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# POTENTIALLY TOXIC ALGAE DETECTION AND MONITORING IN KAZAKHSTANI LAKES AND RIVERS

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A THESIS SUBMITTED
FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGICAL SCIENCES AND TECHNOLOGIES DEPARTMENT OF BIOLOGY SCHOOL OF SCIENCES AND HUMANITIES NAZARBAYEV UNIVERSITY
2021

# DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis. This thesis has also not been submitted for any degree in any university previously.

Aigerim Abdimanova
26 April 2021

## ACKNOWLEDGEMENTS

I would like to use this opportunity to express my gratitude to everyone involved in the process of thesis work completion.

First of all, I am profoundly grateful to Professor Natalie Barteneva, who supported, guided and assisted me in many ways during my graduate degree. I thank you for the opportunity to learn from you and explore the world of environmental research. Thank you for all your motivational lessons.

I had many teachers during these two years. Therefore, I would like to thank all of my laboratory colleagues, especially Veronika Dashkova and Dmitriy Malashenkov, who shared invaluable experience and expertise and provided assistance in the faced challenges. Many thanks to Darya Potashnikova, who spent her time teaching us innovative molecular biology techniques and guiding me further through the experiments. I am also grateful to Professor Vorobyev, who shared his honest feedback to make this work better.

I send my gratitude to Aidyn Abilkas, who kindly agreed to conduct ion chromatography experiments for my thesis and shared the results with me. Aidyn always was open to help with the experiments and answer any questions.

I cannot miss out on my colleagues and friends Yersultan Mirasbekov and Kuanysh Sarkytbayev, with whom I partnered to conduct many experiments. As we learned together, I want to thank you for your support and for making this path more engaging.

Many thanks to the Young Researchers Alliance, who funded the greatest part of the project and provided an opportunity to develop my research potential.

Thanks to the Nazarbayev University Core Facilities, who kindly provided many necessary instruments without which my work could not be possible.

Thank you, the University of Aarhus, LMWE-mesocosm, for providing the control samples with microcystin genes positive probe.

Finally, I want to thank my family for their constant belief in me.

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Master's Degree Program of Science in Biological Sciences and Technologies

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Master of Science thesis; 62 pages, 11 appendices
Supervisors: Natalie Barteneva, Professor, Nazarbayev University, SSH, Department of Biology, natalie.barteneva@nu.edu.kz
26.04.2021

Keywords: CyanoHAB, Imaging flow cytometry, FlowCAM, Real-Time PCR

## ABSTRACT

An increasing scale of emerging cases of Harmful Algal Blooms (HABs) around the world requires a time-effective and facile methodology of toxic algae detection and quantification. The traditional methods such as light microscopy, ELISA (enzyme-linked immunosorbent assay), HPLC (high-performance liquid chromatography), and LC-MS (liquid chromatography-mass spectrometry) take cost and effort to produce an outcome while novel remote sensing technique can be partly sensitive at the early stages of HABs development. The proposed methodology of rapid detection of blooms using imaging flow cytometer FlowCAM integrated with PCR-based methods was adapted to the eleven water bodies in different regions of Kazakhstan.

The analysis of algal samples from the Ural River's delta up to 100 km inland was carried out in August–December 2019 using imaging flow cytometry (IFC), molecular biological, and microscopic techniques. As dominant phytoplankton species, we identified the filamentous cyanobacteria *Cuspidothrix issatschenkoi*, *Dolichospermum* cf. *flos-aquae, Pseudanabaena limnetica,* and we found minor quantities of *Cylindrospermopsis raciborskii*. Real-time PCR analysis and Sanger sequencing indicated the presence in the field samples containing *Microcystis* spp. of genes associated with the production of microcystin (*mcyE*). Moreover, we identified potentially toxic cyanobacteria by IFC in seven other Kazakhstani water bodies. The present study describes the advantages and suggests the particular effectiveness of imaging flow cytometer FlowCAM in the fast detection of potentially toxic algae in rivers and lakes. Developed combined IFC and PCR-based approach can be useful in early detection and monitoring of toxic algae in Kazakhstani rivers and lakes.

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## ABBREVIATIONS

| | |
|---|---|
| HAB | harmful algal bloom |
| IFC | imaging flow cytometry |
| CyanoHAB | cyanobacterial harmful algal bloom |
| ELISA | enzyme linked immunosorbent assay |
| HPLC | high performance liquid chromatography |
| LC-MS | liquid chromatography-mass spectrometry |
| PCR | polymerase chain reaction |
| RT-PCR | real-time polymerase chain reaction |
| DNA | deoxyribonucleic acid |
| $ddH_2O$ | double distilled water |
| DO | dissolved oxygen |
| Chl-a | chlorophyll-a |
| P | phosphorus |
| N | nitrogen |
| TP | total phosphorus |
| TDS | total dissolved solids |
| PF | paraformaldehyde |
| BLAST | Basic Local Alignment Search Tool |
| WHO | World Health Organization |

# 1 INTRODUCTION

## 1.1 Harmful Algal Blooms and climate change

Climate change pressures planktonic systems globally, leading to an increase in harmful algal blooms (HABs) in frequency and severity (O'Hearn & Baker, 2018). HABs are massive aggregates of phytoplankton that respond to such signals as global and local temperature increase, water flow and nutrient supply, especially heightened nitrogen and phosphorus content. While eutrophic, nutrient-rich conditions are the most convenient for algal growth, harmful algae can take advantage of water acidification and pollution to maintain its growth at high levels (Watson et al., 2015). Rapid growth manifests its unpleasant consequences to the balance of the water ecosystem such as hypoxia, discharge of toxic compounds (algal toxins) and gases ($CH_4$, $H_2S$, $NH_3$), all of which have pernicious effects on aquatic fauna. The cases of massive fish kills, shellfish contamination, and death of marine mammals are associated worldwide with the blooming of toxic algae (Huisman et al., 2018). Toxic algae contamination also poses a threat to domestic animals, seafood farms, fisheries and recreational tourism (Wood et al., 2010). Suggested economic losses and public health issues such as drinking water contamination and human algal poisoning from harmful blooms require substantial modifications in the engineering and infrastructure of the water municipality system (Watson et al., 2015).

## 1.2 Cyanobacterial HABs (CyanoHABs)

CyanoHABs is the term that refers to the algal blooms composed mainly of Cyanobacteria (*Cyanophyta*), a phylum of prokaryotic, photosynthetic, blue-green algae which primarily belong to the domain of Bacteria. Cyanobacterial phylum is often characterised by distinct blue-green colour, provided by the dominance of cyanobacterial pigments phycobilin and phycocyanin; however, the abundance of yellow carotenoids and red phycoerythrin gives cyanobacteria a wide range of colours forming blue-green, brown, red and pink-tinged blooms (Bellinger & Sigee, 2015). The organisms range from unicellular forms to big colonial forms. Typically, cyanobacterial cells tend to fit the size < 10 μm in diameter; however, they can be larger in case of complex multicellular structures such as filamentous or colonial organisms (Bellinger & Sigee, 2015). Cyanobacteria equally tolerate saline, brackish and freshwater environments. The cases of *Nodularia* spp.-dominated bloom incidents were regularly encountered in the Baltic Sea from the 1990s (Kahru et al., 2018) and in the

Caspian Sea (Nasrollazadeh et al., 2011). Still, cyanobacterial blooming is more persistent in inland waters compared to marine environments due to the factors such as warmer temperature in shallow waters, high level of anthropogenic pollution and fewer grazing potential by predators (Bukaveckas et al., 2018). Since intense growth in lentic waters tends to produce highly turbid and dense scum, occurrences raise a public concern for freshwater availability. For example, in Lake Taihu in China, cyanobacteria proliferated into a dense green bloom from the 1980s with a detrimental effect on water quality, water ecosystem, and local infrastructure (Ma et al., 2014). The nearby located city Wuxi suffered from a weeklong water crisis due to a toxic blooming of *Microcystis* spp (Huisman et al., 2018). Expanding geographic distribution of CyanoHABs induces a cascade of events such as excessive growth, interference with a living of biological species, oxygen depletion, toxin release, and animal/fish kills in the worst-case scenario around the world (Haellegraf et al., 2018). The examples of water bodies invaded by CyanoHABs include Great Lakes in northern America, Lake Balaton in Europe, Lake Victoria in Africa, which are the vital sources of fresh water for their geographic locations (Paerl, 2017). Economic losses from fish kills, termination of recreational tourism, and healthcare costs expose a detrimental effect on the financial status of the states (Huisman et al., 2018). Therefore, the focus on the CyanoHAB cases in lakes and rivers, which serve as the primary source of freshwater for residential regions, increases.

