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THE BLOOM AND TOXICITY OF CYANOBACTERIA IN LAKE SEVAN

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Seasonal hydrochemical, hydrophysical, and phytoplankton studies were conducted in a littoral zone of Lake Sevan in 2019. It is known that in recent years widespread algal blooms were usually started from the littoral zone of Big Sevan, and then spread towards the pelagic zone of the lake. The bloom was mainly caused by species belonging to the genus *Dolichospermum* (previously *Anabaena)*. Our study has revealed that the physical and chemical properties of water were changed as a result of algal bloom. Particularly, the transparency of water and concentration of dissolved oxygen decreased, while the concentrations of ammonium, nitrite and phosphate ions increased. For the first time, the presence of the toxins microcystin and anatoxin-a was recorded in the lake.

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Introduction. Lake Sevan (with surface elevation of 1900 *m* AMSL, water volume of 36 km^3, and surface area of 1250 km^2) is the largest freshwater body in the South Caucasus region. It is of strategic importance as a freshwater reserve and, therefore, is invaluable for the social and economic life of Armenia [1]. The main reasons for the eutrophication of Lake Sevan are fluctuations in its water level, wastewater discharge from the drainage basin, and outdoor activities in the summer. According to the study by Hambaryan et al. [2], the water quality of Lake Sevan deteriorated periodically, and algal blooms caused by toxic algae species such as *Aphanizomenon flos-aquae*, *Microcystis aeruginosa*, *Dolichospermum*/*Anabaena flos-aquae* took place in the lake [2]. In general, 50–75% of algal bloom isolates can produce toxins. Toxic and non-toxic bloom of the same algae species can arise simultaneously [3, 4]. Production of cyanobacterial toxins (cyanotoxins) can threaten human and animal health and in environmentally relevant concentrations it can even lead to mortality [5].

The aim of the present study was to reveal the changes in water quality under the conditions of toxic algal blooms in Lake Sevan.

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Materials and Methods. In order to reveal cyanobacterial toxins as well as changes in the structure of phytoplankton community and in hydro-chemical parameters (pH, concentrations of dissolved oxygen (DO) and nitrate, nitrite, ammonium, and phosphate ions), we conducted investigations in a littoral zone of Lake Sevan in two periods of 2019: prior to harmful algal bloom (early June) and during harmful algal bloom (in July). The first set of phytoplankton samples was collected from the littoral zones adjacent to Lchashen, Tsovazard, Lichq and Artanish communities. The second set was broader and covered eight stations near Lchashen, Tsovazard, Hayrivanq, Drakhtik, Shorja, Karchaghbyur, Artanish and Lichq communities.

Water samples for hydro-chemical analysis were collected from the littoral zones adjacent to Lchashen, Tsovazard and Lichq communities in June and from the zones adjacent to Lchashen, Tsovazard (less polluted) and Karchaghbyur (probably, the algal bloom started from this site) communities in July. These stations was chosen on the assumption that the bays are the most vulnerable parts of Lake Sevan.

Water samples collected for phytoplankton study were preserved with 1 *mL* of a 40% formaldehyde solution per 20 *mL* of lake water (2% final concentration) and stored in a dark place for 10–12 days. Then, after the sedimentation of plankton and removal of supernatant water, the volume of the sample was decreased from 1000 *mL* to 100 *mL*. Repeating the same process, the volume of the experimental samples was reduced to 10 *mL* [6]. The qualitative and quantitative analyses of phytoplankton were conducted under a microscope in the Nageotte chamber (0,1 *mL*). The taxonomic groups of phytoplankton were identified by using the key determinants [7–11].

