








S.N. Seilbek , A. Konisbai , N.R. Akmukhanova* ,
B.K. Zayadan , A.B. Yelamanova , I.B. Abibullayeva ,
N.E. Bidagulova 

Al-Farabi Kazakh National University, Kazakhstan, Almaty

*e-mail: akmukhanova.nurziya@gmail.com

ALGOFLORA BIODIVERSITY OF THE SORBULAK SEDIMENTATION LAKE AND THE RELEASE OF TOXIN-PRODUCING CYANOBACTERIA

The vast diversity of microorganisms offers an immense and untapped source for uncovering potentially valuable new species. The purpose of this article is to study the abundance of algoflora and the isolation of potential toxin producers from the Sorbulak reservoir. 153 species of microalgae have been identified in the Sorbulak reservoir. The greatest diversity is observed among green algae (44%), which is typical for polluted aquatic ecosystems. In terms of quantitative composition, diatoms occupy the second place (24%). Of the certain species in the ecosystem of the Sorbulak reservoir, 20% are cyanobacteria and 11% are eugenic algae. Among certain cyanobacteria, 15 are potentially toxigenic organisms. 4 pure cyanobacteria cultures have been isolated from the Sorbulak reservoir. Out of the various strains of cyanobacteria that were isolated, it was found that the *Microcystis aeruginosa* strain exhibited the highest toxicity towards the *A.salina* test organism. In the biomass extract of the cyanobacterium *Microcystis aeruginosa*, three structural variants of toxins—microcystins with specific molecular weights: microcystine-LA – 910.61 m/z, microcystine-RR – 1038.52 m/z, microcystine-YR – 1045.47 m/z were detected.

Key words: biodiversity, cyanobacteria, algae, algoflora, toxin-producing cyanobacteria.

С.Н. Сейілбек, А. Қонысбай, Н.Р. Акмуханова*, Б.К. Заядан,
А.Б. Еламанова, И.Б. Абибуллаева, Н.Е. Бидағұлова

Әл-Фараби атындағы Қазақ Ұлттық Университеті, Қазақстан, Алматы қ.
*e-mail: akmukhanova.nurziya@gmail.com

Сорбұлақ көлінің альгофлорасының биоалуантүрлілігі және токсин түзетін цианобактерияларды бөліп алу

Микроорганизмдердің биоалуантүрлілігі жаңа перспективті микроорганизмдердің түрлерін анықтау үшін пайдаланылмаған ең үлкен резервуар болып саналады. Мақаланың мақсаты Сорбұлақ көлінің альгофлорасын зерттеу және токсин түзуге қабілетті цианобактерияларды бөліп алу. Сорбұлақ көлінен микробалдырлардың 153 түрі анықталды. Ластанған су экожүйелеріне тән жоғары алуантүрлілік (44%) жасыл балдырларда анықталды. Сандық құрамы бойынша екінші орынды диатомды балдырлар (24 %) алады. Сорбұлақ су қоймасының экожүйесінен анықталған түрлердің 20% цианобактериялар және 11% эвгленалы балдырлар. Анықталған цианобактериялардың ішінен 15 түрі потенциалды токсигенді организмдер болып саналады. Сорбұлақ көлінен 4 таза цианобактерия дақылдары бөлініп алынды. Цианобактериялардың оқшауланған штамдарының ішінде *A. salina* сынақ объектісіне қатысты ең улы цианобактерия *Microcystis aeruginosa* штаммы болды. *Microcystis aeruginosa* цианобактериясының биомасса сығындысынан токсиндердің 3 құрылымдық нұсқасы анықталды. Олардың молекулалық салмағы: микроцистин-LA – 910,61 m/z, микроцистин-RR – 1038,52 m/z, микроцистин-YR – 1045,47 m/z.

Түйін сөздер: биоалуантүрлілік, цианобактериялар, балдырлар, альгофлора, токсин түзетін цианобактериялар.

С.Н. Сейілбек, А. Қонысбай, Н.Р. Акмуханова*, Б.К. Заядан,
А.Б. Еламанова, И.Б. Абибуллаева, Н.Е. Бидағұлова

Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы

*e-mail: akmukhanova.nurziya@gmail.com

Биоразнообразие альгофлоры озера-отстойника Сорбулак и выделение токсинообразующих цианобактерий

Биоразнообразие микроорганизмов представляет собой огромный и неиспользованный источник для возможного обнаружения новых перспективных видов микроорганизмов. Цель данной статьи изучение численности альгофлоры и выделение потенциальных продуцентов токсина из водохранилища Сорбулак. В водохранилище Сорбулак определено 153 видов микроводорослей. Наибольшее разнообразие отмечается среди зеленых водорослей (44 %), что характерно для загрязненных водных экосистем. По количественному составу второе место занимают диатомовые водоросли (24 %). Из определенных видов в экосистеме водоема Сорбулак 20 % цианобактерий и 11 % эвгленовые водоросли. Среди определенных цианобактерий 15 являются потенциально токсигенными организмами. Из водохранилища Сорбулак выделены 4 чистых культур цианобактерий. Среди изолированных штаммов цианобактерий выявлено, что штамм *Microcystis aeruginosa* является наиболее токсичным для тест-организма *A. salina*. В экстракте биомассы цианобактерии *Microcystis aeruginosa* идентифицированы 3 структурных варианта токсинов-микроцистинов с молекулярной массой: микроцистина-LA – 910,61 m/z, микроцистина-RR – 1038,52 m/z, микроцистин-YR – 1045,47 m/z.

Ключевые слова: биоразнообразие, цианобактерии, водоросли, альгофлора, токсинообразующие цианобактерий.