### 1.3 Cyanobacteria toxins

The toxic effect of cyanobacteria was described as early as the 1700s when an English traveller Cristopher Kirkby described the massive death of dogs, poultry, and cattle connecting it to the green biomass accumulated in the lake Treehomskie in Poland (Huisman et al., 2018). The toxicity of cyanobacteria is enabled by the production of cyanobacterial toxins, divided into five types: neurotoxins, hepatotoxins, cytotoxins, dermatoxins, and irritant toxins. The role of cyanotoxins in cell signalling and possible protection mechanisms and their effect on human health is intensively researched.

**Microcystin** is considered the most prevalent and the most potent hepatotoxin among cyanobacterial toxins. It is produced by a range of different cyanobacterial species such as *Microcystis* spp*., Aphanizomenon* spp*., Nostoc* spp*., Dolichospermum* spp*., Planktothrix* spp. and others. The toxin expression is regulated by a cluster of microcystin synthetase genes (*mcy)*, which can be regulated by complex signalling processes and possibly environmental factors (Kaebernick et al., 2000). The main mechanism of the microcystin toxic effect is

phosphatase inhibition in liver cells, leading to the destruction of the cytoskeleton, membrane disorganisation, and necrosis. Moreover, microcystins increase the production of reactive oxygen species, which induce lipid peroxidation and DNA damage. **Anatoxin-a** acts as a neurotoxin inhibitor of acetylcholinesterase which induces muscular fasciculations and hypersalivation with death from respiratory constraints. Anatoxin-a synthesis is coded by a cluster of genes *ana*, expressed in a range of toxic cyanobacteria such as *Microcystis*, *Anabaena* (*Dolichospermum*), *Oscillatoria*, and *Aphanizomenon* (Codd, Ward, & Bell, 1997). Another cyanobacterial toxin, **cylindrospermopsin**, produced by cyanobacterial genera *Cylindrospermopsis, Aphanizomenon, Raphidiopsis, Umezakia* and *Lyngbya*, and widely recognised as a hepatotoxin. The mechanism of toxicity of cylindrospermopsin includes protein synthesis inhibition, which leads to tissue necrosis effect (Kinnear, 2010). The studies mentioned above described the toxins' effect in mammalian organisms. However, studies on the chronic exposure of seafood to cyanobacterial toxins demonstrate bioaccumulation and possible transfer in the food chain (Codd et al., 1997). Furthermore, acute exposure of fish to the cyanobacterial toxins revealed modifications in protein expression, which play vital roles in lipid metabolism, glycogen storage, and cytoskeleton regulation (Marie et al., 2012). The toxicity effect in fish is augmented by depletion of oxygen due to excessive growth of algal blooms, followed by its rapid decay, which consumes more of the spare reserves of oxygen (Paerl & Otten, 2013).

### 1.4 Environmental factors affecting HABs

#### Temperature

Increasing temperature around the globe in terms of climate change and global warming severely affects aquatic ecosystems as it favours the growth of phytoplankton. In detail, temperature warming intervenes with the thermal regime of the water bodies, influencing nutrient distribution across the layers of water depth, also named water bands. Bloom-forming cyanobacteria, in its turn, are able to adapt to transformations in vertical stratification due to the formation of gas vesicles, which allow regulating its buoyancy (Paerl & Huisman, 2009). The process promotes the proliferation of cyanobacterial species and leads to bloom formation. An invasive bloom-forming species of cyanobacteria *Cylindrospermopsis raciborskii* was incubated in the temperature range from 15º to 40ºC (Briand et al., 2004). Cyanobacterial growth continued logarithmically at 15ºC - 35ºC with a growth arrest at 40ºC. The seasonality of the bloom outbreaks is also connected with the

preferences for warmer temperatures (Djurhuus et al., 2015). Thus, an increase in the temperature favours the expansion of the toxic species of cyanobacteria on the global scale (Paerl & Huisman, 2009). Meanwhile, some studies observed the adaptability of bloom-forming cyanobacteria under extreme drought conditions (Oliveira et al., 2019).

With a high demand for photosynthesis, bloom-forming cyanobacteria require carbon dioxide, limited in the dissolved form underwater. Surface cyanobacterial blooms take advantage of the buoyancy regulation by sequestering carbon dioxide directly from the air, thus eliminating the limitation for growth (Paerl & Huisman, 2009).

**Nutrient supply**

Eutrophication is an excessive nutrient supply to the water body from diverse sources of contamination, which appeared as a consequence of the development of intense civil, industrial, and agricultural life (Baig et al., 2016). Examples of water reservoirs, located near agricultural and industrial plants, which suffered dense cyanobacterial blooms are lakes Taihu (China), Peipsi (Europe), Vortsjarv (Estonia), and Great Lakes on the border of the USA and Canada (Baig et al., 2016; Munawar & Fitzpatrick, 2018; Nõges et al., 2010). Wastewaters, fertilisers, pesticides, and detergents, which started to be utilised by humanity quite recently, are prone to decomposition into simple compounds and serve as a supplementary of essential ions (Drugă et al., 2019). For growth, Cyanobacteria need nitrogen, phosphorus, silicon, ammonia and many other compounds (Glibert, 2020). The significance is framed by the abilities of some cyanobacterial species to fix nitrogen from additional inputs for photosynthesis and utilise phosphorus for senescence initiation (Schoffelen et al., 2018).

Phosphorus (P) enrichment along with nitrogen input was traditionally considered a primary factor of bloom formation. Nitrogen (N) enrichment, often in the form of ammonia, in the water basin signified sewage water emission. Meanwhile, N and P loads in the form of nitrate and phosphate ions were connected to agricultural run-off (Hamilton et al., 2019).

In addition to N and P enrichment, the growth of HABs in eutrophic water reservoirs are observed to correlate with heightened iron ion content. Assumably, iron served as a regulating element in the nitrogen-phosphorus cycle (Du et al., 2019). In summary, N and P input cover cyanobacterial physiological needs, which are essential for blooming productivity and toxicity.

**Light intensity**

Light intensity gives an additional competitive advantage of surface blooming cyanobacteria compared to other species of algae. Specifically, irradiance with bright light is negated by cyanobacteria which utilise it for photosynthesis needs. Moreover, accessory cyanobacterial photosynthetic pigments such as carotenoids or phycobiliproteins exhibit photoprotective functions which warrant the development of the HABs in the long-term perspective (Paerl & Huisman, 2009). In addition, buoyancy regulation via gas vesicles allows bloom-forming species to migrate along the vertical column of water, thus gaining a convenient position in the water ecosystem. Mixing up and down in the water column is also facilitated by wind and water flow, providing additional access to nutrients in the deeper bands of water and light in the surface waters. Thus, cyanobacterial blooming can distribute along several meters in depth. Locating at the surface waters, blooms can create highly turbid environments in which eukaryotic phytoplankton can experience a lack of sunlight, thus suppressing the proliferation of the other species of aquatic phytoplankton (Huisman et al., 2004).

## 1.5 Methods of detection of HABs

**Light microscopy**

Understanding the HABs dynamics and mechanisms of blooming is essential for effective management of water resources. Monitoring and early detection is the best option for prophylaxis of the negative consequences of HABs. One of the earliest and traditional methods for visualisation and identification of harmful algal species is light microscopy. Imaging of bloom-forming algal cells with light microscopy can be conducted with live or fixed samples under the objective, adapted to the size of the microalgae. For example, a microplankton of size < 20 μm is recommended to be visualised under 10x objective. The method possesses good spatial resolution even with organisms with a fragile structure such as spines, scales, and flagella. A simple “drop on the slide” of a concentrated sample can also provide quite a precise estimation of the cell count. Cell quantitation using microscopy methods is broad in alternatives such as inverted microscopy, counting cells, counting on filters (Hallegraeff et al., 2004) but limited in statistics and subjectivity of researchers. Moreover, researchers who perform counting and imaging using microscopy can experience fatigue from experiments with multiple samples. Since microscopy cannot cover large volumes of the samples, it is challenging to produce statistically significant output from the

studies. In addition to this, the inability of microscopy results to manipulate large datasets about blooming can potentially impede the successful monitoring of HABs (Wu et al., 2013).

**Imaging flow cytometry (IFC)**

Developed in the last several decades, IFC was applied for phytoplankton research (Dashkova et al., 2017). The method provided a possibility of high-throughput analysis, which captures simultaneously thousands of events compensating for the gaps in results of microscopic research in HAB monitoring (Olson & Sosik, 2007). For HAB early warning and understanding of dynamics, continuous automated methods like IFC provide both morphological and fluorescence data for temporal evaluation of a single cell and a community regardless of the heterogeneity of the sample (Campbell et al., 2010). Statistical information collected on each individual event provides multiple morphological parameters such as length, width, a diameter of cells. In addition to this, cell count and fluorescence data can be collected (Park et al., 2018). Sample preparation for IFC analysis does not always require fixation and labelling, so live samples can also be manipulated. Thus, methodology holds a great promise for the water municipalities and protection agencies that require fast and effortless monitoring of water quality in HAB detection.