Determination of microcystin was conducted in the laboratory of medical biotechnology of the Institute of Biochemistry NAS RA. A water sample (20 *mL*) was centrifuged, the supernatant was retained (supernatant 1), 2 *mL* of the residue was homogenized in 2 *mL* physiological saline and placed in a refrigerator. The detection of cyanobacterial toxins, in particular microcystin, was conducted by the hemagglutination method [12]. For this purpose, human erythrocytes were taken and washed with a physiological saline. The erythrocytes were then trypsinized with a trypsin concentration of 1 *mg*/*mL*. After incubation, the suspension of cyanobacteria was centrifuged, and the supernatant solution (supernatant 2), which was blue, was taken.

It is known from the literature that the presence of toxins can be controlled by their effect on the alkaline phosphatase activity [13]. The phosphatase activity was determined according to the method recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology [14]. First, alkaline phosphatase activity in human blood serum was determined using p-nitrophenylphosphate as a substrate in the amount of 152.32 UI/*L*. Then, the same amount of blood serum was incubated with the cyanobacterial supernatant 2 for 30 *min* and the enzyme activity was checked. Quantitative analysis of anatoxin-a was done in the STANDART DIALOG LLC testing laboratory. Dissolved oxygen (DO) was measured *in situ* by a Milwaukee dissolved oxygen meter. The concentrations of nitrate, nitrite, ammonium and phosphate ions were measured according to the

ISOspecifications using a SF-26 spectrophotometer [15]. Temperature and pH measurements were carried out *in situ* by a Milwaukee Waterproof pH meter.

Results and Discussion. Phytoplankton community in the littoral zone of Lake Sevan in June was composed of 4 groups: Chlorophyta, Bacillariophyta, Cyanophyta and Euglenophyta. The highest quantity was recorded for Chlorophyta (green algae) (Fig. 1). The dominant species were *Binuclearia lauterbornii*, *Characium acuminatum*, *Oocystis solitaria* and *Trebouxia humicola*. The highest quantity of algae was recorded at the Lichq sampling site, were *Binuclearia lauterbornii* was the dominant species (3,856,000 cells/*L*), which can be mentioned as a weak algal bloom. At the same time, the species of Bacillariophyta (diatoms) prevailed in biomass (Fig. 1). The main species were *Stephanodiscus astraea*, *Diatoma hiemale*, *Navicula cryptocephala* and *Navicula radiosa*.

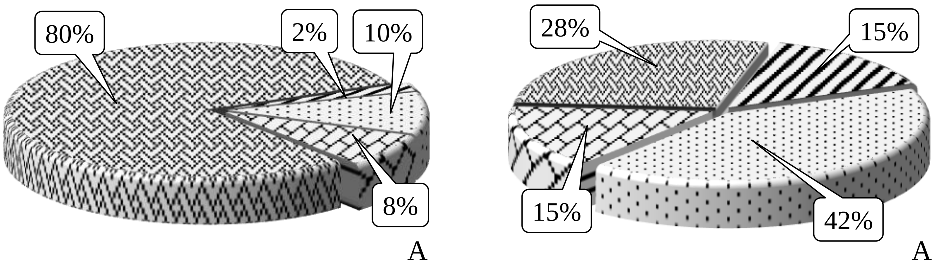

Fig. 1. The structure of the phytoplankton community of the studied parts of Lake Sevan in terms of quantity (A) and biomass (B) in the beginning of June 2019.

Cyanophyta (blue-green algae) accounted for 8% of the quantity and 15% of the biomass of the total phytoplankton community (Fig. 1). Of this group, *Merismopedia elegans*, *Aphanothece clathrata*, *Microcystis aeruginosa*, *Microcystis wessenbergii*, *Aphanizomenon flos-aquae*, *Dolichospermum*/*Anabaena flos-aquae* were the dominant species. Qualitative and quantitative weak development was recorded for Euglenophyta (euglena algae). The main species were *Trachelomonas oblonga* and *Trachelomonas volvocina*.

The highest quantitative values of algae were recorded near Lichq community and the least development of algae was recorded adjacent to Tsovazard community (Tab. 1).

