

# ***Dolichospermum lemmermannii*** **(Nostocales) bloom in world's deepest Lake Baikal (East Siberia): abundance, toxicity and factors influencing growth**

Bondarenko N.A.<sup>1</sup>, Tomberg I.V.<sup>1</sup>, Shirokaya A.A.<sup>1\*</sup>, Belykh O.I.<sup>1</sup>, Tikhonova I.V.<sup>1</sup>,  
Fedorova G.A.<sup>1,2</sup>, Netsvetaeva O.G.<sup>1</sup>, Eletskaia E.V.<sup>1</sup>, Timoshkin O.A.<sup>1</sup>

<sup>1</sup> Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Ulan-Batorskaya Str., 3, Irkutsk, 664033, Russia

<sup>2</sup> Russian State University of Justice, Ivana Franko Str., 23a, Irkutsk, 664074, Russia

**ABSTRACT.** Mass development of the cyanobacteria *Dolichospermum* (*D. lemmermannii* as the dominant species) was reported in the coastal zone of Bol'shye Koty Bay (western coast of the southern basin), towards the end of July to the beginning of August in 2019. Blooms were observed as 1–1.5-m wide bands adjoining the shoreline and stretched uninterrupted over 2 km. Abundance of cyanobacteria in blooms varied within 7.2–71.9 thousand cell mL<sup>-1</sup>, with 0.73–7.20 g m<sup>-3</sup> biomass attained. Maximal concentration was observed opposite the biological station of Irkutsk State University, a frequently visited place that was hosting participants of several conferences at that time. Hydrochemical analysis of samples collected four days after continuous heavy rains showed much higher concentrations of nutrients in contrast with the data obtained a week before (24 July), which is normal for that season. Nutrient concentrations were elevated relative to long term averages by 3 to 30 fold: with phosphate concentrations up to 0.200 mg L<sup>-1</sup>, ammonium ions 0.29 mg L<sup>-1</sup>, and nitrates 0.31 mg L<sup>-1</sup>. Possible reasons for the harmful freshwater cyanobacterial bloom that is unusual for this part of the lake are discussed. Excessive proliferation of common cyanobacteria *D. lemmermannii* in the open lake areas was affected by several factors: long-lasting heavy rains, zero wind, high air (from 13–15°C at night to 29°C in daytime) and water temperatures (from 15°C to 19.2°C); and absence of isolated septic tanks in the nearshore zone.

**Keywords:** harmful freshwater cyanobacterial bloom, *Dolichospermum lemmermannii*, cyanobacterial ecology, environmental factors, saxitoxin, biotic changes, Lake Baikal

## **1. Introduction**

Cyanobacteria (Cyanophyta, blue-green algae, Cyanoprokaryota) are attributed to prokaryotes, which are organisms with no true nucleus but with oxygenic photosynthesis (Andreyuk et al., 1990; Ecology of cyanobacteria..., 2012). The Cyanobacteria includes over 3,000 ancient unicellular or colonial species inhabiting various environments: fresh and salt waters, soils, barren deserts, hot springs and the Arctic glaciers (Komárek and Anagnostidis, 1998; Briand et al., 2002; Belyakova et al., 2006; Bondarenko and Shchur, 2007; Ecology of cyanobacteria..., 2012; Salmaso et al., 2015).

Cyanobacteria are highly adaptive organisms that proliferate in large amounts, causing water blooms (Paerl et al., 2001; Belyakova et al., 2006; Bondarenko and Shchur, 2007; Sitoki et al., 2012; Ho and Michalak, 2015; Korneva, 2015; Salmaso et al., 2015; Kurashov et al., 2018). Cyanobacterial blooms deteriorate water

quality, thus threatening water supplies, decreasing in fish production and impacting recreational use. Most of the Cyanobacteria are considered potentially toxic, endangering both human and animal health (Sirenko and Gavrilenko, 1978; Briand et al., 2002; Belyakova et al., 2006; Sitoki et al., 2012; Belykh et al., 2015a; 2015b; 2016; Ho and Michalak, 2015; Salmaso et al., 2015). In the last few decades, cyanobacteria have received increasing attention from researchers, especially in regard to their toxin production. Several types of cyanotoxins, among which microcystins were the most common, have been identified. These toxins are produced by *Microcystis*, *Anabaena* (presently *Dolichospermum*), *Planktothrix* and *Oscillatoria* genera. Neurotropic saxitoxins (STX) and their analogies called paralytic shellfish toxins (PST) are regarded as the most poisonous (Chorus and Bartram, 1999). In seas, PST are synthesised by dinoflagellates, while in freshwaters by cyanobacteria.

Long-term investigations of Baikal plankton

\*Corresponding author.

E-mail address: [shirokaya@bk.ru](mailto:shirokaya@bk.ru) (A.A. Shirokaya)

have found cyanobacteria among the summer plankton (Meyer, 1930; Kozhova, 1959; Popovskaya, 1991). Common colonial Baikal forms include species of *Gloeotrichia* and one of the most species-rich genera *Anabaena* (13 species and their variations). In the middle of the last century, Kozhova (1959) wrote that blue-green algae inhabit only shallow areas of Baikal, including the southern part of Maloe More Strait, nearshore areas of Chivyrkuy and Barguzin bays, Selenga shallows, North Baikal Sor and other small, shallow, well-warmed bays, where one litre of water contains several dozens of algal colonies. For example, the high concentrations of blue-green algae were reported in Bol'shye Koty Bay after strong winds from Selenga shallows during the warming period for the lake (Kozhova, 1959). According to Popovskaya (1991), during summer months, algal blooms occurred only in the nearshore zone of Middle and Northern Baikal where the surface water temperature was the highest (up to 12–15°C) and most occurrences of windless days. Hence, in such days, the abundance of *Anabaena* and *Gloeotrichia* genera reached 30 thousand cell mL<sup>-1</sup> and biomass 3 to 6 g m<sup>-3</sup>. In the 1980s, *Anabaena* species showed rapid growth in their biomass (Popovskaya, 1991). Blooms visible to the naked eye also covered large areas in the pelagic zone of the middle and northern lake basins from July to August. However, this phenomenon did not last long and was evidently caused by intensive proliferation of different species of *Anabaena*; most of all *A. lemmermannii* (Bondarenko, 2009).