Another advantage of IFC is the relative portability of instruments. Constant monitoring can keep control over different potentially blooming species, reveal new organisms prone to blooming, and in case of an outbreak, collect samples for focused microscopic and molecular research (Campbell et al., 2013). The previous research using IFC for HABs detection has shown diverse findings: unnoticed cases of blooms and bloom-forming organisms, cell division, cell life cycle, and HAB prediction mechanisms via interspecies interaction studies (Henrichs et al., 2011; Harred & Campbell, 2014). Dunker and co-authors (2018) in their work found a way to identify the life-stage of the algae according to the fluorescence data produced by IFC. The study also demonstrated that the integration of IFC with deep learning neural networks could augment image classification and species identification efficiency.

Although many studies were conducted with IFC as a method of monitoring phytoplankton and recognition of blooms, the traditional routine of microscopy is prevalent in most world environmental laboratories. However, considering the time required to analyse and identify bloom using this conventional strategy, the chances to detect the bloom at its early stage of development are decreasing (Antonella & Luca, 2012).

Present newly-designed imaging flow cytometers share the magnification ability similar to one in microscopy. However, the spatial resolution of the cells is slightly decreased compared to microscopy. Since the time of passing through the flow cell is short, the amount of light captured by the image is lower (Barteneva & Vorobjev, 2016). This is the limiting factor of identification of species contributing to HABs using only IFC. The analysis is better to be supported by the microscopical identification of HABs to the species or genus level (Olson & Sosik, 2007).

FlowCAM (Yokogawa Fluid Imaging Technologies, USA) is an imaging flow cytometer focused on the analysis of microplankton in size range from 2 μm to 1000 μm with a semi-automated classification of cells. Comparison studies demonstrated that FlowCAM provides matching patterns in cell abundance and biomass as manual calculation using light microscopy (Álvarez et al., 2013). The instrument participated in several studies aimed at bloom characterization (Lehman et al., 2013, 2014; Buskey & Hyatt, 2006).

**Remote sensing**

The recent addition to technologies for HABs detection is remote sensing methods, namely, satellite analysis. Remote sensing methods can be used for spatio-temporal monitoring of the HAB development at the early and late stages of HABs (Jing et al., 2019). Specifically, remote sensing utilises multispectral properties of satellites to detect temperature anomalies, colour anomalies, chlorophyll-a spectral intensity, and density (Isenstein et al., 2014). For example, in Australia, these parameters could be successfully used for the detection of anatoxin-a spreading across Lake Victoria (John et al., 2019). To make detection more effective in time and space, a combination of remote sensing and molecular techniques of HAB detection as stated by Zhao and co-authors (2018).

**Detection of microcystin-producing Cyanobacteria with PCR-based methods**

The initial phase of HABs detection includes determination of the bloom, identification of bloom-forming species, and quantitation of cell abundance using image-based methods like IFC or microscopy. However, imaging-based methods cannot distinguish between toxic and non-toxic strains of cyanobacteria, thus missing out on the validation of the toxicity of the bloom (Medlin, 2013). While it is known that toxic blooms are far more dangerous than non-toxic blooms, implementation of a highly sensitive method like RT-PCR as a part of HABs detection strategy might be an effective decision for a rapid response on the bloom

formation. RT-PCR is able to determine the toxin-encoding genotype of cyanobacteria at any stage of the bloom (Martins & Vasconcelos, 2011) and helps to identify potentially toxic algal species. The method consists of the amplification of the genomic sequences of cyanobacteria, characterised as ribosomal genes or genes regulating certain protein expressions like toxin synthesis, nitrogen fixation, and others (Antonella & Luca, 2012). While the process of amplification continues, amplified sequences emit fluorescence signals which are collected by a detector. The quantity of fluorescence can be correlated with the number of cycles and amount of DNA input at the beginning of the reaction. Thus, the reaction of RT-PCR may help both identification and enumeration of toxin-producing species in the sample of water (Coyne et al., 2005). The correlation of the presence of toxin-producing genes and expression of cyanotoxins was validated by numerous works which demonstrated that PCR-based analysis can be as sensitive as ELISA, LC-MS and HPLC (Baker et al., 2002; Anjos et al., 2006; Al-Tebrineh et al., 2011). Moreover, RT-PCR assay can be more sensitive to small amounts of toxin-bearing strains, thus revealing the toxin gene presence not validated by the ELISA technique Al-Tebrineh et al., 2011.

### 1.6 Potentially toxic cyanobacteria in the world and in Kazakhstan

The geographic distribution of CyanoHABs is expanding and new cases are reported every year. According to Svirčev et al., 2019, *Microcystis* spp. was reported most frequently, thus being the most abundant potentially toxic cyanobacteria around the world. However, *Anabaena* spp. (now *Dolichospermum* spp.) and *Aphanizomenon* spp. (now *Cuspidothrix* spp.) were also reported commonly with the most often recorded cyanotoxins microcystin(63%), cylindrospermopsin (10%) and anatoxin-a (9%). According to Harke et al., 2016, the analysis of literature suggested the presence of *Microcystis* spp. blooming in 108 countries. 79 countries validated the presence of hepatotoxin microcystin. In Kazakhstan, the study on the investigation of endorheic lakes and river basins were conducted by research groups from Nazarbayev University, however, this work was not related to potentially toxic algae (Yapiyev et al., 2017). Potentially toxic species *Microcystis aeruginosa* was listed in the works by Barinova and colleagues (2011) in the small lakes of Northern Kazakhstan and Shardara reservoir in Southern Kazakhstan (Barinova and Krupa, 2017). However, the toxicity of the species was not investigated or validated. The only case of blooming was reported in Lake Chebachiye, Central Kazakhstan caused by *Anabaena flos-aquae (*Nurashov and Sametova, 2015). No analysis of toxicity was conducted. The cases of water blooming

were reported in the media multiple times, still no consequences other than odour and colour such as fish and animal kills, drinking water contamination, human poisoning were not described in the context of the problem (lenta.inform.kz).

### 1.7 Overall goal

In this work, integration of a high-throughput IFC and molecular analysis using RT-PCR is suggested for effective detection of HABs at the early stages of HAB development. IFC can provide an initial analysis of cell taxonomic resolution (supported by light microscopy) and abundance of potentially toxic algae, while toxicity validation can be performed by detection of toxin-producing algal strains. The work provides the full experience of the method development and its application on the field samples from the lakes and rivers of Kazakhstan.

# 2 MATERIAL AND METHODS

## 2.1 Sampling

There were two sets of water samples collected for the present study. Initially, water samples from the river Ural were collected in Atyrau province in August 2019. Next year, the sampling process was extended to different lakes and rivers across the territory of Kazakhstan. Ural river, flowing through two Kazakhstani provinces, is the 3rd longest river in Europe. The choice of the Ural river as a study place related to the massive fish die-off reported on the river Ural in December 2018-March 2019 (https://informburo.kz/). In river Ural, the total number of sampling locations was fourteen. Specifically, three samples of water were taken downstream closer to the river estuary, where the Ural meets the Caspian Sea. Upstream the river, there were five sampling sites distributed along with a distance of 100 km from the city boundary. Within Atyrau city boundaries, the river water was collected at six sampling sites, including an artificial channel diverted from the Ural river named Peretaska. In December 2018, the channel Peretaska was the first place where the fish kill was encountered. The distribution of sampling sites from the river estuary to the most upstream point: T1 Delta> T2 Channel> T3 Ship> T4 Low Peretaska> T5 Balykshy> T6 Peretaska> T7 Oil Refinery Water Intake ANPZ> T8 Atyrau Su Arnasy> T9 Geolog> T10 Almaly> T11 Saraishyk> T12 Low Mahambet> T13 High Mahambet> T14 100 km. Figure 1 illustrates the distribution on the map.