Introduction

Wastewater is the result of most human activities that require the use of water. According to the data from the UN World Report, the amount of produced wastewater and its total pollution is continuously increasing worldwide. The problems of wastewater disposal fully apply to Kazakhstan with a climate-related shortage of water resources. One of the largest artificial reservoirs for technical purposes not only in the Republic, but also in the world are wastewater storage facilities in Almaty and the region (Sorbulak and ponds of the Right-Bank Sorbulak Canal – Pskov). They store large reserves of fresh, but insufficiently clean water, which can potentially be used for economic purposes. However, wastewater storage facilities may contain nutrients of natural and anthropogenic origin, creating conditions for eutrophication and exponential growth of microalgae.

Blooming occurs when the ecological balance is disturbed, as in uncontaminated natural reservoirs and also in reservoirs under the influence of anthropogenic eutrophication. One of the primary and perilous agents in the occurrence of water “blooms” is cyanobacteria. Usually, the proliferation of cyanobacteria in water is associated with the emission and buildup of various bioactive substances and toxins. [1]. These toxins can be categorized into two groups based on their activity: biotoxins and cytotoxins. In terms of chemical structure and

mode of action, biotoxins are further classified into hepatotoxic cyclic peptides (hepatotoxins) and neurotoxic alkaloids (neurotoxins) [2]. Hepatotoxins exert their influence on hepatocytes and exhibit carcinogenic properties. Neurotoxins interfere with the functions of the nervous system, housing market to experience rapid death within minutes due to respiratory muscle paralysis. Cytotoxins affect individual cell functions, primarily by inhibiting enzymes, without lethally affecting multicellular organisms. Cyanobacterial toxins are categorized structurally into three main groups: peptides, alkaloids, and lipopolysaccharides. The mechanisms of action of cyanobacteria toxins encompass a spectrum of effects, ranging from hepatotoxic and neurotoxic consequences to genotoxicity [3].

However, despite the fact that these secondary metabolites of cyanobacteria pose a danger to humans and animals, some of them at the same time may be interesting for agrobiotechnology as a source of various agrochemical preparations. For example, *Microcystis* strains are known to produce cyclic heptapeptides that have algicidal, larvicidal and herbicidal activity [4]. Anatoxin-a(c) is a potent acetylcholinesterase inhibitor belonging to the organophosphate class, and it is produced by strains of *Anabaena flos-aquae* and *A. lemmermannii*. This particular form of endotoxin induces hypersalivation and bloody tearing in vertebrates [5]. This is the only natural organophosphate with an insecticidal effect and a new generation of pesticides can be

created on its basis. It is noteworthy that synthetic organophosphates, employed over an extended period, exhibit a propensity to dissolve in lipids and accumulate within cell membranes across diverse organs in both humans and animals. In contrast, anatoxin-a(c) demonstrates solubility in water, rendering it more amenable to biodegradation.

The use of these compounds as biocides (such as algicides, herbicides and insecticides) is predicted to be more beneficial compared to synthetic biocides from an environmental point of view. Due to the numerous problems caused by synthetic pesticides, the development of biogenic pesticides with a reduced risk potential is desirable [6]. Drugs with biological origin have one undeniable advantage over “artificial” ones. It consists in the fact that natural compounds are “honed” by evolution in terms of the qualitative and quantitative correspondence of their components to each other and are easily biodegradable and safe for the environment [7].

The biodiversity of the algaeflora of the Sorbulak Lakes and ponds of the Right-Bank Sorbulak Canal was studied in detail in the laboratory of Photobiotechnology of the Department of Biotechnology of the Al-Farabi Kazakh National University. But little is known about toxic cyanobacteria, as cases of cyanobacteria blooming in Lake Sorbulak have been repeatedly recorded in the summer, leading to the appearance of toxins. In this regard, the purpose of this article is to study the abundance of cyanobacteria and the isolation of toxin producers from the Sorbulak reservoir.

Materials and methods

The object of the study – the Sorbulak settling lake (43.675830, 76.576168) located in the Ili district Almaty region (Figure 1).

Water samples were taken in the summer months of 2022. A total of 40 algological samples were collected, including samples of plankton, benthos, and periphyton. Samples were collected at shallow depths, in places with pronounced vegetation of algae. All collected samples were carefully labelled. The labels indicated the sample number, the time and place of collection, and the name of the collector. In the course of the study, field collections and laboratory analyses were carried out using methods generally accepted in algological practice, quantitative samples were taken using a frame ($S = 0.01 \text{ m}^2$). The fouling was scraped off the substrate with a brush, fixed with Lugol's solution modified by G.V. Kuzmin [1]. Cyanobacteria were studied using Premere and MicrosAustria light

microscopes with magnification from x40 to x100. About 30-40 fields of view were viewed from each water sample on at least 5 preparations. The results obtained were expressed in the number of cells per 1 ml of water. Cyanobacteria species were determined in the native and fixed cell states. At the same time, formaldehyde and iodine solutions were used as a fixative. Cyanobacteria species were determined using determinants x 8-9]. The number of organisms was estimated on a frequency scale after enumeration into 100 visual fields. The frequency of occurrence was taken into account according to a nine-point six-step frequency scale with the following designations: 1 – very rare; 2 – rare; 3 – often; 5 – often; 7 – very often; 9 – mass. The types of microalgae indicators were determined according to the determinants indicated in the list of references. The saprobity index of the reservoir was calculated using the Pantle and Bucca method [10].