Table 1

Distribution of algae groups by quantity and biomass in Lake Sevan in the beginning of June 2019

Sampling site	Bacillariophyta		Cyanophyta		Chlorophyta		Euglenophyta	
	cells/*L*	g/m^3	cells/*L*	g/m^3	cells/*L*	g/m^3	cells/*L*	g/m^3
Lchashen	248000	2.1752	176000	0.28032	1400000	0.74648	0	0
Tsovazard	16000	0.1824	200000	0.95872	8000	0.16	0	0
Lichq	264000	1.6488	8000	0.0024	4080000	2.50032	136000	1.768
Artanish	208000	1.8912	160000	0.82272	256000	0.45824	24000	0.312
Average	184000	1.4744	136000	0.51604	1436000	0.96626	40000	0.52

The determination of temperature and pH was performed along with the phytoplankton sampling. The results are shown in Tab. 2. The pH was at the optimal level for the lake [16].

Table 2

Temperature and pH values in the littoral zone of Lake Sevan in the beginning of June 2019

Parameter	Lchashen	Tsovazard	Lichq
Temperature, °C	22	20	18
pH	8.4	8.6	8.45

High values of DO concentration were observed in the littoral zone of the lake in June (Fig. 2).

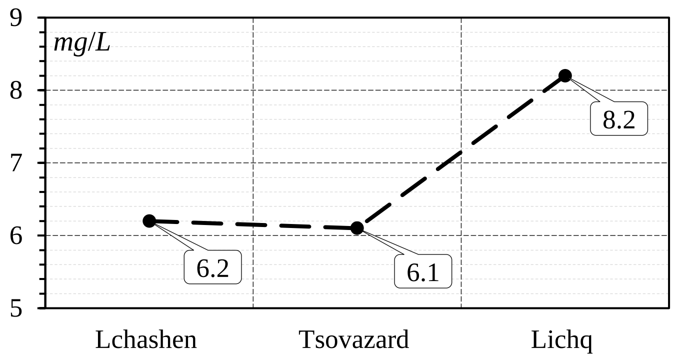

Fig. 2. DO content in Lake Sevan in June 2019.

The nutrient concentrations were at the background level in all observed sites of the lake in the beginning of June (Fig. 3). The lowest concentrations of ammonium, nitrate, nitrite and phosphate ions were observed near Tsovazard community

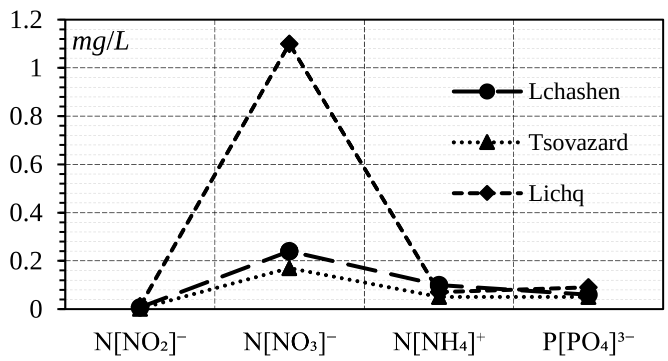

Fig. 3. Quantitative values of nutrients in Lake Sevan in the beginning of June 2019.

Significant changes in the phytoplankton community occurred after June 20. Harmful algal blooms were recorded in different parts of the lake. A change in the color of water, an accumulation of long green filaments on the surface of the water, and a sharp decrease in water transparency were observed. High quantitative values (up to 12,000,000 cells/*L* and 72 g/m^3) were recorded for species belonging to the genus *Dolichospermum*/*Anabaena* in the water samples near Hayrivanq community. A widespread greening of water, an unpleasant odor and an accumulation of algae on the water surface were noticed during July at all observation points. Due to the blue-green algal bloom in the lake, the quantitative parameters of phytoplankton increased, and cyanobacteria became the dominant group in phytoplankton community (Fig. 4). As a result, the regular development of the remaining groups was suppressed.