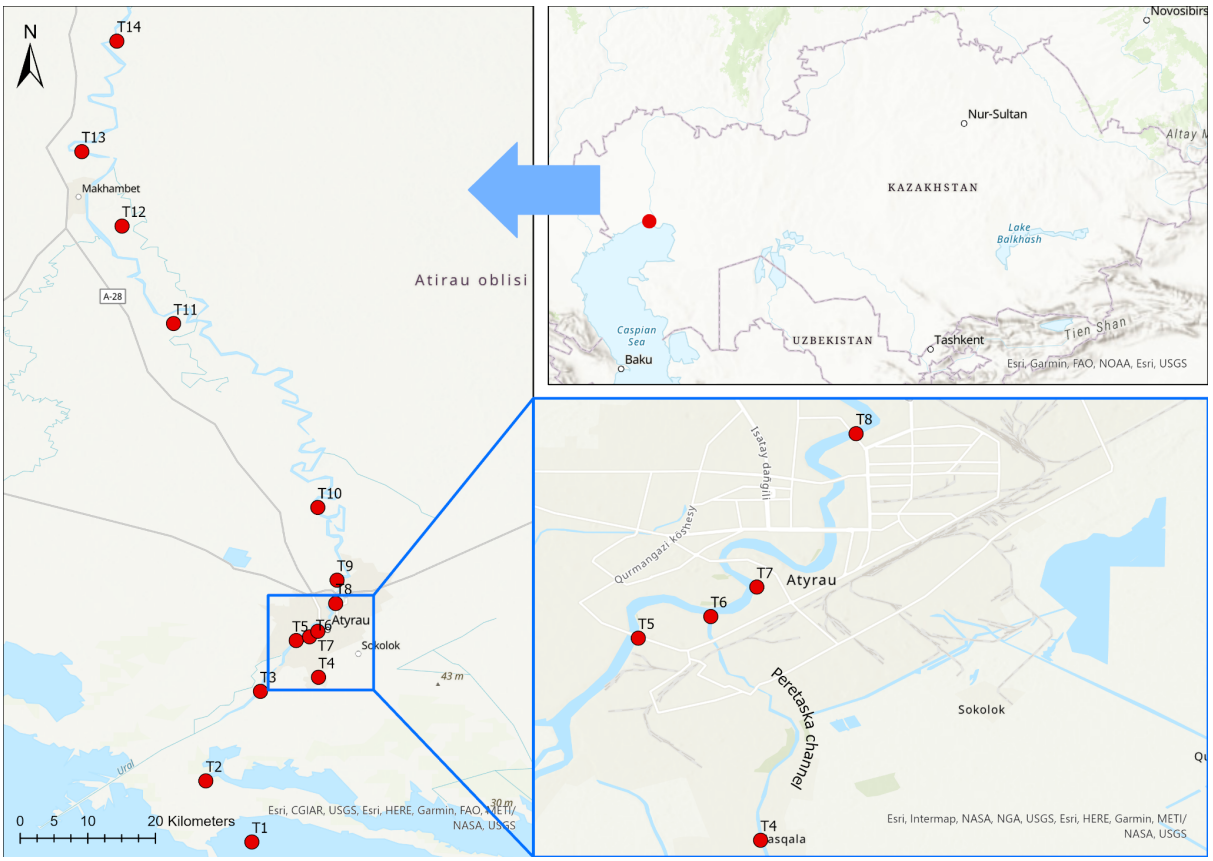


Figure 1. Map representation of the sampling sites on Ural river, Western Kazakhstan. Samples were collected closer to the delta of Ural river (T1-T3), within the city boundaries (T4-T9) at the main points of the fish kill in December 2018, and upstream the river up to 100 km from the city boundary (T10-T14).

Furthermore, water samples were also collected from Kazakhstani lakes and rivers near the residential areas across the country's entire territory. The following rivers and lakes were selected: river Ishim in Akmola province, Fedorov reservoir in Karagandy province, river Irtysh in East Kazakhstan province, lake Parchach in Almaty province, river Syr-Darya in Kyzylorda province, river Jigirgen in Turkestan province, river Ural in Atyrau province, Aktobe reservoir in Aktobe province, river Chagan in Western Kazakhstan province, river Badam in Southern Kazakhstan province and lake Bakpyldak in Pavlodar province. The distribution on the map is shown in Figure 2. The selection was performed related to the known cases of massive fish kills and possible anthropogenic pollution from the nearby located populated centers. Sampling was conducted in the period of three months, September-November 2020. The samples of water were collected at each sampling point each week during a four-week period, excluding three sampling sites: river Irtysh (Semey province), river Syr-Darya (Kyzylorda province), and lake Bakpyldak (Pavlodar province).

At the aforementioned places, the number of samples was lessened due to inclement weather conditions.

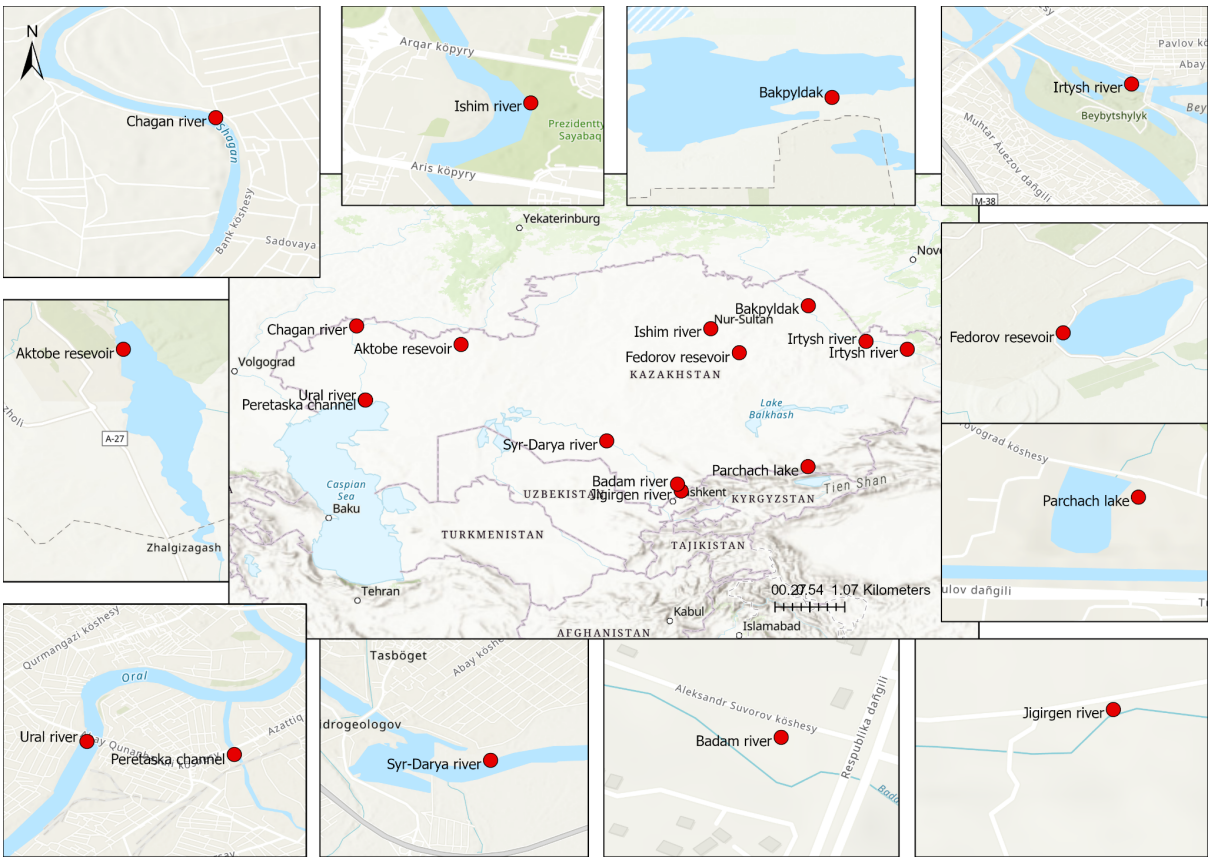


Figure 2. Map representation of sampling sites in the Kazakhstani lakes and rivers from different regions of Kazakhstan. Sampling included eleven water bodies: river Ishim, Fedorov reservoir, river Irtysh, lake Parchach, river Syr-Darya, river Jigirgen, river Ural, Aktobe reservoir, river Chagan, river Badam, and lake Bakpyldak. Locations of the sampling places were illustrated on the national map. Smaller maps on the sides demonstrate positions of sampling at each water body.