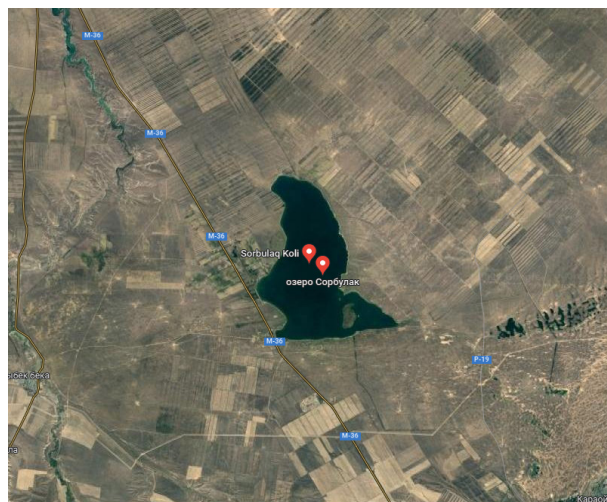


Figure 1 – Map of Sorbulak lake (43.675830, 76.576168)

The accumulation culture of cyanobacteria was obtained according to the traditional method. Standard microbiological methods were used to isolate an algologically pure culture from accumulation cultures [11]. Algologically pure crops were obtained from the obtained accumulative crops by the method of repeated successive replanting into appropriate nutrient media. Cyanobacteria monocultures were obtained by inoculation with a stroke and using a micropipette. The algological purity of the isolated cultures was checked by microscopy [11]. Cyanobacteria were grown in 500 ml flasks under sterile conditions. The mineral media of Gromov and Zarruka were used.

To determine the toxicity of cyanobacteria biomass, lyophilic drying was performed in a Telstar lyophilizer. The toxicity of cyanobacteria was studied by short-term experimentation with a test object on an *Artemia salina* test object [12]. For the experiment, three-day mature female crustaceans of medium size without parthenogenetic embryos were selected and the experiment was carried out according to the following scheme: a series of concentrations (10.0 mg/ml, 1.0 and 0.1 mg/ml) of lyophilized cyanobacteria biomass in glass glasses (100 ml) with a medium volume of 20 ml were compiled. The control was the option without adding biomass [13]. The degree of toxicity (percentage of death of test organisms A%) was determined according to the method [14], where $A \leq 10$ is non-toxic, $10 < A \leq 25$ is slightly toxic, $25 < A \leq 35$ is low toxic, $35 < A \leq 50$ is medium toxic, and $A < 50$ is highly toxic;

Cyanobacteria extracts underwent separation and analysis employing a high-performance liquid chromatograph, specifically the HP 1100 Mass Spectrometer MSDSL-IonTrap [15]. The separation of cyclic peptides occurred on a Zorbax XDB C8 analytical column (4.6 x 150 mm). The mobile phase comprised a methanol-water mixture, with a linear gradient ranging from 30% to 100% methanol over a 30-minute period, and a flow rate set at 0.6 ml/min-1, maintained at 30°C. An aliquot of 20 µl from the analyzed extract was utilized. The peaks at the column outlet were recorded using two detectors: an ion-trap type mass spectrometer and an ultraviolet polychromatic detector (PDA). Detection of cyclic peptides transpired at 230 nm with a retention time falling between 10 and 25 minutes. The mass charges (m/z) of ionized molecules (MSI) were ascertained through tandem mass spectrometry. Toxin identification was achieved by comparing the molecular weights (mass charges) of compounds and aligning them with their retention times on the chromatogram.

Results and discussion

Algoflora of the Sorbulak settling lake and isolation of pure cyanobacteria cultures

The Sorbulak settling lake is a natural closed basin northwest of the city of Almaty, used for collecting, post-treatment and storage of the city's wastewater. The deterioration of water quality and the ecological state of Lake Sorbulak settler has been repeatedly noted. A disaster for water users are situations that arise in the summer period of the year associated with intense "blooming" of water, which causes an increase in the color, tastes and smells of

water, as well as the incidence and death of fish in the reservoir.

The analysis of the dynamics of algoflora development did not differ significantly from the data obtained in 2001–2005, the biodiversity of algoflora was within the range of average values over a long period. 153 species of microalgae have been identified in the Sorbulak storage lake. The greatest diversity is observed among green algae (45%), which is typical for polluted aquatic ecosystems (Fig. 2). Diatoms occupy the second place in terms of quantitative composition (Fig. 2). Of these, the leading role is played by species of the *Pennatophyceae* class, in addition, it should be noted the great species diversity of the genera *Navicula*, *Nitzschia*. Species of the order *Discoidalis* developed in large numbers: *Cyclotella Kuetzingiana* Thm., *Stephanodiscus Hantzschii*, *Pennatae: Stauroinies anceps* Ehr, *Synedra ulna* (Nitzsch) Ehr. 20% are cyanobacteria from all the certain species in the ecosystem of the Sorbulak reservoir. In addition to them, euglenic algae (17 species) were noted in the waters of the Sorbulak, which played a secondary role in the formation of algocenosis of water.

Of the certain species of cyanobacteria in the ecosystem of the Sorbulak accumulator, there are species of *Chroococcales*: *Gloeopcapsa minor* (Kutz) Hollerb, *Microcystis aeruginosa f pseudofilamentosa*, *Microcystis aeruginosa f sphaerodictyoides* Elenk, *Microcystis aeruginosa f flos-aquae*, *Microcystis aeruginosa f aeruginosa*, *Merismopedia glauca* (Ehr)Naeg (Table 1). *Anabaena variabilis* Kutz, *Anabaena flos-aquae f minor* (Lyngh) Breb, *Anabaena constricta*, as well as species of *Oscillatoriales* developed from *Nostocales*: *Oscillatoria irrigua* (Kutz), *Oscillatoria brevis* (Kutz) Gom., *Oscillatoria planctonica* Wolosz, *Oscillatoria angustissima* W. et. G.S. West, *Oscillatoria Villa* Garden., *Spirulina major* Kutz, *Spirulina meneghiniana*, *Spirulina maxima* A. Wurtz. According to the results of our study, 31 species of cyanobacteria were identified in the Sorbulak storage lake (Table 1).