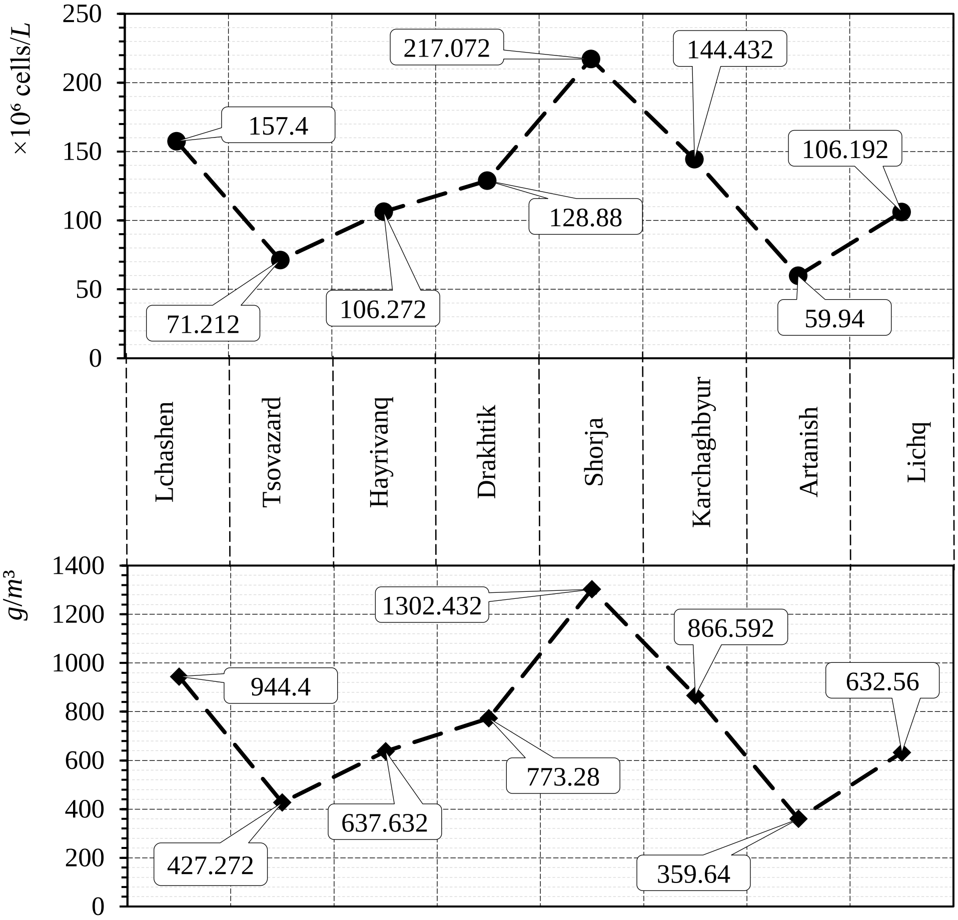

Fig. 4. Dynamics of Cyanophyta quantity (a) and biomass (b) in Lake Sevan in July 2019.

The highest quantity of Cyanophyta was observed adjacent to Shorja community (217,072,000 cells/*L* and 1302 g/m^3) (Fig. 4). The blue-green algal bloom was due to the massive growth of the species *Dolichospermum*/*Anabaena lemmermannii*, *D*/*A. abbreviata*, *D*/*A. flos-aquae*, *D*/*A. cylindrica*, *D*/*A. circinalis*.

Weak development was recorded for some diatom species such as *Amphora ovalis*, *Fragilaria crotonensis*, *Stephanodiscus astraea* and *S. hantzschii* at the stations near Lichq and Lchashen communities. Green alga *Binuclearia lauterbornii* was again recorded at the station near Lchashen community but with quantitative values reduced to 96,000 cells/*L* and 0.04 g/m^3.