Recently, various constituents of the Baikal nearshore algal community have undergone changes (Kravtsova et al., 2014; Timoshkin et al., 2016). Long-term studies (Kozhova, 1959; Antipova, 1974; Votintsev et al., 1975; Izmet'eva, 1988; Popovskaya, 1991; Bondarenko et al., 2012; Bondarenko and Logacheva, 2017) revealed significant restructuring of the lake's phytoplankton community, including the littoral zone. So far none of the publications reported mass blooms of *Anabaena* (currently termed *Dolichospermum*) under dominance of *A. lemmermannii* in the nearshore area of Bol'shye Koty Bay, although small numbers of the algae occurred early and now in the pelagic zone of this area and, in some years, its abundance was as many as 10 cells mL<sup>-1</sup> (Antipova, 1974).

In late July to early August 2019, high concentrations of planktonic organisms, resembling mass proliferation of cyanobacteria on their colour and visual features, were observed in some locations of Bol'shye Koty Bay. Here we present our findings as indicators of ecological changes in Lake Baikal with special attention to the possible impact in the nutrient loading.

## 2. Materials and methods

### 2.1. Study sites and sampling

Bol'shye Koty Bay (51°54'10.44"N, 105°04'14.06"E) stretches along the western side of the southern basin of Lake Baikal (Fig. 1). There

is a settlement by the same name on the shore with a Biological station of Irkutsk State University (ISU hereafter), and another station of a Research Institute of Biology (ISU). Both stations provide facilities to the university students and the staff of the Biology Institute participating in summer practice and field work. Approximately 1 km south of the Biological station is a field station of the Limnological Institute SB RAS (LIN hereafter). The University Biological station regularly organises international workshops, scientific conferences and meetings in summer. In July 2019, the station was frequently visited: it welcomed the 19<sup>th</sup> International Baikal Summer School on Physics of Elementary Particles and Astrophysics, Russian-American Summer School on Biology and a refresher course in teaching Russian as a foreign language (not less than two hundred visitors in total). In the summer, the small settlement also attracts many tourists.

To delineate the bloom-affected areas and determine the dominant organisms, we made visual observations of the nearshore zone within the limits of the bay (Fig. 1), starting from the field station of LIN (the southernmost point, site No. 2) to hotel Mayak (the northernmost point, site No. 3). The total length of the zone studied was 2.5–3 km. Samples for organism identification were directly collected from the blooms at the water's edge, and conditionally non-bloom locations were chosen as reference sites (site No. 3).

Microphotographs of the microorganisms dominating the samples were taken using a Meiji Techno Co. microscope with x40, x100 and x400 magnification by an attached ToupView 3.7 (ToupTek) digital camera.

Water for chemical analysis was sampled twice at two sites on the 24<sup>th</sup> and 31<sup>st</sup> of July, covering the distance of nearly 2.5–3 km along the shoreline (Fig. 1): opposite the ISU (sampling point No. 1) and the LIN (sampling point No. 2) field stations. Site No. 3 was located north of the first two points at the end of the settlement (Fig. 1).

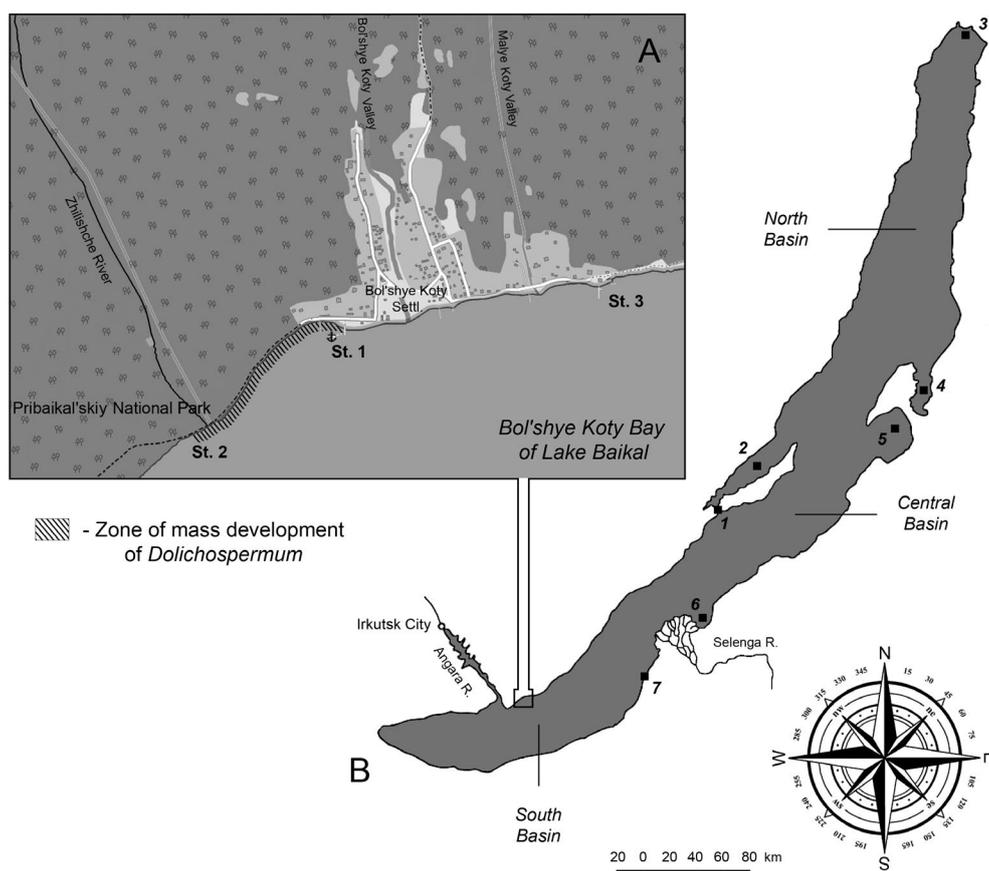
Sampling of rainfall was carried out using an automatic rain collector "US-320" (Japan), funnel diameter 357 mm in accordance with the manual (Technical manual..., 2000).

