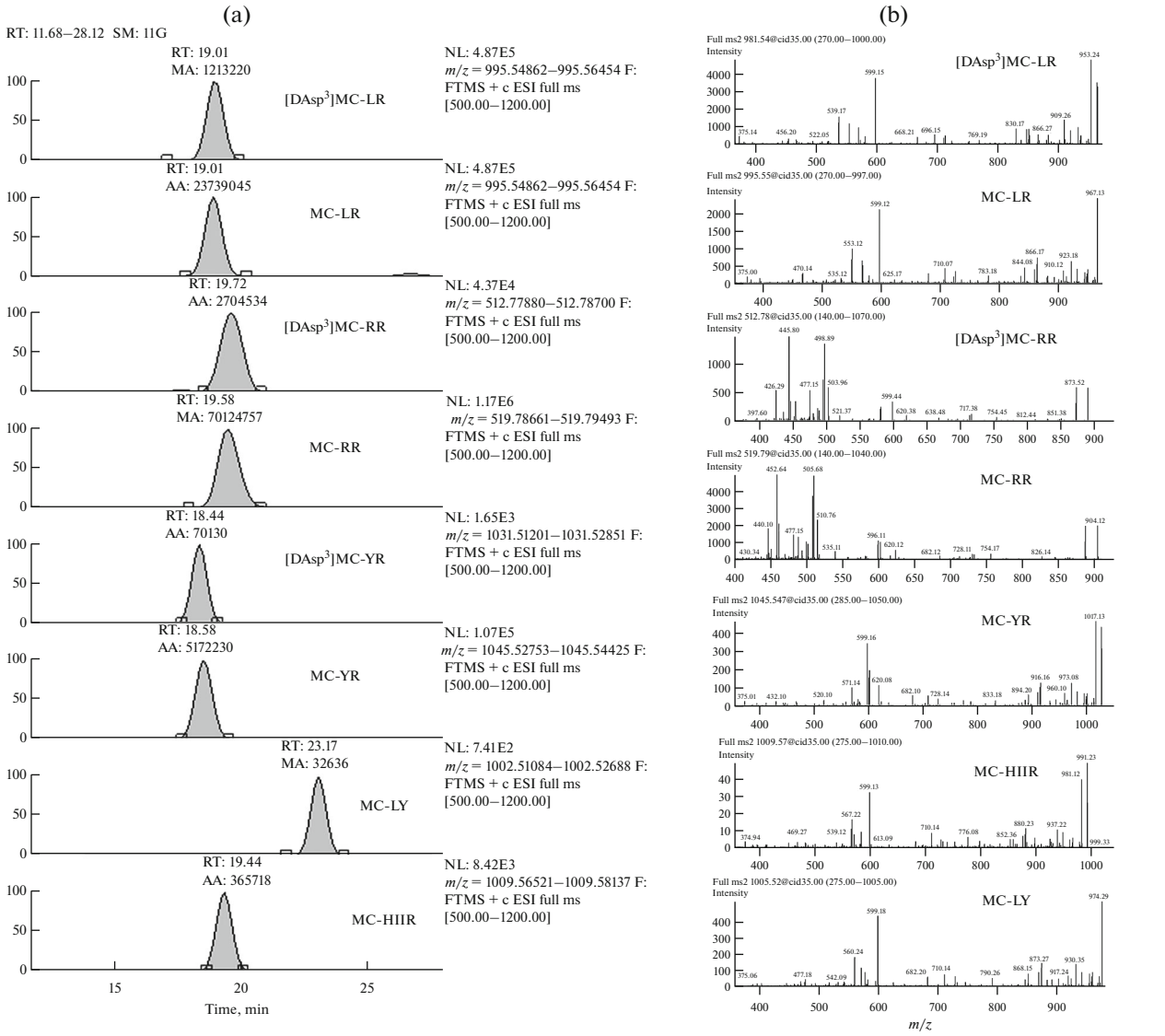

Fig. 3. Mass chromatogram of the isolated current (a) and tandem mass spectra (b) for detected structural variants of MC in the extract of the sample from Ytyk-Kyuyol Lake.

The intracellular concentration of MCs was the highest in the sample from Ytyk-Kyuyol Lake (803 ng/L), and trace amounts of MCs were present in Dachnoye, Leontiyevskoye, Log, and Nal-Tyungyulyu lakes. Quotas of MCs in the studied lakes were low and varied within 0.002–0.22 μg/mg (Table 5).