As is known, the determination of the biodiversity of microalgae of various reservoirs allows us to assess the degree of contamination of each reservoir with a high degree of reliability. Along with the numerous functions of algoflora, algae, due to the stenotopy of many species, their high sensitivity to environmental conditions, significantly contribute to the biological analysis of water. Thus, the change in their state from highly polluted to moderately polluted is accompanied by quantitative shifts in the species composition of algae, i.e. The development

of various types of microalgae largely depends on changes in environmental conditions. Therefore, investigating the microalgae biodiversity within the reservoir and assessing the saprobity index enables the determination of its ecological condition. Representatives of β – mesosaprobic microalgae species such as *Ankistrodesmus minutissimus*, *Oocystis*

crassa, *Nitzschia Hantzschiana*, *Microcystis aeruginosa*, indicator microalgae of oligosaprobic zones are found in smaller numbers – *Synedra pulchella*, *Achnantes minutissima*, *Cyclotella Kuetzingiana*, *Scourfilla complanata*, the saprobity index is 2.5, i.e. the reservoir is characterized by the type of organic pollution as β -mesosaprobic.

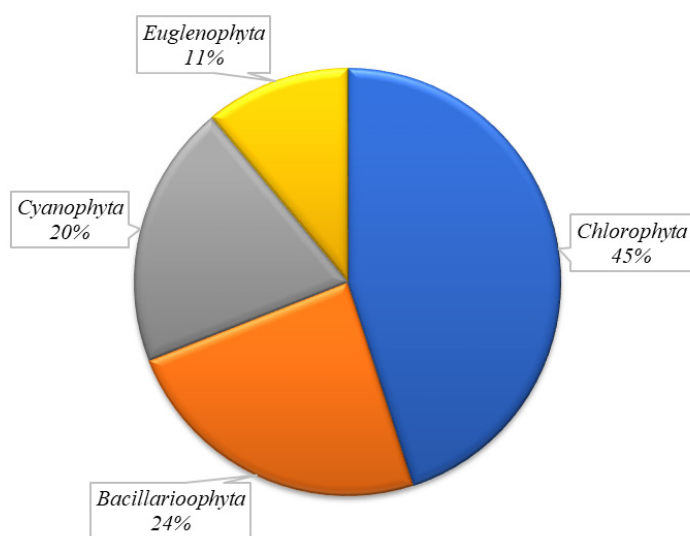


Figure 2 – Quantitative ratio of microalgae species in the Sorbulak storage lake

Table 1 – Species composition of cyanobacteria of the Sorbulak storage lake

№	Composition of cyanobacteria	Occurrence				Saprobity	Toxicity
		january	april	july	october		
	<i>Aphanizomenon flos-aquae</i>	1	3	5	2	-	+
	<i>Anabaena variabilis</i> Kutz	2	2	9	2	-	+
	<i>Anabaena flos-aquae f minor</i> (Lyngh) Breb	2	7	9	3	β	+
	<i>Anabaena constricta</i>	1	3	3	3	-	+
	<i>Dolichospermum sp.</i>	1	1	2	1		-
	<i>Gloeopcapsa cohaerens</i> (Breb) Hollerb	1	1	3	1	-	-
	<i>Gloeopcapsa minor</i> (Kutz) Hollerb.	1	1	1	1	-	-
	<i>Nostoc commune</i>	2	5	9	5	-	+
	<i>Nostoc muscorum</i>	2	3	5	3	-	-
	<i>Nostoc calcicola</i>	2	7	9	5	-	+
	<i>Microcystis aeruginosa f sphaerodictyoides</i> Elenk	1	5	9	3	β	+
	<i>Microcystis aeruginosa f flos-aquae</i> (Wittz)Elenk.	1	7	5	5	β	+
	<i>Microcystis aeruginosa f aeruginosa</i> Kutz Elenk	1	5	9	5	β	+
	<i>Microcystis aeruginosa f pseudofilamentosa</i> (Crow. Elenk),	1	5	9	5	β	+
	<i>Merismopedia glauca</i> (Ehr)Naeg.	3	5	5	2	β	-
	<i>Merismopedia punctata</i>	2	2	3	2	β	-

Table continuation

№	Composition of cyanobacteria	Occurrence				Saprobity	Toxicogenicity
		january	april	july	october		
	<i>Merismopedia tenuissima</i> Lemm.	2	2	3	2	$\alpha - \beta$	-
	<i>Phormidium foveolarum</i> (Mont) Gom.	2	2	5	3	α	-
	<i>Phormidium ambiguum</i>	1	5	5	3	-	-
	<i>Phormidium tenue</i> (Menegh) Gom	1	5	7	3	-	+
	<i>Pseudanabaena</i> sp.	2	5	3	5	-	-
	<i>Oscillatoria irrigua</i> (Kutz)	2	2	5	2	-	-
	<i>Oscillatoria brevis</i> (Kutz)	3	2	2	3	α	+
	<i>Oscillatoria planctonica</i> Wolosz	2	2	3	2	-	-
	<i>Oscillatoria tenuis</i> Ag	3	2	7	5	$p - \alpha$	+
	<i>Oscillatoria willei</i> Garen	3	3	2	3	-	-
	<i>Spirulina major</i> Kutz	2	2	2	3	-	-
	<i>Spirulina meneghiniana</i>	1	2	2	1	$\alpha - \beta$	-
	<i>Spirulina minima</i> A. Wurtz	3	2	5	2	-	-
	<i>Synechocystis</i> sp	2	5	7	5	-	+
	<i>Synechococcus</i> sp	2	5	7	5	-	+

In comparison with the spring period, the species composition and frequency of cyanobacteria increased in summer (Table 1). The frequency of occurrence of all certain cyanobacteria in the summer period was higher. If in the spring, representatives of the genus *Microcystis* in the algoflora were very rare, then in the summer, representatives of this genus dominate the frequency of occurrence over the rest of the species. Cyanobacteria stand out as pivotal producers of allelochemical substances and toxins in freshwater environments [16]. As a result, they have the potential to shape the dynamics of competition and the prevalence of algal communities in aquatic environments by selectively inhibiting the growth of other algae [17]. The phytoplankton composition in freshwater ecosystems, particularly in eutrophic waters, exhibits variability and commonly encompasses cyanobacteria and green microalgae as predominant constituents [17]. In eutrophic lakes such as Lake Taihu, cyanobacteria and green algae exhibit seasonal succession [18]. Our study also showed that seasonal dynamics is observed between cyanobacteria and green algae in the Sorbulak reservoir, and *Microcystis aeruginosa* actively develops during the summer season [19].