The sampling procedure is divided into water collection, water preservation, and measurement of water and phytoplankton parameters. Water was collected into a clean 1.5 L plastic bottle, preliminarily rinsed by river water to minimise the contaminants, at a depth of 50 cm or the length of an extended arm. 50 ml of the sample were fixed instantly by Lugol's solution, 1% glutaraldehyde, or 0.5% paraformaldehyde solution for microscopy and IFC. Afterwards, water and phytoplankton samples were stored in a cooler box until delivered to the laboratory and then kept at 4ºC in the refrigerator. The following water parameters were measured at a Ural river in 2019 at each sampling site: temperature, pH, conductivity, Total Dissolved Solids (TDS), salinity, and dissolved oxygen. Sampling in different Kazakhstani lakes and rivers involved volunteer help; therefore, the number of parameters was limited by

temperature and pH (estimated by Litmus paper strips). Sampling measurements and calibration of multimeters were conducted according to a Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes by WHO (1996). Shortly, acquisition of temperature replicates and other water parameters was not indicated as a mandatory step of the protocol. Instead, through examination and calibration of multimeters a week before the sampling was performed. The measurement of each parameter can take several minutes for the multimeter to stabilize the values, therefore, repetition can significantly prolong the sampling process. Initial training in field sampling was obtained during the international expedition Aral 2019 (under the supervision of Professor Lajos Voros Balaton Limnological Research Institute, Hungarian Academy of Sciences).

### 2.2 FlowCAM-based Imaging flow cytometry

All samples were analysed using an imaging flow cytometer FlowCAM from Yokogawa Fluid Imaging Technologies, USA. The instrument is equipped with 10x and 20X objectives and can capture multiple events simultaneously. FlowCAM was calibrated using focus beads provided by Yokogawa Fluid Imaging Technologies. The cytometer has two modes of recording: an autoimage mode and laser-triggered mode. An autoimage mode, used in this study, detects events with fluorescence without any threshold and is convenient to use with the samples of high concentrations of algae. The instrument is affiliated with the software Visual Spreadsheet 4.0. Shortly, the sample was filtered using 100 µm filter tissue while the flow cell is being washed by ultrapure water at the velocity of 2 ml/min. The focus and flow speed were set manually depending on phytoplankton sample density.

### 2.3 Light microscopy

The microscope used in the study is Leica DM 2500, equipped with Leica Application Suite Vs. 4.9.0 software. Firstly, the sample was sedimented overnight before visualising it under the microscope. The drop of the sedimented sample was taken by a plastic Pasteur pipette from the bottom of the tube, minimising disturbance of the cells, put onto a clean slide, and covered with a coverslip. Initially, objective x4 was used. For detailed identification, the objective was switched to higher magnification. The images were taken under x40 objective.

### 2.4 PCR-based analysis

The DNA extraction was performed using PowerWater DNA Isolation Kit from MO BIO Laboratories Inc (USA). The extraction was performed according to the instructions from the manufacturer, and DNA concentration was determined using the NanoDrop 8000 spectrophotometer. Polymerase Chain Reaction was performed according to the New England Biolabs protocol with Phusion High Fidelity PCR Master Mix (New England Biolabs, USA). A set of primers was designed for detecting the genes responsible for toxin production. A set of common freshwater cyanobacterial strains and their produced toxins were selected from the literature according to the geographic location of the strain, which caused the contamination. The primers were initially examined using an OligoAnalyzer software from Integrated DNA Technologies.

The reaction was set by adding 1 µl of each primer, 2-3 µl of template DNA (20-30 ng of DNA as measured by NanoDrop 8000), 25 µl of Phusion High Fidelity PCR Master Mix and filled up to 50 µl of total reaction volume. The reaction settings on the BioRad T100 Thermal Cycler were following the routine: denaturation step at 95°C for 3 min and continued with 40 cycles of denaturation at $95^0$C for 10 sec, annealing at $T_m$ of primers for 30 sec and extension at $72^0$C for 30 sec. The first trials were conducted using temperature gradients from $49^0$C to $57^0$C which allowed to determine an optimal annealing temperature of primers.

**Table 1. Primers for PCR detection of toxin genes.**

| Strain | Target gene | Toxin synthetase specific primers (5' → 3') |
|---|---|---|
| *M. aeruginosa* PCC7806 | *mcyE/ndaF* specific for microcystin/nodularin toxins gene | F: GAAATTTGTGTAGAAGGTGC |
| | | R: CAATGGGAGCATAACGAG |
| *C. raciborskii* AWT205 | *cyrA* specific for cylindrospermopsin gene | F:GTCTGCCCACGTGATGTTATGAT |
| | | R:CGTGACCGCCGTGACA |

Positive controls for the microcystin gene (*mcyE*) were obtained from the mesocosm experiment (Mirasbekov et al., 2021). Positive control for cylindrospermopsin were supplied by Diagnostic Technologies (USA). Gel for gel electrophoresis was stained by SYBR Safe DNA Gel Stain from Bio-Rad (USA).

**Real-Time PCR**

The same set of primers was used for Real-Time PCR as for end-point PCR reaction. The reaction of total volume 20 µl was set up with 2.5 µl of SYBR Green Buffer from Bio-Rad, 2.5 µl of dNTPs, 2.5 µl of $MgCl_2$, 1 µl pf forward primer, and 1 µl of reverse primer 0.5 µ of Taq polymerase, 5µl of $ddH_2O$ and 5 µl of template DNA. The volume of the DNA template was calculated according to the concentration of template DNA for each sample as defined by NanoDrop from 0.5 ng/µl to 10 ng/µl. The reaction was set in the Bio-Rad CFX96 Touch Real-Time Thermocycler from Bio-Rad (USA). The PCR reaction started from the denaturation step at $95^0$C for 3 min and continued with 40 cycles of denaturation at $95^0$C for 10 sec, annealing at $T_m$ of primers for 30 sec, and extension at $70^0$C for 30 sec. The melting curve was set from $50^0$C to $95^0$C. The reactions were set as triplicates for each sample with $ddH_2O$ as a negative control.

**Sanger sequencing**

The samples which have shown a positive band for toxin-producing genes after PCR were sent to Evrogen (Russian Federation) for Sanger sequencing. Sequencing would provide awareness on the precise structure of the toxin gene sequence. A forward primer of *mcyE* designed by Vaitomaa and co-authors (2003) was used for sequencing. Sequences were analysed for homologs in other organisms using the BLAST (Basic Local Alignment Search Tool) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as described by Pearson, 2013.

**2.5 Chlorophyll extraction**

The filter papers with a cell material on them were cut into pieces and dissolved in 4 ml of 99% methanol in glass tubes. The sample was ground gently with a glass rod and left for 30 minutes at room temperature in the dark for extraction of the cell residues. Then, the glass tubes were inserted into a hot water bath at 74ºC for several minutes while shaking and grinding gently to facilitate extraction of the pigment. The samples were transferred to a 1.5 ml Eppendorf tube and centrifuged for 1-2 minutes to sediment filter paper residues. 330 ul of supernatant was removed and placed into 96-well plates. 99% methanol was used as a blank sample. Absorbance was measured at 653 nm, 666nm, and 750 nm using a Thermofisher Varioskan multimode reader. The final result of Chl-a content in the sample was obtained according to this formula:

$$Chl\ -\ a\ (\mu g/L)\ = \frac{Chl-a(mg/L)*4ml*1000}{filtered\ volume\ (ml)}$$

### 2.6 Ionic chromatography

The ionic composition of water was estimated by the ion chromatography technique (IC). Samples were firstly filtered through 0.22 μm cellulose acetate filters. Dionex ICS-6000 HPIC system (Thermo Scientific, USA) connected to Chameleon 6.0 software was used to analyse the levels of chloride, phosphate, nitrite, fluoride, bromide, nitrate, and sulfate ions. Separation of anions was performed with the help of Dionex IonPac AS11-HC-4 μm capillary column (2x250 mm). 30 mmol potassium hydroxide solution was used as an eluent. Dionex IonPac CS12A-5 mSm IC column (3x150 mm) determined the cations. A calibration graph was ranged from 1 to 30 ppb with the correlation coefficient equal to 0.998. 40 times-diluted samples went through the quality control at 15 ppb. In total, an average of three measurements was deduced as a result of split samples (sub-samples from the same aliquot of 1000 ml). The procedure was performed at Nazarbayev University Core facilities.