From one to eight structural variants of MCs were revealed in the samples; the contribution of arginine-containing variants MC-RR, MC-LR, and MC-YR was the greatest; the content of demethylated variants was lower. Mass chromatograms of ion currents (Fig. 3a) and tandem mass spectra (Fig. 3b) were obtained by analyzing the sample extract from Ytyk-Kyuyol Lake. We chose fragments $[Arg\text{-}Adda\text{-}Glu+H]^+$ (corresponding to the signal with m/z 599) and $[C_{11}H_{15}O\text{-}Glu\text{-}Mdha]^+$ (m/z 375) as characteristic ions for arginine-containing MC (Fig. 3b), as well as ions characteristic of each structural variant. The most toxic MC-LR was present in all samples, and its concentration in plankton was maximal in Ytyk-Kyuyol Lake (300 ng/L). Intracellular neurotoxic AN-a, SXT, and hepatotoxic NOD were not detected in the samples.

DISCUSSION

All the studied lakes apparently underwent anthropogenic impact consisting of the input of organic matter and nutrients from the catchment area, which resulted in a high nitrogen and phosphorus concentration, intensive color, and great COD. This caused high trophic status of the lakes. According to the classification by R.G. Wetzel (2001), Tyungyulyu Lake was assigned to hypertrophic reservoirs with respect to the

content of mineral nitrogen, and the rest of the lakes were β-eutrophic. All the reservoirs were hypertrophic according to the total phosphorus concentration. The ratio of the content of mineral nitrogen to the total phosphorus also testifies that the reservoirs were hypertrophic. Phytoplankton reached the greatest biomass only in Ytyk-Kyuyol, Leontiyevskoye, and Dachnoye lakes, where the water bloom of reservoirs was seen by the greenish tint of water. With respect to the phytoplankton biomass according to the classification by G.K. Nürnberg (1996), Tyungyulyu, Log, and Nal-Tyungyulyu lakes were α-β-mesotrophic, and the rest of the reservoirs were β-eutrophic. According to the classification of the trophic status of lakes by G.K. Nürnberg (1996) based on the concentration of chlorophyll *a,* Tyungyulyu Lake was assigned to α-β-mesotrophic, Ytyk-Kyuyol and Nal-Tyungyulyu lakes were polytrophic, and the rest of the lakes were α-β-eutrophic. All the lakes were characterized by a high content of total iron. It is known that the portion of biologically available phosphorus in waters rich in oxygen and iron may comprise only some part of its total amount (Nurnberg and Peters, 1984). Therefore, despite the increased content of nutrients in the studied reservoirs, their bioavailability could be limited.

The reason for the relatively poor development of cyanobacteria in Tyungyulyu Lake may be high pH. There is an opinion that the growth of cyanobacteria significantly decreases at pH > 9.5 (Fontes et al., 1987; Wang et al., 2011).

Cryogenic lakes are drainageless, which results in an increase in the water salinity at various stages of their development. This explains the high salt content in Tyungyulyu Lake, which is assigned to brackish lakes according to the classification by V.S. Samarina (1977). The increased salt concentration in water of Tyungyulyu Lake was probably another reason for the lowest biomass of cyanobacteria and of the concentration of chlorophylls among the lakes studied, despite the high trophic status and a significant content of nitrogen and phosphorus compounds. The decrease in phytoplankton biomass contrary to water salinity was described by the example of reservoirs of the Chany Lake system (Safonova and Ermolaev, 1983). The experiment with *Microcystis* cultures exposed to different salt concentrations (Tonk et al., 2007) showed that MCs were no longer detected at salinity higher than 10‰, but *Microcystis* could still grow at salinity to 17‰. The high concentration of chlorophylls *b* and *c* in Dachnoye, Log, and (especially) Nal-Tyungyulyu lakes was explained by the significant development of diatoms and green algae: their biomass reached from 15 to 40% of the total phytoplankton biomass.

It is known from the available data that cyanobacteria occur in the studied lakes from June to September, but their biomass becomes maximal in the second half of July and the beginning of August (Ivanova, 2000). Thus, the peak of the mass development of cyanobacteria is confined to a rather short time period, which is related to a limited vegetation period in the lakes studied. There are data on phytoplankton of 1964 for Ytyk-Kyuyol Lake, which testify that the mass development of cyanobacteria was previously typical for the reservoir: their biomass reached 8.5 mg/L in July (Vasil'eva, 1968). However, the composition of mass species differed from the modern one: there were no representatives of the genus *Microcystis* among them, and they were predominated by *Dolichospermum affine* (Lemmermann) Wacklin, L.Hoffmann & J.Komárek, *D. flos-aquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, *Aphanizomenon flos-aquae,* and *Trichodesmium lacustre* Klebahn.