The taxonomic composition and plankton abundance were recorded after sampling at all these sites on the 31<sup>st</sup> of July at the moment of the cyanobacterial bloom.

The air and water temperature dynamics in this area in June–July 2019 were registered by Optic StowAway Temp (C) ONSET Tid Bit Loggers and HOBO Loggers. The measuring frequency was 1 in 30 minutes and they were installed near the lakeshore opposite the field station of LIN. The surface water temperature was measured on the 31<sup>st</sup> of July, using Checktemp °C thermometer.

### 2.2. Water chemistry analyses

Chemical analyses were performed using conventional freshwater water chemistry methods (Wetzel and Likens, 2000; Khodzher et al., 2017) and



**Fig.1.** A. Schematic map of Bol'shye Koty Bay (Lake Baikal), showing the sampling points (Stations 1–3). B. Other locations where cyanobacterial blooms have been observed (1—Malye Ol'khonskiye Vorota Strait, 2—Maloe More Strait, 3—North Baikal Sor, 4—Chivyrkuy Bay, 5—Barguzin Bay, 6—Proval Bay, 7—Istok Sor).

carried out at the Laboratory of Hydrochemistry and Atmosphere Chemistry (LIN) using the equipment of the Shared Research Facilities for Physical and Chemical Ultramicroanalysis LIN SB RAS. The samples were filtered through a cellulose acetate membrane filter, with a pore diameter of 0.45  $\mu\text{m}$ , and the nutrient content in the filtered water was measured using the following colorimetric methods: indophenol blue method for  $\text{NH}_4^+$ , Griss's method for  $\text{NO}_2^-$ , Deniges's method for  $\text{PO}_4^{3-}$  and the nitrate was measured with sodium salicylate (Khodzher et al., 2017).

### 2.3. Plankton identification

For plankton identification, we fixed 1 L of water with Lugol's solution and then concentrated it by settling. Plankton organisms were counted twice in a 0.1 mL Nageotte chamber under a Peraval light microscope with x720 magnification. The biomass was calculated from the organism number using individual cell volumes (Kiselev, 1956). To determine the biovolume, 100 cells of the species were measured. Cyanobacteria were identified according to Komárek and Anagnostidis (1998).

### 2.4. Saxitoxins

The presence of STX was recognised by ELISA, using the Abraxis Saxitoxin ELISA kit Abraxis LLC, USA) in according to the manufacturer's protocol. Enzyme-

linked immunosorbent assay was carried out by the Stylab Company (Moscow, Russia). The water samples 1 mL in volume and plankton samples (cyanobacteria surface scum) were dried by a vacuum centrifuge at 60°C. Then the plankton dried samples were weighed. The ELISA data were processed by RIDA® SOFT Win.

The genetic and ELISA analyses were carried out after sampling surface water on the 31<sup>st</sup> of July 2019 and on the 5<sup>th</sup> of August 2019 during the summer stratification.

## 3. Results

### 3.1. Air and water temperature

The air got warmed up to mean diurnal temperatures of 20°C and higher in the study area in July over the last decade (and during 10 days before the bloom), rising to 25–29°C in daytime and never below 13°C at night (13 to 15°C). Late July is the time of intense warming of the water and results in diurnal temperature fluctuations in the Baikal nearshore zone. In this period, even the bottom water temperature at the depth of 3m varied within the range of 15 to 19.2°C (except two cases, the 30<sup>th</sup> of July and the 1<sup>st</sup> of August, when morning temperatures felt to 6 and 7°C) during the 10 days preceding the cyanobacteria bloom (Fig. 2). The surface-water temperature, measured at the time of sampling along the nearshore blue-green bloom, was 14.4°C across all four measurement points.

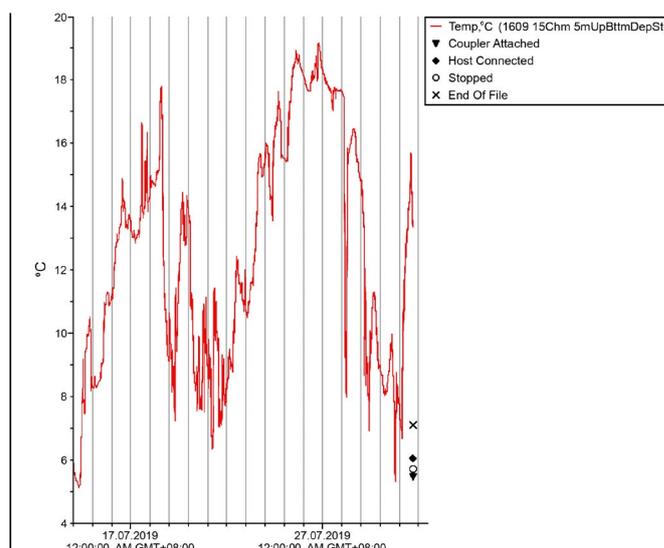
### 3.2. Hydrochemistry

Hydrochemical data have been collected annually since 2014 in the nearshore area of Bol'shye Koty Bay during the summer (data of I.V. Tomberg, LIN). Long-term dynamics (2014–2019) show a drop to the minimal values of the nutrients (mostly below detection limit) in July–August, induced by the consumption of mineral nitrogen and phosphorus by phytoplankton in the nearshore zone of the bay, similar to the entire lake (Table 1). As shown in Table 1, the station 3 significantly differs from the other stations 1 and 2 by ammonium and nitrate nitrogen concentrations, which could indicate less intensive anthropogenic pressure at this station because the Baikalian pure waters always contain trace concentrations of these ions (Votintsev et al., 1975; Khodzher et al., 2017).