Presently, approximately 50 cyanobacterial species are recognized for their capability to produce toxins [20]. However, toxigenicity is a characteristic specific to individual strains rather than the entire species [21]. Field observations and laboratory investigations of cultures have revealed that certain cyanobacterial types

may encompass both toxigenic and non-toxigenic strains [22]. Within the cyanobacteria of the Sorbulak accumulator, 15 organisms are identified as potentially toxigenic (Table 1). 4 algologically pure cyanobacteria cultures were obtained from the Sorbulak accumulator (Figure 2).

Cyanobacteria toxicity assessment at the Artemia salina test facility

To assess the toxicity of cyanobacteria, an experiment to determine acute toxicity was conducted at the *Artemia salina* test facility. Acute toxicity testing is the main method that can provide information about the effects of cyanobacteria on test subjects. This method does not provide information about the mechanism of inhibition, but the death of the tested organisms is quantified. Unlike other invertebrates used in toxicity tests, *Artemia salina* larvae do not require feeding during the first 72 hours, so any change in their growth during testing can be attributed to exposure to a toxic substance. By testing for acute toxicity with the *Artemia salina* test strain, we can determine the concentration of the toxin at which 50% of the test strains die (LC_{50}).

According to the experiment to assess the biotoxicity of isolated cyanobacteria, in the case of *Phormidium foveolarum* and *Spirulina minima* at biomass concentrations of 0.1 mg/ml, 1 mg/ml, 10 mg/ml, no changes in the appearance of the test object and mortality were recorded within 12-24 hours (Table 2).

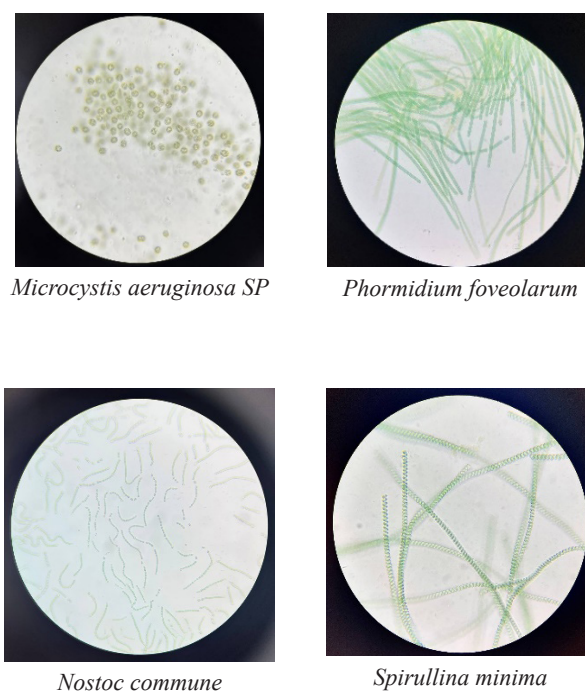


Figure 2 – Algological pure cyanobacteria cultures

Table 2 – *Artemia salina* survival test at various concentrations (mg/ml) of dry cyanobacteria biomass, %

№	Culture	Test time, h					
		12	24	12	24	12	24
		Concentrations of dry cyanobacteria biomass (mg/ml)					
		0,1		1		10	
1	<i>Phormidium foveolarum</i>	0	0	0	0	0	0
2	<i>Spirulina minima</i>	0	0	0	0	10	0
3	<i>Microcystis aeruginosa</i>	10	20	30	60	80	100
4	<i>Nostoc commune</i>	0	10	10	20	30	40

At a concentration of 10 mg/ml of dry cells in *Nostoc commune* cultures, 40% *A. salina* was observed to die within 24 hours. At a biomass concentration of 1 mg/ml for 24 hours, more than 50% of *A. salina* mortality was detected in *Microcystis aeruginosa* variants (Table 2). *Microcystis aeruginosa* culture showed high toxicity, causing 100% mortality of *A. salina* at a biomass concentration of 10 mg/ml for 24 hours. Morphological changes were also observed in the test object (Figure 3).

Cyanobacteria cultures subjected to biotesting using *A. salina* were classified according to the degree of toxicity [14] as follows:

Class I – refers to crops with high toxicity. This includes a dedicated culture of *Microcystis aeruginosa*, which caused the death of 100% of test objects within 24 hours at a biomass concentration of 10 mg/ml.

Class IV – represents cultures with moderate toxicity. This class includes *Nostoc commune* with moderate toxicity, where $35 < A \leq 50$ during biotesting.

Class V – refers to non-toxic crops. This includes cultures of *Phormidium foveolarum* and *Spirulina maxima*, during biotesting of which no changes in the concentration of these cultures in the vital activity of the test object were detected.