Cyanobacteria in the lakes studied included species whose toxigenic properties were previously confirmed based on the study of particular cultivated strains: *Microcystis aeruginosa* and *M. flos-aquae* (producers of MC), as well as *Aphanizomenon flos-aquae* and *Dolichospermum spiroides* (producers of AN-a) (Bernard et al., 2017). Some other cyanobacteria found in the plankton of these lakes are now assigned to potentially toxic species, because their frequency of occurrence in reservoirs with the presence of cyanotoxins around the world is high and/or the toxic effect of their extracts on animals is known (without the identification of toxins). We revealed probable producers of NOD (*Aphanocapsa incerta*) (Jakubowska and Szeląg-Wasielewska, 2015), of MC and AN-a (*Woronichinia naegeliana)* (Willame et al., 2005; Voloshko et al., 2008; Bober and Bialczyk, 2017), of MC (*Snowella lacustris* (Humpage, 2008) and *Dolichospermum viguieri (*Mariani et al., 2015)), and of SXT (*Aphanizomenon flos-aquae* (Cires and Ballot, 2016; Lyon-Colbert et al., 2018)).

The combined study by PCR and liquid chromatography–mass spectrometry methods showed that cyanobacteria capable of producing neurotoxins SXT and AN-a, as well as hepatotoxic NOD, were absent in the studied lakes during their survey. However, we obtained the first evidence of the development of MC-producing cyanobacteria in plankton in five of the six lakes studied. The amplification of the *mcyE* gene region, using HEP primers universal to hepatotoxic cyanobacteria, and the detection of intracellular MC confirmed the ability of cyanobacteria in the lakes of Yakutia to produce these cyanotoxins. Further molecular analysis was aimed at identifying MC producers with the use of primers specific to the *mcyE* gene. *Microcystis* was the main producer of MCs in all the lakes, since PCR products of 250 bp in size were obtained using mcyE-F2/MicmcyE-R8 primers specific to MC-producing *Microcystis*. The results of light microscopy were in good agreement with the PCR data. Two species *M. aeruginosa* and *M. flos-aquae* known from published works as producers of MCs were found in plankton of five of the six lakes (Bernard

et al., 2017). It was shown that *Microcystis* species mainly produce MC-RR, MC-LR, and MC-YR (Furey et al., 2008), and these arginine-containing variants of MCs predominated in the lakes. We revealed *mcyE* gene-containing populations of *Dolichospermum* in Ytyk-Kyuyol Lake: the PCR product of the expected size (250 bp) was amplified with genus-specific primers mcyE-F2/AnamcyE-12R. The microscopic analysis of phytoplankton and the method of liquid chromatography–mass spectrometry showed that *Dolichospermum* species were present in this lake, and intracellular MCs were also detected. Cyanobacteria in phytoplankton were present in trace amounts in Tyungyulyu Lake, and the development of potentially toxic *Woronichinia naegeliana* and *Snowella lacustris* was insignificant. However, both intracellular cyanotoxins and genes of their biosynthesis were not detected in samples from Tyungyulyu Lake, which confirmed the inability of *Woronichinia naegeliana* and *Snowella lacustris* populations in the lake to synthesize cyanotoxins.