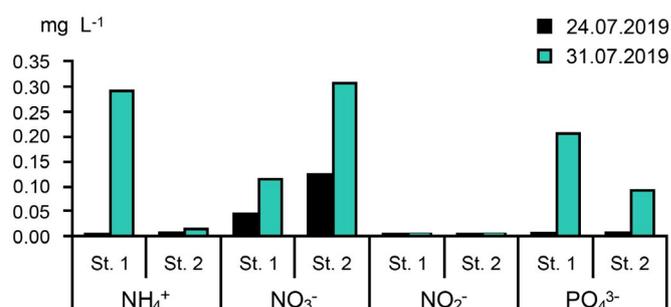
The chemical composition of the water collected on the 24<sup>th</sup> of July 2019 did not deviate from the long-term average reports—only trace quantities of ammonium ions, nitrates and phosphates were registered in the nearshore waters (Fig. 3). In the vicinity of the field station of LIN, the concentrations of nitrates were slightly higher ( $0.12 \text{ mg L}^{-1}$ ), due to the effect of the small Zhilishche River running into the lake 50 m north of the LIN station. The concentration of silicon here was also higher ( $0.84 \text{ mg L}^{-1}$ ), as compared to the water near the university station ( $0.53 \text{ mg L}^{-1}$ ).

The samples collected on the 31<sup>st</sup> of July during the cyanobacterial bloom showed concentrations of phosphates, ammonium and nitrates to be much higher than those registered a week before (Fig. 3). We observed that a heavy rainfall on the 26–29<sup>th</sup> of July 2019 may have caused such changes in the chemical composition of the lake water. During this period, over 51 mm of precipitation was reported, making up half the monthly norm of July. Showers washed away large quantities of soil organics and, likely, domestic wastes from non-isolated septic tanks of the neighbouring buildings. The rains also affected the inflow of nitrogen, phosphorus and silicon.

Chemical analysis of the rainwater showed the possible source of nutrients to the lake (Table 2). The phosphate concentration in the rainwater reached  $0.131 \text{ mg L}^{-1}$ , nitrates up to  $3.65 \text{ mg L}^{-1}$ , and ammonium  $10 \text{ mg L}^{-1}$ . The noteworthy indications of source for these nutrients are the high concentrations of potassium (up to  $0.64 \text{ mg L}^{-1}$ ) and sulphates (up to  $8.53 \text{ mg L}^{-1}$ ) and extremely low pH values (3.93–4.77). The atmosphere was evidently contaminated at the time by large-scale



**Fig.2.** Water temperature near the bottom at a depth of 3 m, a fragment of a plot showing the dynamics of water temperature 10 days before the bloom.



**Fig.3.** Concentration of nutrients in the water near the shore of Bol'shye Koty Bay in July 2019 at Site 1—the biological station of Irkutsk State University—and Site 2—field station of Limnological Institute SB RAS. Although replicate sampling for nutrients was not conducted, extensive sampling before and after the work reported here shows that coefficients of variation for lake water nutrient analyses are typical < 10% with high values ranging to 20% (Khodzher et al., 2017; Tomberg et al., 2012).

forest fires registered in the Krasnoyarsk Region and to the north of the Irkutsk Region. The rise of nitrate ( $0.31 \text{ mg L}^{-1}$ ) and silicon concentrations ( $1.41 \text{ mg L}^{-1}$ ) near the LIN station, possibly, was a consequence of excessive inflow of river water raised after rain.

**Table 1.** Long-term average concentrations of nutrients ( $\text{mg L}^{-1}$ ) during July–August 2014–2019.

	$\text{NH}_4^+$	$\text{NO}_2^-$	$\text{NO}_3^-$	$\text{PO}_4^{3-}$	Si
			C ± S, $\text{mg L}^{-1}$		
Site 1	$0.009 \pm 0.008$	$0.003 \pm 0.002$	$0.05 \pm 0.02$	$0.009 \pm 0.007$	$0.38 \pm 0.13$
Site 2	$0.011 \pm 0.009$	$0.002 \pm 0.002$	$0.11 \pm 0.06$	$0.007 \pm 0.004$	$0.50 \pm 0.33$
Site 3	$0.005 \pm 0.005$	$0.001 \pm 0.001$	$0.04 \pm 0.02$	$0.009 \pm 0.002$	$0.41 \pm 0.14$

C: average concentration; S: standard deviation.

**Table 2.** Elemental composition of precipitation in 2019 in Bol'shye Koty Settlement (station of Limnological Institute SB RAS).

Date	N	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	K <sup>+</sup>	Σ <sub>и</sub>	pH
		C ± S, mg L <sup>-1</sup>						
26.07.2019	1	1.01	0.43	0.131	2.13	0.14	5.2	4.53
27.07.2019	5	1.20 ± 1.04 (2.88)	0.77 ± 0.65 (1.72)	0.034 ± 0.027 (0.069)	2.93 ± 2.09 (6.11)	0.06 ± 0.05 (0.14)	5.8 ± 4.6 (13.0)	4.45 ± 0.36 (4.03)
28.07.2019	4	0.57 ± 0.20 (0.57)	0.47 ± 0.33 (0.95)	0.012 ± 0.007 (0.021)	2.13 ± 0.76 (3.06)	0.03 ± 0.01 (0.05)	3.6 ± 1.1 (4.6)	4.38 ± 0.10 (4.27)
29.07.2019	2	2.28 ± 0.47 (2.61)	3.62 ± 3.49 (6.1)	0.018 ± 0.010 (0.026)	5.10 ± 1.23 (5.97)	0.16 ± 0.01 (0.16)	11.9 ± 5.3 (15.6)	4.01 ± 0.04 (3.98)
30.07.2019	1	3.65	10.1	0.012	8.53	0.64	24.1	4.33

N: number of samples collected per day; Σ<sub>и</sub>: sum of ions; C: mean daily concentration; S: standard deviation; maximal concentrations observed in a day mentioned in brackets.