### 2.7 Total Phosphorus (TP) measurement

The protocol of Total Phosphorus (TP) measurement by Motomizu and co-authors (1983) was adapted to our laboratory in smaller volumes to prevent reagent spoiling. All reagents were freshly made because the period of usage of most reagents is limited to 2 weeks. Calibration solutions with the known concentrations of phosphorus from 0 to 0.5 mg/L were prepared from 1 mg/L potassium dihydrogen phosphate $KH_2PO_4$ standard solution (50 mg/L) by diluting the stock in dd$H_2O$. Molybdate antimony solution (R2) was prepared by mixing 130 g/L ammonium heptamolybdate $(NH_4)_6Mo_7O_{24}$ solution with 3.5 g/L of potassium antimon(III)oxide tartrate $K(SbO)C_4H_4O_6$ solution with the addition of small portion of 4M sulphuric acid. Samples of water were filtered through 0.45 μm and mixed with 100 μl of 4M sulphuric acid. From this step on, the calibration solutions and samples will be processed simultaneously. 10 ml of the calibration solution or sample is mixed with 2 ml of 50 g/L potassium peroxidisulphate ($K_2S_2O_8$). The mixture was put into an autoclave for 30 minutes. When the solutions cool down, 400 μl of R1 reagent (50 g/L ascorbic acid) was added. Having mixed for 30 seconds, 400 μl were of R2 (molybdate/antimony solution) were added. In the end, samples were incubated for 15-30 minutes at room temperature and measured using an Evolution 300 Uv-Vis spectrophotometer (ThermoFisher Scientific) at 880 nm. The

intensity of absorbance is proportional to the concentration of molybdenblue complex formed from the source of phosphorus in the water sample. The calibration curve of absorbance at 880 nm was plotted against known concentrations of phosphorus calibration solutions. The equation obtained from the linear relationship was used to determine TP content in water samples.

### 2.8 Statistical Analysis

Multivariate statistical analysis of relationships between the abundance data and environmental parameters was conducted using Primer 7 software. The data was square root transformed and normalised. PCA (Principal Component Analysis) was used to generate a comparison of environmental parameters over sampling sites. Spearman correlation test ($p<0.05$) was used to interpret the statistically significant relationship between abundance data and environmental parameters. The datasets of environmental parameters included temperature, pH, TDS, conductivity, dissolved oxygen and ionic composition (nitrates, ammonia). Biological datasets included abundances of five potentially toxic cyanobacterial species.

# 3 AIMS OF THE THESIS PROJECT

I. To identify the species of potentially toxic cyanobacteria in phytoplankton samples from the Ural river using FlowCAM-based imaging flow cytometry (IFC) and light microscopy.

II. To detect microcystin-producing cyanobacteria with genus-specific microcystin-synthetase gene E (*mcy E*) PCR in phytoplankton samples from river Ural.

III. To apply combined IFC- and PCR-based methodology to detect potentially toxic cyanobacteria in other Kazakhstani lakes and rivers.

# 4 RESULTS

## 4.1 Description of sampling sites

Sampling sites from T1 to T3 were scattered downstream of the Ural river. Sampling sites T4-T9 were scattered within the city boundaries, while T10-T14 were located upstream of the river. The river estuary contained brackish and turbid water with a wide animal diversity: valuable species of migratory birds, turtles, raccoon dogs, and wild boars. Sample T1 was taken at the estuary, while sample T2 was collected in the artificial channel which connected the Ural and the Caspian Sea. Sample T3 was taken downstream of the river Ural lower the city boundary. Samples taken within the city boundary match the places of the highest fish mortality in December 2018. Thus, samples were taken from the river Ural at the city district (T5-T9) and artificial channel Peretaska at two points: the higher (T6) and lower part (T4) of Peretaska. The sampling sites were located near the residential and industrial areas. The sampling sites upstream of the river (T10 - T14) were scattered along with the small villages such as Almaly (T10), Saraishyk (T11), Mahambet (T12-T13), Kyzyluy(T14). The residential life of Atyrau and its satellite municipalities are dependent on the river as a source of water for agricultural, industrial, and domestic purposes and may pollute the river and influence the quality of water. The industrial, municipal, and residential entities withdraw water from the river and return it back into specialised areas. Specifically, the electric power plant of Atyrau city as well as the oil refinery plant withdraw water from the upper part of the Peretaska channel and throw processed water back to the lower part of Peretaska channel. Therefore, the temperature measurements at the lower part of the Peretaska channel were observed to be higher than the upper part of Peretaska during the winter season when water temperature stays low or goes under the freezing point.

## 4.2 Environmental parameters

### Temperature, pH, salinity and DO

Water parameters such as salinity, pH, and temperature were measured. Salinity measurements demonstrated slightly increased values in the estuary of river Ural falling into the Caspian Sea, where brackish waters flow (0.42%). The salinity declined at the channel (0.15%) connecting the river and Caspian Sea and further until it turned into fresh water in the river Ural. At the same time, temperature values were stable for the summer season ranging from $23.2^0$C to $25.6^0$C across the sampling sites, excluding a significant deviation at

sampling site T4 Low Peretaska, where temperature peaked at 29.9$^0$C. An additional measurement of temperature in the Low Peretaska during the winter season (12/2019) also showed an outstandingly high temperature of 25.9$^0$C while surrounding water in the Ural river was at 0.1$^0$C. At the same time, the majority of sampling sites were characterised by pH 8, slightly higher than the normal pH of water. It was observed that pH lowered at several sampling sites such as T4 Low Peretaska (pH 7.54), T5 Balykshy (pH 7.06) and T14 100 km (pH 7.03). In addition, dissolved oxygen was measured at sampling sites T4-T14. It was found that saturation of the upstream part of the river water was sufficient with the measurements ranging from 10.05 to 13.01 mg/L, whereas saturation of water within the city boundaries was significantly lowered. The lowest level of dissolved oxygen encountered at the Ural river sample sites was 6.4 mg/L at T7 Oil Refinery Water Intake. Further, measurements at the artificial channel from the Ural river named Peretaska detected the level of DO 3.7 mg/L at T4 Low Peretaska and 6.5 mg/L at T6 Peretaska. Since T6 Peretaska sample was collected at the beginning of the channel, where the waters of the river and a channel mix, the measurements are similar, whereas the lower part of Peretaska demonstrates extreme deprivation of dissolved oxygen.

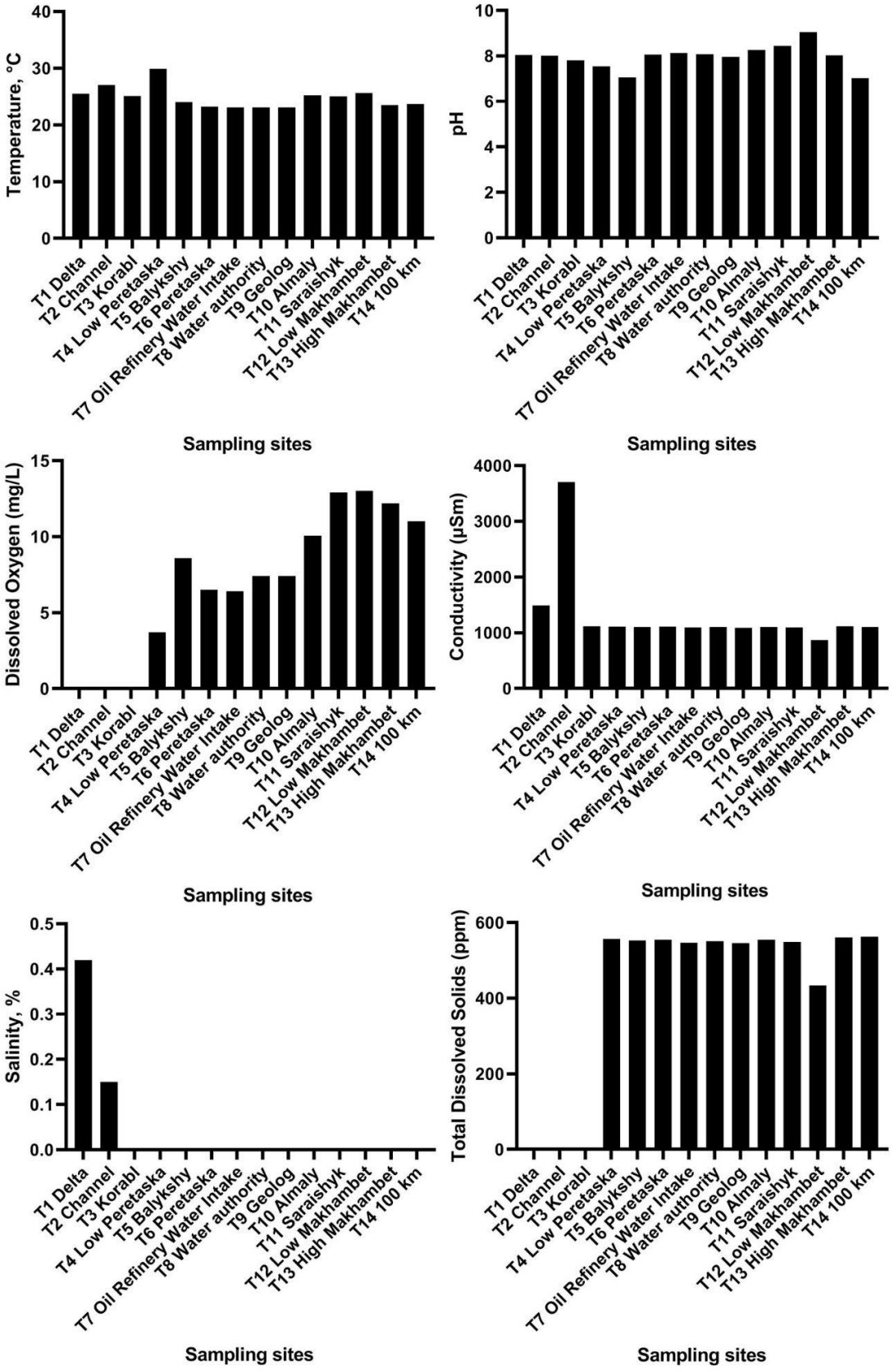


Figure 3. Environmental parameters measured in the Ural river at the sampling sites T1-T14. Six water parameters included temperature ($^0$C), pH, Dissolved oxygen (mg/L), salinity (%), Total Dissolved Solids (ppm), conductivity (µS/m).