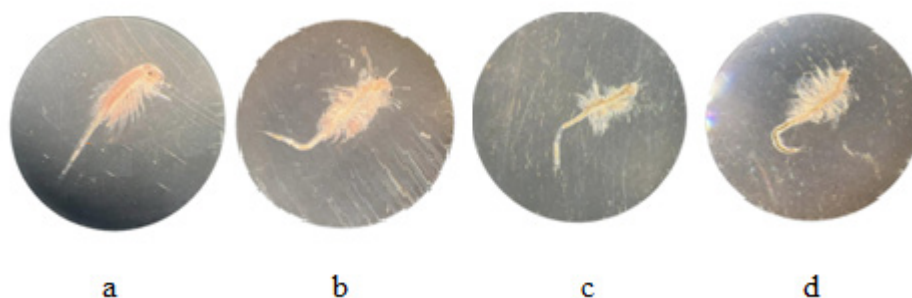


Figure 3 – Morphological changes in crustaceans *Artemia salina* after testing the dry biomass of *Microcystis aeruginosa*, a – control, b, c, d – at a concentration of *Microcystis aeruginosa* biomass of 10 mg/ml. Optical microscope with x10 magnification

Thus, among the cyanobacteria strains isolated by us, the *Microcystis aeruginosa* strain turned out to be the most toxic in relation to the *A. salina* test object.

The composition of toxins produced by the cyanobacterium Microcystis aeruginosa

Extracts from the biomass of the cyanobacterium *Microcystis aeruginosa* contained three clearly recognized compounds, microcystin-LA – molecular formula – $C_{46}H_{67}N_7O_{12}$ molecular mass – 910,61 m/z., microcystin -RR – molecular formula – $C_{49}H_{75}N_{13}O_{12}$ molecular mass – 1038,52 m/z.

microcystin -YR – molecular formula – $C_{52}H_{72}N_{10}O_{13}$ molecular mass – 1045,47 m/z (Figure 4).

Microcystins are the most common cyanotoxins in freshwater. More than 65 microcystin derivatives have been identified. Structurally, these molecules are cyclic heptapeptides with a special amino acid characteristic of this group of molecules, as well as nodularin. Microcystins are powerful inhibitors of protein phosphatases, this inhibition causes a violation of the cellular architecture, the main target of which is hepatocytes due to the active transport of this compound by bile acid transporters [23].

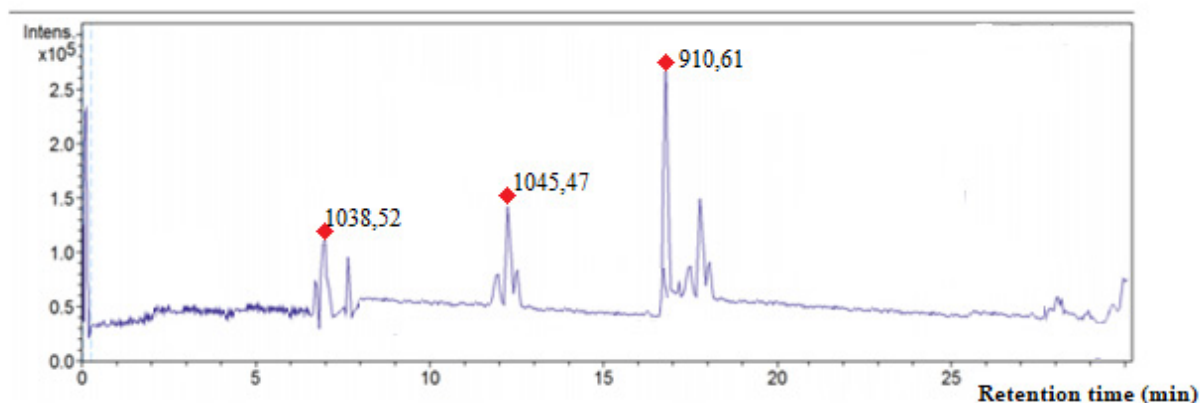


Figure 4 – HPLC chromatogram of extracts of lyophilized biomass of *Microcystis aeruginosa*. The numbers indicate the molecular weights of toxins (m/z): microcystin -LA – 910,61 m/z, microcystin -RR – 1038,52 m/z, microcystin -YR – 1045,47 m/z

These compounds are pervasive in the natural environment and play a crucial role in maintaining ecological equilibrium through interspecific interactions, and their symbiotic relationships, spanning across different trophic levels [24, 25].

Exhibiting a diverse array of biological properties [26], these metabolites actively engage in numerous processes within aquatic reservoirs [27].

According to the results of the study, 153 species of microalgae were identified in the Sorbulak storage

lake. The greatest diversity is observed among green algae (44%), which is typical for polluted aquatic ecosystems. In terms of quantitative composition, diatoms occupy the second place (24%). Of the certain species in the ecosystem of the Sorbulak reservoir, 20% are cyanobacteria and 11% are eugenic algae. In the spring, representatives of cyanobacteria in the algoflora were very rare, but in the summer, cyanobacteria dominate the frequency of occurrence over the rest of the microalgae, especially *Microcystis aeruginosa* actively develops in the summer season. Among certain cyanobacteria, 15 are potentially toxigenic organisms. 4 pure strains of cyanobacteria have been isolated from the Sorbulak reservoir. Among the isolated strains of cyanobacteria, the *Microcystis aeruginosa* strain turned out to be the most toxic in relation to the *A. salina* test object. Within the biomass extract of the cyanobacterium *Microcystis aeruginosa*, three structural variants of

toxins, specifically microcystins with discernible molecular weights, were successfully identified: microcystin-LA with a molecular weight of 910.61 m/z, microcystin-RR with a molecular weight of 1038.52 m/z, and microcystin-YR with a molecular weight of 1045.47 m/z. The exploration of cyanotoxins as potential reservoirs of biocidal agents is of interest in the context of advancing novel and effective methodologies for pest and pathogen control in agricultural, aquatic, medical, and industrial domains.

Acknowledgments

This research was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan under these projects: AP14870201 “Search and study of new secondary metabolites of cyanobacteria promising for use in agricultural biotechnology”.