### 3.3. Plankton

On the 24<sup>th</sup> of July 2019, the surface water in the nearshore zone of the bay was clear, whereas on the 31<sup>st</sup> of July, after four days of continuous showers that caused the rise in nutrient concentrations (Fig. 3), we observed massive abundance of cyanobacteria (Fig. 4). Substantial amounts of cyanobacteria were observed along the shoreline between the field station of LIN in the south and a pier of the Research Institute of Biology in the north. Maximal blooms were registered opposite the hostels of the Institute and University which were visited by approximately 200 people in the two weeks before the study. The distance from the water's edge to the housings was about 20–30 m. The shoreline zone in Bol'shye Koty Bay north and south of these buildings was free of the visible blooms of nuisance cyanobacteria. The cyanobacteria formed a solid stripe 1–1.5-m wide and approximately 1-km long adjoining the water's edge, reducing the transparency of water to 20–30-cm depths. The day after, on the 1<sup>st</sup> of August (windless, sunny, no more rain), visual observations revealed no cyanobacterial masses along the shoreline area. An expedition of microbiologists collecting samples on the 13<sup>th</sup> of August found these cyanobacteria deep in the water nearly 15 m away from the shore.

Microscopic analysis of the “blooming” organisms revealed massive abundance of *Dolichospermum* cyanobacteria during collection, with a dominance over 98% of the total abundance of *D. lemmermannii* (Fig. 5). The concentration of *Dolichospermum* along the shore of the bay varied from 7.2–71.9 thousand cell mL<sup>-1</sup> and biomass 0.73–7.20 g m<sup>-3</sup>. Maximal concentration was recorded at the site opposite the canteen of the University Biological station, with a minimum at Site No. 3 at the northern end of the bay (Fig. 1). The maximal abundance and biomass in this case were higher than those reported by Popovskaya (1991) at the end of 1980–90s, 30 thousand cell mL<sup>-1</sup> and 3–6 g m<sup>-3</sup>, respectively. The colonies included actively dividing cells, heterocysts and akinetes, i.e. cyanobacteria were not at the initial but a mature vegetation stage. These colonies were densely covered by attached ciliates of the

genus *Vorticella*, their abundance varying from 10–20 cells mL<sup>-1</sup> indicating the bloom was likely terminating.

### 3.4. Saxitoxins

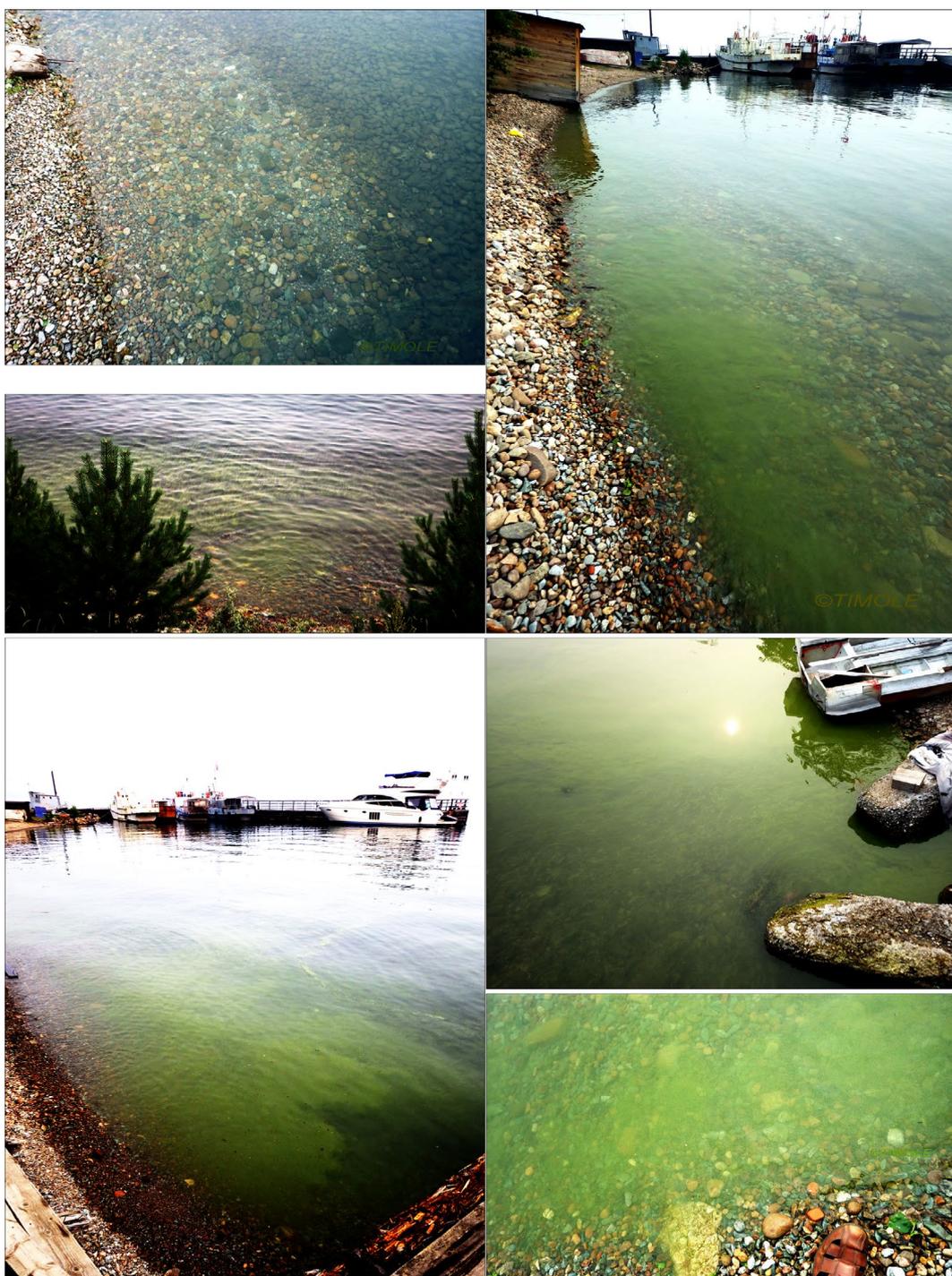
Saxitoxin-producing genes were present across all samples under this study, as shown by the PCR screening with *stxA*-gene primers. The concentration of saxitoxin in the water samples collected on the 31<sup>st</sup> of July was 0.45 ± 0.05 µg L<sup>-1</sup> and 0.025 ± 0.01 µg L<sup>-1</sup> on the 13<sup>th</sup> of August, respectively. Saxitoxin content in cyanobacteria cells (intracellular) was 7.900 ± 200 µg g<sup>-1</sup> of dry weight.