**Nutrients**

Using ionic chromatography, nitrogen in the form of nitrates and ammonia was measured in the water. The lowest nitrates (NO3) level in the Ural river was encountered in the estuary of the Ural river (T1 Delta) with the Caspian Sea and a channel connecting those parts (T2 Channel), 0.22 and 0.13 mg/L, respectively. The trendline increases closer to the city, where the level of nitrates range from 1.26 mg/L to 4.69 mg/L. The highest concentrations were detected at the T6 Peretaska, T5 Balykshy region, and T7 Oil Refinery Water Intake with the values of 4.69, 3.92, and 3.5 mg/L, respectively. Upstream the river, the average concentration of nitrates was reported to be 0.5 mg/L, excluding the sampling site near the village Almaly (T10 Almaly). Nitrogen in the form of ammonia was increased at T12 Low Mahambet, peaking at 3.25 mg/L.

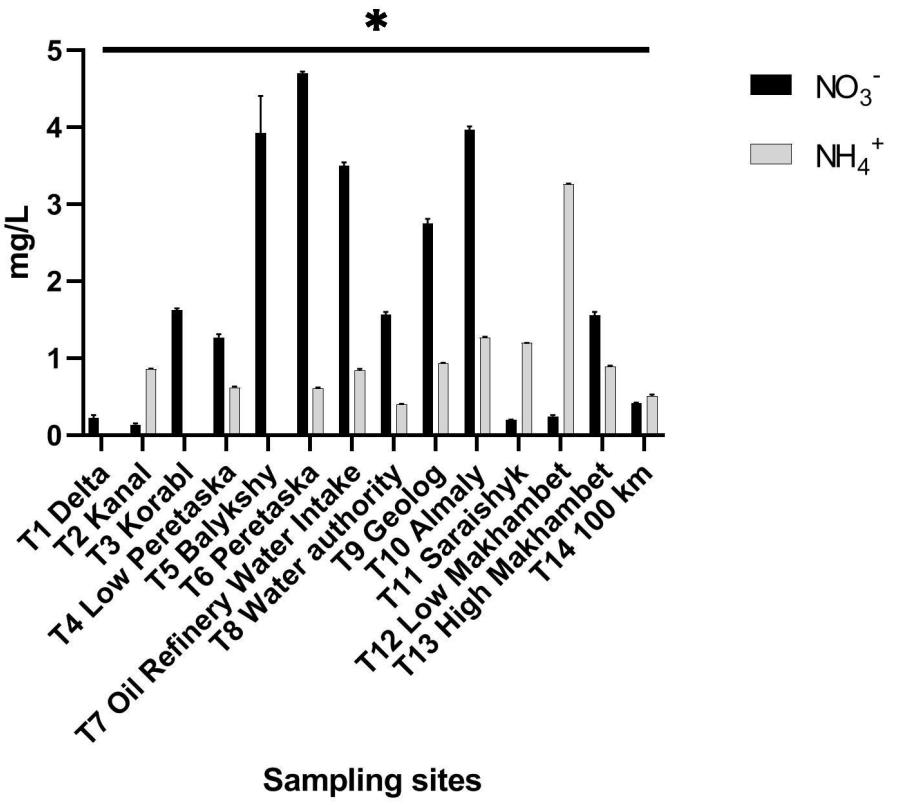


Figure 4. Analysing replicates of subsampling of nitrogen content in the form of ammonia and nitrates in the river Ural. The error bars show standard deviation. One-way ANOVA was applied separately to nitrates and ammonia to deduce significant difference among sampling sites. The samples are statistically different with $p < 0.0001$. Measurements were conducted in August 2019.

**Total Phosphorus (TP)**

Total Phosphorus was measured using the adapted protocol of Motomizu and co-authors (1983). The complex reaction between inorganic phosphate and organic-bound phosphorus which converted in a chain of reactions to the molybdene blue substance, which can be measured at spectrophotometer at 880 nm. Three samples from the Upper Peretaska (T6), Lower Peretaska (T4), and Ural river (T5) were selected to measure the level of inorganic phosphates and organic bound phosphorus (TP). A standard curve with known concentration solutions was plotted against the level of absorbance at 880 nm to determine the concentrations of the unknown water samples. The results of the measurement are indicated in Table 2.

**Table 2. Total Phosphorus (TP) concentrations in selected samples from the Ural river.**

| № | Sample Name | Absorbance 880 nm | Concentration (mg/L) |
|---|---|---|---|
| 1 | Upper Peretaska (T6) | 0.051 | 0.03078 |
| 2 | Lower Peretaska (T4) | 0.044 | 0.01832 |
| 3 | Ural river (T5) | 0.008 | ND |

The TP concentration detected in the upper part of Peretaska was higher than in the lower part of the channel. Meanwhile, the phosphorus level in the river Ural was too low for the method to detect it.

* Photometric accuracy of Evolution 300 UV-Vis Spectrophotometer ±0.004.

**4.3 Abundance of potentially toxic species of cyanobacteria in river Ural**

Using imaging flow cytometer FlowCAM, we have encountered an abundance of filamentous cyanobacteria of coiled and straight morphology in the river Ural. After classification and identification of these images with expert-taxonomist (> 10 years of experience), five species of potentially toxic cyanobacteria were distinguished in the river Ural in August 2019. Species that have been identified are *Cuspidothrix issatschenkoi*., *Dolichospermum flos-aquae*, *Pseudanabaena limnetica, Cylindrospermopsis raciborskii, Sphaerospermopsis aphanizomenoides*, and *Microcystis aeruginosa.* The dominant genera encountered were *Cuspidothrix* sp., *Dolichospermum* spp., and *Pseudanabaena limnetica*. The sampling started from T1 Delta, an estuary of the river Ural with the Caspian Sea, where waters were brackish

(0.42%) with a correspondingly low abundance of cyanobacteria. The abundance rises slightly in the shallow channel with brackish (0.15%) and turbid water connecting the Caspian Sea with the river Ural (T2 Channel). T3 Ship is the sampling point located at the edge of the city where a low amount of *Pseudanabaena limnetica* and *Cuspidothrix* spp. were detected. Within the city boundaries, the lower part of Peretaska (T4) and Balykshy region (T5) contained low concentrations of cells whereas the upper part of Peretaska (T6) concentrated the majority of potentially toxic species such as *Cuspidothrix* spp., *Pseudanabaena limnetica* and *Dolichospermum* spp. with minute quantities of *Cylindrospermopsis raciborskii* and *Sphaerospermopsis* sp. Cell density peaked at 198 particles/ml for *Cuspidothrix* spp., 199 particles/ml for *Pseudanabaena limnetica* and 79 particles/ml for *Dolichospermum* spp. at T6 Peretaska. The nearby located sampling site Oil Refinery Water Intake (T7) encountered lesser amounts of *Pseudanabaena limnetica* (143 particles/ml*) and Cuspidothrix* spp. (61 particles/ml). Predominant genera were detected primarily at the sampling sites upstream of the river. The highest densities of 885 and 634 cyanobacterial cells of the genus *Cuspidothrix* spp. per ml of water were reported at the sampling sites T11 Saraishyk and T10 Almaly, respectively. Other species such as *Pseudanabaena limnetica* and *Dolichospermum* spp. were also detected at a density of 100-200 particles/ml upstream of the river. Similar cell densities were detected in the nearly located sampling sites T10 Almaly, T12 Low Mahambet, T14 100 km. The activity of a modest amount of *Microcystis aeruginosa* was revealed at a single sampling site T12 Low Mahambet.