It is known that some species of blue-green algae (species of the *genera Anabaena*, *Microcystis*, *Oscillatoria* (*Planktothrix*), *Nostoc*, and *Anabaenopsis*) produce secondary metabolites, i.e., toxins that contain hazards by altering the habitat and environmental conditions. In the studied samples, two types of toxins were detected: microcystin and anatoxin-a [17–19]. These toxins are characteristic of species of the *Anabaena* genus [20].

The presence of toxins in two supernatant solutions was tested on erythrocytes for their agglutination activity. Supernatant 1 showed no agglutination activity. The test with supernatant 2 immediately indicated agglutination, which revealed the presence of microcystin. The presence of microcystins controlled by their effect on alkaline phosphatase activity was evident from their inhibitory activity, with a decrease in phosphatase activity from 152.32 to 70.72 UI/*L*.

By our order, in the STANDART DIALOG LLC testing laboratory, there was carried out an analysis of the water samples submitted by us for the presence of anatoxin-a, which showed its presence in Lake Sevan for the first time. The amount of anatoxin-a in 0.5 *mL* of water was 0.147 $\mu g/L$ (test protocol N1827, product code 1/1720; dated 08.06.2019).

The occurrence of a cyanobacterial bloom could cause changes in the chemical and hydro-physical parameters of water [20]. The results of our study indicated a slightly acidic pH level at the station near Karchaghbyur community in July (Tab. 3).

Table 3

Temperature and pH values in the littoral zone of Lake Sevan in the beginning of July 2019

Parameter	Lchashen	Tsovazard	Karchaghbyur
Temperature, °C	18	18	17
pH	7.3	8.3	6.8

The water vertical transparency decreased and became approximately zero.

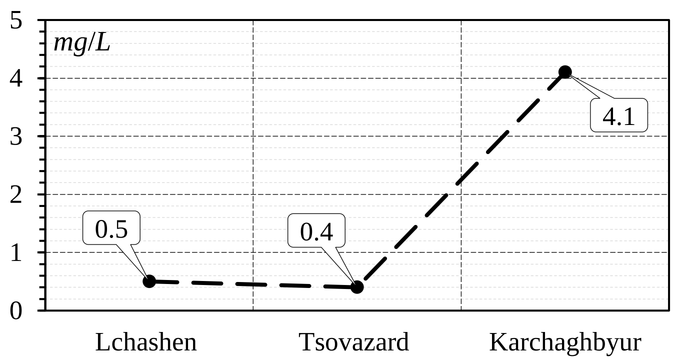

Fig. 5. DO content in Lake Sevan in July 2019.

In all the study areas, the DO content was lower than the environmental standard [16], which indicated the eutrophication of the lake (Fig. 5) [21].

High concentrations of the nutrients were recorded in all the study areas of the lake in July (Fig. 6).

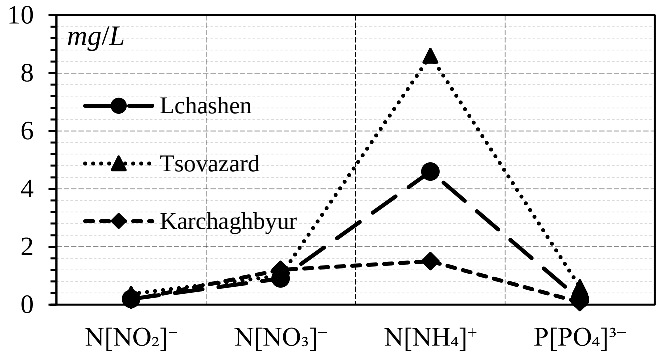

Fig. 6. Quantitative values of nutrients in Lake Sevan in July 2019.