## 4. Discussion

Notwithstanding that cyanobacteria are common inhabitants of shallow, warm, calm and eutrophicated waters, their recent mass development in many large lakes and water reservoirs of the world has become a matter of deep concern for the research community due to their ability to produce toxins that are hazardous to human and animal health (Paerl et al., 2001; Sitoki et al., 2012; Ho and Michalak, 2015; Korneva, 2015; Kurashov et al., 2018; Sterner et al., 2020). For example, Lake Erie suffered from a burst of cyanobacterial bloom, leading to an extension of dead zones at the lake bottom, a decrease in fish populations and the contamination of beaches, negatively affecting the local tourism industry (Ho and Michalak, 2015; Chaffin et al., 2019). High microcystin was registered in the drinking water of Toledo City, Ohio, located at the shallowest of Lake Erie after a cyanobacteria bloom in the municipal water supply system (Ho and Michalak, 2015). The cause of this massive cyanobacterial growth was the discharge of phosphorus into the lake from agricultural fields and municipal sewage treatment plants. Lake Erie receives the largest loading of phosphorus than any North American Great Lakes. As a result, the residents of Toledo (over 200.000) were prohibited from using tap water for domestic needs and had to buy bottled water.

Cyanobacterial blooms are often associated with eutrophic environments, but they sometimes occur in oligotrophic, for example, in Lake Superior, there have been observed *Dolichospermum* blooms along the southern shoreline, a region where human recreational contact often is high (Sternier et al., 2020). In oligotrophic Lake Baikal over the last decades, investigators have attributed the cyanobacteria encountered not only in bays and shallows as well as the pelagic zone of the lake to the dominant forms of the summer plankton population (Izmest'eva, 1988; Popovskaya, 1991; Belykh and Sorokovikova, 2003; Belykh et al., 2006; Bondarenko, 2009). Picoplankton of the genera *Synechococcus* and *Synechocystis* are among

principal primary producers in the summer pelagic zone, and their abundance often reaches several billion per litre. According to the white disc test, the water transparency drops to 3.5–5.0 m in summer which is its annual minimum (Bondarenko, 2009). In years with warmer water and the absence of storms, these species are joined by the representatives of *Dolichospermum*, first by *D. lemmermannii*, the abundance of which sometimes attains millions of cells per litre. The blooms are most frequently observed in Selenga shallows, the nearshore shallow areas of the northern lake basin, and some adjacent locations in the middle and southern basins (Bondarenko, 2009).