References

1. Voloshko L.N., Pljushh A.V., Titova N.N. (2008). Toksiny cianobakterij Cyanophyta, Cyanobacteria [Cyanobacteria toxins Cyanophyta, Cyanobacteria] // Al'gologija. Vol. 18. – №1. P. -3-20. (In Russian)
2. Carmichael, W.W. The cyanotoxins // Adv. Bot. Res. – 1997. – Vol. 27. – P. 211–256. [https://doi.org/10.1016/S0065-2296\(08\)60282-7](https://doi.org/10.1016/S0065-2296(08)60282-7)
3. Voloshko L.N., Pinevich A.V., Kopeckij I., Titova N. N., Hrouzek P., Zelik P. (2010). Produciruemye cianobakterijami toksiny v period «cvetenija» vody v nizhnem Suzdal'skom ozere (Sankt-Peterburg, Rossiya) [Toxins produced by cyanobacteria during the “flowering” of water in the lower Suzdal Lake (St. Petersburg, Russia)]// Al'gologija. – Vol. 20. – №2. – P. 210–233. (In Russian)
4. Ramos D.F., Matthiensen A., Colvara W., Votto A.P.S., Trindade G.S., da Silva P.E.A., Yunes J.E.S. Antimycobacterial and cytotoxicity activity of microcystins // J Venom Anim Toxins Incl Trop Dis. – 2015. – Vol.21. – № 9. <https://doi.org/10.1186/s40409-015-0009-8>
5. Mahmood N.A., Carmichael W.W. The pharmacology of anatoxin-a(s), a neurotoxin produced by the freshwater cyanobacterium *Anabaena flos-aquae* NRC 525–17 // Toxicon. – 1986. – Vol. 24. – №5. – P. 425–34. [https://doi.org/10.1016/0041-0101\(86\)90074-7](https://doi.org/10.1016/0041-0101(86)90074-7)
6. Kumar, J., Ramlal, A., Mallick, D., Mishra, V., 2021. An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance // Plants. – 2021. – Vol. 10. – № 6. – P. 1185. <https://doi.org/10.3390/plants10061185>
7. Olson, S. An Analysis of the Biopesticide Market Now and Where it is Going // Outlooks on Pest Management. –2015. –Vol. 26. – P. 203–206. https://doi.org/10.1564/v26_oct_04
8. Gollerbah M. M., Polyanckij V. N. (2001) Precnovodnye vodorosli ih izuchenie. [Freshwater algae their study]. Sovetskaya nauka, pp. 200. (In Russian)
9. Muzafarov A. M. (1987). Opredelitel' sinezelenyh vodoroslei Srednej Azii:/ A. M. Muzafarov, A. E. Ergashev, S. Halilov [The determinant of blue – green algae of Central Asia].pp. 406-89210. Sirenko L. A., Kozickaja V. N. Biologicheski aktivnye veshhestva vodoroslej i kachestvo vody [Biologically active substances of algae and water quality]. Izd. Nauk. dumka. – 1988. – S. 312. (In Russian)
11. Temraleeva A.D., Dronova S.A., Moskalenko S. V., Didovich S.V. Modern methods for isolation, purification, and cultivation of soil cyanobacteria // Microbiology. – 2016. –Vol. 85. – P.389–399. <https://doi.org/10.1134/S0026261716040159>.
12. Kiviranta J., Sivonen K., Niemelä S.I., Huovinen K., Detection of toxicity of cyanobacteria by *Artemia salina* bioassay // Environ Toxicol Chem. – 1991. – Vol. 6. – P. 363–369. <https://doi.org/10.1002/tox.2530060407>
13. Beatie, K. A., Ressler, J., Wiegand, C., Krause, E., Codd, G. A., Steinberg, Ch. E. Wand Pflugmacher, S. Comparative effects and metabolism of two microcystins and nodularin in the brine shrimp *Artemia salina* // Aqua Toxicol. –2003. – Vol. 62. – P. 219–226. [https://doi.org/10.1016/s0166-445x\(02\)00091-7](https://doi.org/10.1016/s0166-445x(02)00091-7)