Of the mineral forms of nitrogen, ammonium ions prevailed in the lake (Fig. 6). The results of the study showed that ammonium concentration in the most "blooming" part of the lake (adjacent to Karchaghbjur community) exceeded the background level by almost 20 times [16]. High concentrations of ammonium ions in the lake can lead to mass poisoning and cause the loss of hydrobionts, especially fish [22]. A high level of nitrite ions (0.16 *mg*/*L* near Tsovazard community and 0.37 *mg*/*L* near Karchaghbyur community) was observed during this study as well and exceeded the background level by 2–6 times [16]. The amount of nitrate ions in the lake was relatively low: 0.9 *mg*/*L* near Lchashen and 1.2 *mg*/*L* near Tsovazard communities (Fig. 6).

Phosphate is considered most significant among the nutrients responsible for the eutrophication of lakes, as it is the primary initiating factor [23]. The phosphate concentration in water was very high, compared to environmental standards at the station near Karchaghbjur community (0.6 *mg*/*L*) and exceeded the background level by six times [16]. A high concentration of phosphate ions was also recorded at the station near Lchashen community (0.18 *mg*/*L*). Phosphate ions were within the optimal levels at the station near Tsovazard community (0.08 *mg*/*L*) (Fig. 6).

Conclusion. Thus, harmful algal bloom was recorded in the littoral zone of Lake Sevan during our study. The cyanobacterial species of the genus *Dolichospermum*/*Anabaena* were dominant. As a result, the regular development of the remaining groups was suppressed. Cyanobacteria produced microcystin and anatoxin-a, which were observed in water samples. The growth of cyanobacteria and the formation of bloom strongly affected the hydrological and hydro-chemical characteristics of the lake, lowering the pH, water transparency and DO content in the water. Increase in nutrient concentrations, especially ammonium and phosphate ions, were recorded. The results of this study clearly indicates the eutrophication processes in Lake Sevan.

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ՍԵՎԱՆԱ ԼՃՈՒՄ ՑԻԱՆՈԲԱԿՏԵՐԻԱՆԵՐԻ ԾԱՂԿՈՒՄԸ ԵՎ ՏՈՔՍԻԿՈՒԹՅՈՒՆԸ

2019 թ. գարուն–ամառ եղանակներին իրականացվել են Սևանա լճի ափամերձ տարածքների ֆիտոպլանկտոնային, ջրաֆիզիկական և ջրաքիմիական հետազոտություններ: Հայտնի է որ վերջին տարիներին լճի համատարած ծաղկումը սկիզբ է առել Մեծ Սևանի ափամերձ հատվածից՝ տարածվելով խորքային հատվածներ: Ծաղկում հիմնականում առաջացրել են *Dolichospermum/Anabaena* ցեղի տեսակները: Մեր հետազոտությունները պարզել են, որ ջրի ծաղկման հետևանքով փոփոխվել են ջրի ֆիզիկաքիմիական հատկությունները: Մասնավորապես նվազել է ջրի թափանցելիությունը և լուծված թթվածնի պարունակությունը, մինչդեռ ամոնիում, նիտրիտ և ֆոսֆատ իոնների պարունակությունն ավելացել է: Առաջին անգամ բացահայտվել է միկրոցիստին և անատոքսին-a տոքսինների առկայությունը լճում:

Л. Р. ГАМБАРЯН, Л. Г. СТЕПАНЯН, М. В. МИКАЭЛЯН, К. Г. ГЮРДЖЯН

ЦВЕТЕНИЕ И ТОКСИЧНОСТЬ ЦИАНОБАКТЕРИЙ В ОЗЕРЕ СЕВАН

Были проведены гидрохимические, гидрофизические и фитопланктонные исследования в прибрежной части озера Севан в весенне-летний период 2019 г. Известно, что в последние годы масштабное цветение воды начиналось в прибрежной части Большого Севана, распространяясь на глубоководные участки. Цветение в основном вызывали виды рода *Dolichospermum/Anabaena*. Наши исследования выявили, что вследствие цветения изменялись физико-химические показатели воды. В частности уменьшились прозрачность воды и концентрация растворенного кислорода, в то время как концентрации ионов аммония, нитритов и фосфатов увеличились. Впервые в озере обнаружено присутствие токсинов микроцистина и анатоксина-a.