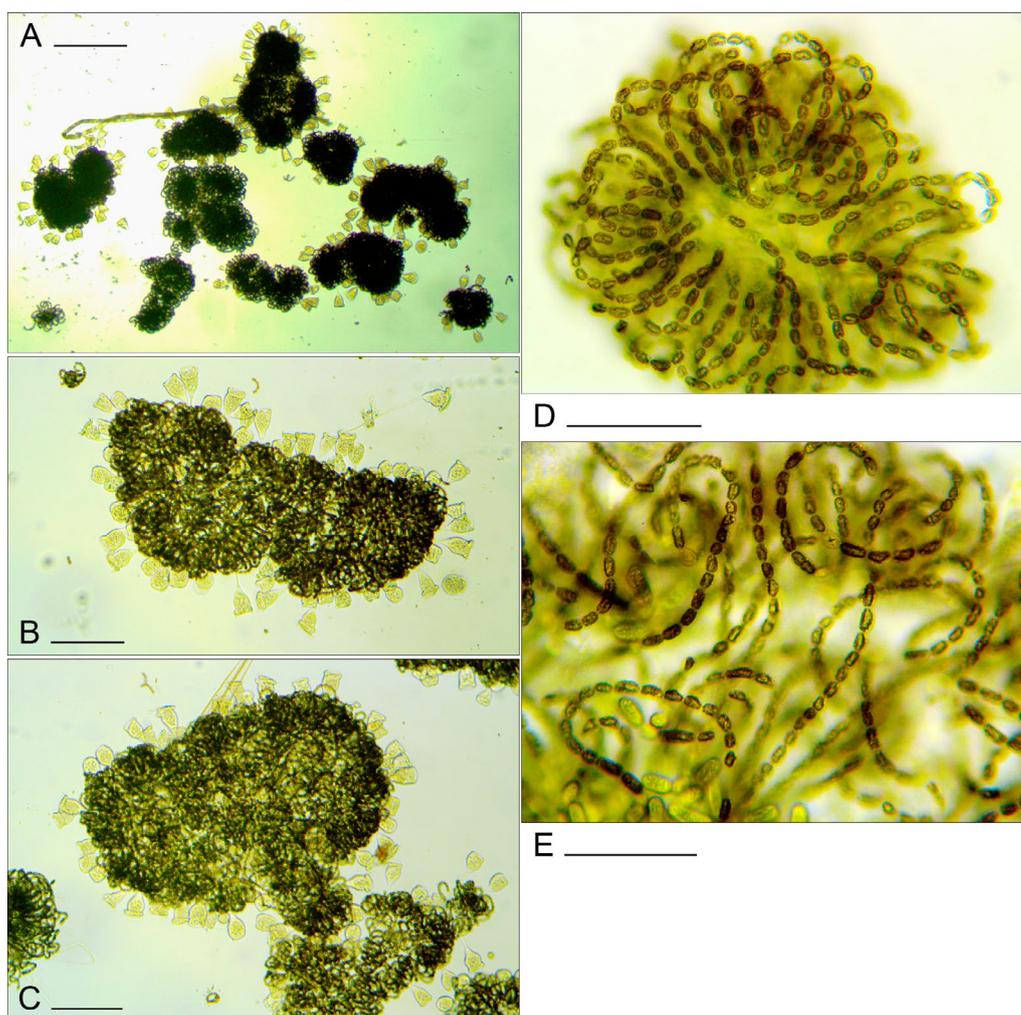


**Fig.4.** Nearshore area of Bol'shye Koty Bay in summer 2019: free of *Dolichospermum lemmermannii* bloom (slightly northeastern of Station 1; left upper photo) and within the bloom (photo images consequently made from Station 1 to Station 2).

Thus, an intriguing question arises: What was the triggering point for the cyanobacterial bloom in Bol'shye Koty Bay in 2019? Some researchers consider the blooming of cyanobacteria in the open parts of Baikal to be a consequence of wind activity (Kozhova, 1959; Votintsev et al., 1975; Popovskaya, 1991) that induced a transfer of the algae from the shallow-water areas, particularly Selenga shallows, Istok Sor and Proval Bay, to other lake regions. It is suggested that cyanobacterial masses in the coastal area of Bol'shye Koty Bay in July 2019 migrated from Selenga River, Maloe More Strait shallows and other places with winds or currents flowing along the shoreline. According to Antipova (1974), Bondarenko et al. (2012) and our personal data, in 1947–2018, the concentration of the algae in Bol'shye Koty Bay did not exceed 5–10 cells mL<sup>-1</sup> in summer; but on the 31<sup>st</sup> of July 2019, their abundance was 7.2–71.9 thousand cells mL<sup>-1</sup>. Thus, one can easily state that the time taken for cyanobacterial masses to develop in this bay, starting from the initial quantity and considering a cell division rate of 1 to 2 per day (Votintsev et al., 1975; Bondarenko, 2009), might vary from 3.3 to 8.9 days. It seems likely that, for a short period, the nearshore zone was populated by *D. lemmermannii* masses with the initial concentration of 5–10 cells mL<sup>-1</sup> under favourable conditions; in other

words, extensive growth of cyanobacteria started there, in the bay. In this context, it would be interesting to know the factors that may have influenced their initiation and growth.

Higher temperature is regarded as one of the driving forces of intensive cyanobacterial development (e.g. Hickel, 1988; Briand et al., 2002; Jann-Para et al., 2004; Belyakova et al., 2006; Bondarenko and Shchur, 2007; Ecology of cyanobacteria..., 2012; Korneva, 2015; Salmaso et al., 2015). Ecologic plankton surveys undertaken in the water bodies and channels of East Siberia confirm this fact. Reports on the blooms in low-mineralised (total ions 36–80 mg L<sup>-1</sup>) mountain and high mountain lakes of the region upon the warming of surface waters in the summer provide us with convincing evidence of the thermal effect on cyanobacteria production (Bondarenko and Shchur, 2007). On the other hand, the largest annual biomass of cyanobacteria was observed in September–October in the lakes around Baikal when the water temperature dropped from 21°C to 6°C (Bondarenko and Shchur, 2007). In January–February, intensive winter vegetation of cyanobacteria in these nearshore lakes shows that the water temperature is an important but not exclusive factor affecting their quantitative characteristics (Bondarenko and Shchur, 2007). This is



**Fig.5.** External view of *Dolichospermum lemmermannii* from the coastal zone of Bol'shye Koty Bay. Scale bars: A—0.30 mm, B–C—0.16 mm, D–E—65.4 μm.

also confirmed by the large diversity and abundance of cyanobacteria in lakes of Yakutia (Komarenko and Vasilyeva, 1975), Taymyr (Ermolayev, 1974) and other lakes of the Extreme North (Osobennosti struktury..., 1994).

According to the available data, including the above-mentioned cases of patchy blooms of *D. lemmermannii* in the open pelagic zone of Baikal, water warming and higher temperatures in calm windless weather, indeed, should be considered as one of the driving forces for this species' mass growth, but barely the leading one. The air and bottom-water temperatures in Bol'shye Koty Bay were very high 10 days before blooming, while the last few days after the bloom were calm. Cyanobacteria started to develop on the 28<sup>th</sup> and 29<sup>th</sup> of July when the temperature of the upper-water layer decreased to 14.4°C due to heavy rain. But warm and stable temperature conditions of surface water may positively affect the harmful cyanobacterial bloom.

Water "blooms" in well-warmed shallow water bodies caused by cyanobacteria are also considered to be related to nutrient enrichment during vegetation (Hickel, 1988; Osobennosti struktury..., 1994; Briand et al., 2002; Jann-Para et al., 2004; Belyakova et al., 2006; Bondarenko and Shchur, 2007; Ecology of cyanobacteria..., 2012; Salmaso et al., 2015), particularly with higher concentrations of nitrogen and phosphorus as reported in this paper. Long-term dynamics of the nutrients analysed using the data obtained in this investigation as well as published (Votintsev et al., 1975) evidence that concentrations of phosphates (up to 0.200 mg L<sup>-1</sup>), ammonium (up to 0.29 mg L<sup>-1</sup>), and nitrates (up to 0.31 mg L<sup>-1</sup>) registered in the nearshore lake water on the 31<sup>st</sup> of July 2019 were in 3 to 30 times higher than average long-term concentrations. Bol'shye Koty Settlement located at the bay shore is not equipped with centralised sewage treatment utilities or isolated septic tanks, inevitably leading to increase in nutrients in the coastal waters, especially in summer when the number of visitors is high. These nutrients would be supplemented by the extra inflow of nutrients, mainly nitrogen and phosphorus, from the heavy and continuous four-day rainfall in July 2019. These heavy rains promoted the nutrient load to the lake. Maximal concentrations were confined to the area near the water's edge, which receives the flow of surface and ground waters enriched by nutrients (Fig. 3; Table 2). It seems hard to find another reason, if any, for the cyanobacterial bloom precisely in the water's edge zone 0.2–0.5m deep after four days of calm but rainy weather.