Data on possible concentrations of MCs in plankton of the northern circumpolar and polar lakes are scarce. The main attention during the study of toxic cyanobacteria in Arctic and Antarctic reservoirs has been paid to benthic species from the genera *Nostoc, Scytonema,* and *Oscillatoria*, which are part of cyanobacterial mats (Hitzfeld et al., 2000; Jungblut et al., 2006; Wood et al., 2008; Kleinteich et al., 2012; Chrapusta et al., 2015). Therefore, all published concentrations of MCs are given as the toxin amount (in ng or μg) relative to 1 g of dry organic matter (of cyanobacterial mat), which is incomparable with the concentrations of MCs determined in water and plankton, because they are usually calculated as the toxin amount in 1 L of filtered water (ng/L or μg/L). There are data in the work by J.V. Trout-Haney et al. (2016) that the maximal total content of MCs (including intracellular and extracellular fractions) did not exceed 400 ng/L in water of the Arctic lakes of southwestern Greenland during 2 years of observations. This is comparable to the concentration of intracellular MCs in Ytyk-Kyuyol Lake detected in this study. In general, it is concluded that MC concentrations in northern reservoirs under severe climatic conditions are orders of magnitude lower than in many eutrophic freshwater ecosystems of temperate or tropical regions (Cirés et al., 2017). However, concentrations of MCs in bloom spots in the circumpolar and Arctic lakes of Northwestern Russia may reach from 2500 ng/L in Lake Imandra (Denisov et al., 2021) to 12500 ng/L in Svyatozero Lake of the Onega basin (Smirnova et al., 2021). Thus, in the case of the formation of blooming spots in the studied lakes and others in the permafrost zone of Yakutia, we should expect a significant exceeding of the MPC of MC-LR (1000 ng/L) recently introduced for surface water bodies of domestic water consumption and cultural water use in the Russian Federation according to *SanPiN* 1.2.3685-21.

The MC quota is the amount of cyanotoxin in a unit biomass (or in one cell) of cyanobacteria producers. The MC quota is used as an indirect indicator of the toxicity of natural cyanobacterial blooms of reservoirs to assess potential risks to humans (Fastner et al., 2001; Chorus and Welker, 2021).

The calculated quotas of MCs for the studied lakes of the permafrost zone were extremely low and varied in the range from 0.002 to 0.22 μg MC/mg B. The quota of MCs in the studies of polar Lake Imandra was also low (0.005–0.069 μg MC/mg B) and significantly varied from year to year during the survey period (Denisov et al., 2021). The quotas of MCs in reservoirs of the temperate zone are on the contrary much higher: 0.05–3.8 μg MC/mg B in lakes of Germany (Fastner et al., 2001) and 0.14–5.6 μg MC/mg B in reservoirs of the Volga, the Kama, and the Don (Chernova et al., 2020). Although the data are insufficient, it may be assumed that the specific content of MCs per unit biomass of cyanobacteria producers is apparently significantly lower in reservoirs of the northern regions as compared to fresh waters of temperate latitudes. Further studies are needed to determine whether such low quotas of MCs in northern populations of cyanobacteria are related to the smaller production of MCs (the expression of genes of MC biosynthesis) under severe climatic conditions or to a small portion of strains capable of producing MCs. It was recently shown that only about 50% of the analyzed colonies of *Microcystis flos-aquae*—one of the most common producers of MCs in fresh waters of Russia—contained *mcy* genes of MC biosynthesis and were potentially capable of producing the toxin (Sidelev et al., 2020). This species was the main source of MCs in Ytyk-Kyuyol Lake during our study, and its biomass was significant (3.2 mg/L); however, the intracellular concentration of MCs and, consequently, the toxin quota were low.

CONCLUSIONS

Data on the presence of cyanotoxins of MCs and their potential producers in plankton of some lakes on permafrost in Yakutia (Yakutsk and its environs) were obtained for the first time. Most of the lakes under study were characterized by high concentrations of nitrogen and phosphorus compounds, an intensive water color, and an increased content of organic matter, which may be explained by a strong anthropogenic load. As a result, cyanobacterial blooming of water was visually detected in August 2021 in Ytyk-Kyuyol, Leontiyevskoye, and Dachnoye lakes. The intracellular concentration of MCs in plankton samples reached 803 ng/L, and they were dominated by MC-LR, the most toxic for organisms. Molecular methods have shown that the main producers of MCs were represented by species of the genus *Microcystis,* as well as by species of *Dolichospermum* in one of the lakes, the presence of which in phytoplankton was confirmed by

light microscopy during quantitative processing of samples. Available data on the phytoplankton of Ytyk-Kyuyol Lake in the middle of the last century enable us to conclude that, though the biomass has not increased, the structure of the species composition and the dominant species have changed. These changes could be caused by the increased anthropogenic load and global climate changes. In this regard, the urgency of studying the spread of cyanotoxins and their producers in the region will increase. Further research is needed to assess the potential danger of cyanotoxins for humans. A number of lakes in Central Yakutia are used for industrial fishing. Some of these reservoirs are characterized by water blooming in summer, with the mass development of cyanobacteria. Taking into account that MCs are very stable compounds capable of accumulation in tissues of living organisms, studies of the presence of cyanotoxins not only in the biomass of cyanobacteria, but also in lake water and in tissues of fish harvested in lakes may be urgent.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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