14. Metcalf J.S., Lindsay J., Beattie K.A., Birmingham S., Saker M.L., Törökne A.K., Codd G.A. Toxicity of cylindrospermopsin to the brine shrimp *Artemia salina*: comparisons with protein synthesis inhibitors and microcystins // *Toxicon*. –2002. –Vol. 40. №8. – P. 1115–20. [https://doi.org/10.1016/s0041-0101\(02\)00105-8](https://doi.org/10.1016/s0041-0101(02)00105-8)
15. Dittman E., Fewer D.P., Neilan B.A. Cyanobacterial toxins: biosynthetic routes and evolutionary routes // *FEMS Microbiol Rev.* – 2013. Vol. –37. P. 23–43. <https://doi.org/10.1111/j.1574-6976.2012.12000.x>
16. Singh R., Parihar P., Singh M., Bajguz A., Kumar J., Singh S., Prasad S.M. Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: Current status and future prospects // *Front Microbiol.* – 2017. – Vol. 8. – P. 1–37. <https://doi.org/10.3389/fmicb.2017.00515>
17. Dong J., Li C., Chang M., Dai D., Liu S., Quan B., Zhang Y., Gao Y. Effects of toxic cyanobacterium *Microcystis aeruginosa* on the morphology of green alga *Chlorella vulgaris* // *Ann. Limnol. Int. J. Lim.* – 2019. – Vol. 55. – P. 7. <https://doi.org/10.1051/limn/2019006>
18. Cai L., Zhu G., Zhu M., Yang G., Zhao L. Succession of phytoplankton structure and its relationship with algae bloom in littoral zone of Meiliang Bay, Taihu Lake // *Eco Sci.* – 2012. – Vol. 31. – P. 345–351 (in Chinese with English abstract). <http://www.ecolsci.com/EN/Y2012/V31/I4/345>
19. Dong J., Gao Y.N., Chang M.Y., Ma H.H., Han K., Tao X., Li Y. Colony formation by the green alga *Chlorella vulgaris* in response to the competitor *Ceratophyllum demersum* // *Hydrobiologia.* – 2018. – Vol. 805. – P. 177–187. <https://doi.org/10.1051/limn/2018003>
20. Żymańczyk-Duda E., Samson S. O., Brzezińska-Rodak M. and Klimek-Ochab M. Versatile Applications of Cyanobacteria in Biotechnology // *Microorganisms.* – 2022. – Vol. 10. – № 12. – P. 2318. <https://doi.org/10.3390/microorganisms10122318>
21. Sivonen K. Cyanobacterial toxins and toxin production // *Phycologia.* — 1996. – Vol. 35. — P. 12–24. <https://doi.org/10.2216/i0031-8884-35-6s-12.1>
22. Rastogi R.P., Sinha R.P. Biotechnological and industrial significance of cyanobacterial secondary metabolites // *Biotechnology Advances.* – 2009. – Vol. 27. – P. 521–539. <https://doi.org/10.1016/j.biotechadv.2009.04.009>
23. Chih Yu Chen. Biosynthesis DEHP и DBP from red algae // *Water Res.* – 2004. – V. 38, № 4. – P. 1014–1018.
24. Babu B., Wu J.T. Production of phthalate esters by nuisance freshwater algae and cyanobacteria // *Science of The Total Environment.* – 2010. – V. 408, № 21. – P. 4969–4975. <https://doi.org/10.1016/j.scitotenv.2010.07.032>
25. Lotfy M.M., Hassan H.M., Hetta M.H., El-Gendy A.O., Mohammed R. Phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from River Nile derived fungus *Aspergillus awamori* // *Beni-Suef University Journal of Basic and Applied Sciences.* – 2018. – Vol. 7. – P. 263–269. <https://doi.org/10.1016/j.bjbas.2018.02.002>
26. Philip D., Kaleena P.K., Valivittan K. GC-MS analysis and antibacterial activity of chromatographically separated pure fractions of leaves of *Sansevieria roxburghiana* // *Asian J. Pharm. Clin. Res.* – 2011. – V. 4, № 4. – P. 130–133.
27. Rajamanikyam M., Vadlapudi V., Parvathaneni S.P. Isolation and characterization of phthalates from *Brevibacterium mcbrellneri* that cause cytotoxicity and cell cycle arrest. // *Excli J.* – 2017. – V. 16. – P. 375–387. <https://doi.org/10.17179/excli2017-145>

Авторлар туралы мәлімет:

Сейілбек Сандугаш Нұрланқызы – әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының «Биотехнология» мамандығының 2 курс PhD докторанты (Алматы, Қазақстан, sseilbek1@gmail.com)

Қонысбай Айгерім Мәлікқызы – әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының «Биотехнология» мамандығы бойынша жаратылыстану ғылымдарының магистрі, (Алматы, Қазақстан, aigerimkonysbay@gmail.com)

Акмуханова Нұрзия Рахмедиевна (корреспонденттік автор) – биология ғылымдарының кандидаты, әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының ассоциирленген профессоры (доцент) (Алматы, Қазақстан, aktukhanova.nurziya@gmail.com)

Заядан Болатхан Қазыханұлы- биология ғылымдарының докторы, , әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының профессоры, ҰҒА-ң Академигі (Алматы, Қазақстан, zbolatkhan@gmail.com)

Еламанова Аделя Байқадамовна – әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының «Биотехнология» мамандығының 2 курс магистранты (Алматы, Қазақстан, elamanovaadelya@mail.com)

Абибуллаева Индира – әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының «Биотехнология» мамандығының 2 курс магистранты (Алматы, Қазақстан, indira_26.07.1994@mail.com)

Бидағұлова Назым Ерболқызы әл-Фараби атындағы Қазақ ұлттық университетінің биотехнология кафедрасының «Биотехнология» мамандығының 2 курс магистранты (Алматы, Қазақстан, nbidagulova@mail.com)

Information about authors:

Seilbek Sandugash – 2nd year PhD student at the Department of Biotechnology of al-Farabi Kazakh National University of Biotechnology major (Almaty, Kazakhstan, sseilbek1@gmail.com)

Konysbay Aigerim – Master in Biotechnology, al-Farabi Kazakh National University (Almaty, Kazakhstan, aigerimkonysbay@gmail.com)

Akmukhanova Nurziya (corresponding author) – Candidate of Biological Sciences, Associate Professor of Biotechnology Department of al-Farabi Kazakh National University (Almaty, Kazakhstan, email: akmukhanova.nurziya@gmail.com)

Zayadan Bolatkhan – Doctor of Biological Sciences, Professor of Biotechnology Department of al-Farabi Kazakh National University, Academician of the National Academy of Sciences of the Republic of Kazakhstan (Almaty, Kazakhstan, email: zbolatkhan@gmail.com)

Adelya Yelamanova – Biotechnology major Master's student at Biotechnology Department of al-Farabi Kazakh National University (Almaty, Kazakhstan, email: elamanovaadelya@mail.com)

Abibullayeva Indira – Biotechnology major Master's student at Biotechnology Department of al-Farabi Kazakh National University (Almaty, Kazakhstan, indira_26.07.1994@mail.com)

Bidagulova Nazym – Biotechnology major Master's student at Biotechnology Department of al-Farabi Kazakh National University (Almaty, Kazakhstan, nbidagulova@mail.com)

Received December 01, 2024

Accepted March 25, 2024