We suggest that the polluted waste waters were the main source increasing ammonium and phosphate concentrations in the nearshore zone at the University station because ammonium and phosphate were usually detected as traced elements in such waters. This is also confirmed by the data obtained in 2016–2018 during studies at the LIN field station in the same bay (Timoshkin et al., 2018). High concentrations of nutrients like those observed in the ground (lysimeter data) and interstitial waters of the beach were found. Convincing evidence has been provided for the

contamination being a result of the continuous inflow of domestic waste waters from the non-isolated sewage pits of the station household, bringing about the mass development of benthic *Spirogyra* (morphotype 1), which is known as an indicator of water pollution of Baikal in the area of underwater discharge of contaminated ground waters. The hydrochemical indicators of the nearshore waters were not different from those given in the long-term average data. It is interesting to note that the heavy rains during the period preceding the bloom also contributed to the accumulation of ground water in lysimeters and the contamination of interstitial waters in summer 2016.

Along with the environmental factors affecting the harmful freshwater cyanobacteria bloom, we should consider the sensitivity of some cyanobacterial forms to mechanical effects, such as waves, turbidity, etc. During our surveys, we observed the same situation: *D. lemmermannii* masses disappeared from the shore area on the 1<sup>st</sup> of August, after a strong night wind; however, cyanobacteria were still occurred in the water until at least the 13<sup>th</sup> of August.

Blooms of saxitoxin-producing cyanobacteria in the littoral part of Bol'shye Koty Bay led to the occurrence of saxitoxins in the water, with concentrations up to  $0.45 \pm 0.05 \mu\text{g L}^{-1}$ . A dominant *D. lemmermannii* species was apparently the producer of saxitoxin. As early as 2010, ELISA and *stxA*-gene sequencing showed that *D. lemmermannii* was responsible for synthesis of saxitoxins in the littoral zone of eastern coast of Lake Baikal in the vicinity of Turka Village (Belykh et al., 2015a).

The presence of saxitoxins in the lake waters of Finland and Denmark was related to *D. lemmermannii* activity because the saxitoxin-positive phytoplankton samples included 95–100% of this species (Lepisto et al., 2005; Rapala et al., 2005). *D. lemmermannii* blooms in Scandinavian lakes appeared to be one of the most toxic among the cases registered, maximal STX (1.070  $\mu\text{g L}^{-1}$ ) concentrations were observed in an oligotrophic lake (Rapala et al., 2005). STX concentrations reported in Lake Baikal were much lower than the minimal values registered in Finnish lakes. It should be noted that the analytical kit used for saxitoxin identification had 0.6–29% cross-reactivity with the analogues: dcSTX, neoSTX, dcneoSTX, GTX2,3, dcGTX2,3, GTX-5B, sulfoGTX1,2 and lynbyatoxin variants, therefore, the samples under this study might contain the above-mentioned paralytic toxins of molluscs. Earlier, MALDI-TOF analysis allowed us to identify several saxitoxin variants in Lake Baikal (Barguzin Bay and Malye Ol'khonskiye Vorota Strait) and reservoirs on the Angara River (Irkutsk, Bratsk and Ust'-Ilimsk) (Belykh et al., 2015b).

Countries with widespread harmful toxic blooms that are hazardous to human health have introduced national guidelines for water use. In New Zealand, Australia and Brazil, the maximum permissible level for drinking water is 3  $\mu\text{g L}^{-1}$  (Current approaches..., 2012). In the shore area of Bol'shye Koty Settlement, the concentration of saxitoxin in the water ( $0.45 \pm 0.05 \mu\text{g L}^{-1}$ ) was below the threshold for drinking and recreational water usage. Our latest observations

showed lower saxitoxin concentration in the water samples from Lake Baikal. Their maximal concentration registered in 2010 in Middle Baikal was  $1.93 \pm 0.64 \mu\text{g L}^{-1}$  (Belykh et al., 2015a). In Maloe More Strait, the concentrations were more similar to those reported here ( $0.59 \pm 0.2$ ) (Belykh et al., 2015b).

A complex set of environmental and anthropogenic factors, such as weather conditions and maximal recreational activity during the period of studies, were found to be involved in the mass development of *D. lemmermannii* in the open part of Bol'shye Koty Bay. This indicates the urgent need of establishing federal regulations on sewage treatment in small nearshore settlements, which are overpopulated during peak tourist season, providing for the lowest possible contamination of the open part of Baikal by untreated household and municipal wastes.

Moreover, our recent findings suggest that the current contribution of heavy precipitation to the late summer (period of maximal warming of the water in the littoral zone of Lake Baikal) in East Siberia combined with other favourable environmental factors might possibly trigger more widespread proliferation of toxic cyanobacteria not only in Bol'shye Koty Bay but also throughout the entire lake perimeter, eventually reaching the pelagic zone. Similar situation was in the large oligotrophic Lake Superior, the most pristine of the Laurentian Great Lakes. In the past decade, there have been observed *Dolichospermum* blooms (Sterner et al., 2020). The two largest of them, in 2012 and 2018, occurred during years of especially extreme rainfall which provided nutrients or living propagules to the blooms from the watershed. The authors consider, if these newly observed blooms are indeed driven by temperature and rainfall as this evidence suggests, blooms may continue.

As far as our present investigation of the harmful toxic cyanobacterial blooms in the coastal zone of Lake Baikal is concerned, we realise how vulnerable this unique water body is because it shelters endemic flora and fauna and holds 20% of the world's non-frozen fresh water reserves, while 2.7 billion people experience water scarcity. Finally, the obtained results, regrettably, provide one more evidence of current negative ecological changes in coastal biota of Lake Baikal.

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## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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