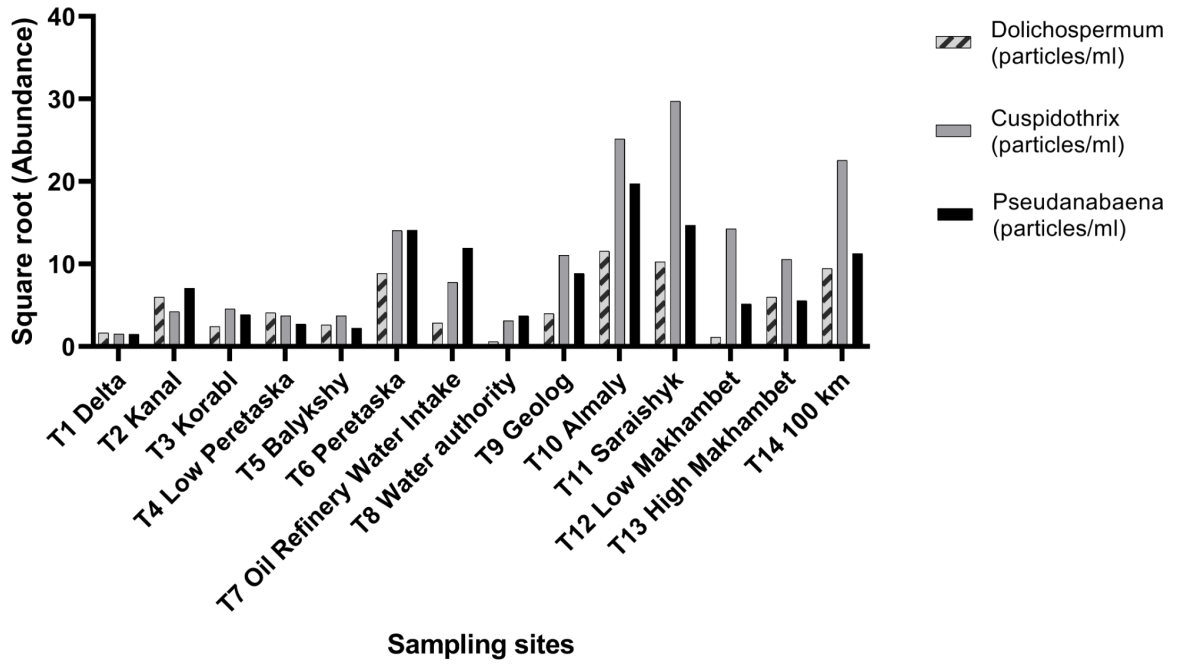


Figure 5. Abundance (particles/ml) of the dominant potentially toxic species of cyanobacteria in the Ural river. Square root transformation was applied. Samples were collected in August 2019.

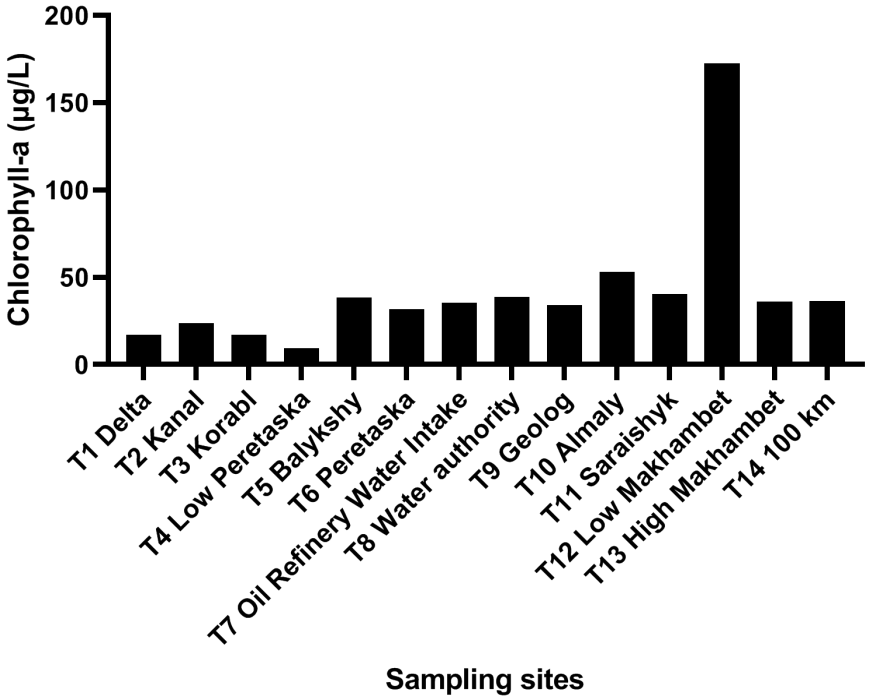


Figure 6. Chlorophyll-a content in the river Ural (µg/L) . Sampling was conducted in August 2019.

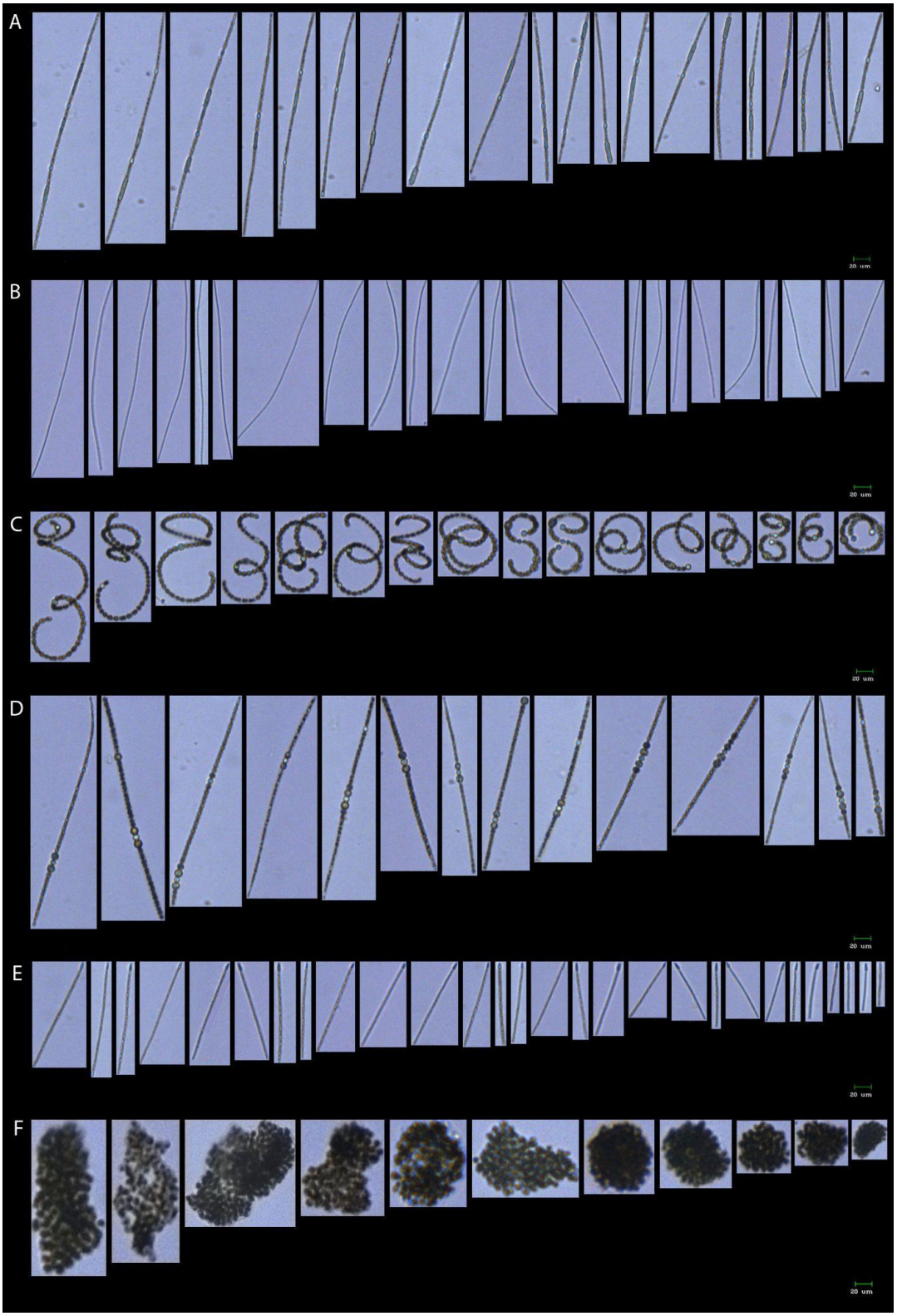


Figure 7. Representation of FlowCAM recorded galleries of the most abundant species of potentially toxic cyanobacteria detected in the river Ural. Sampling time is August 2019. Samples were fixed with 0.5% glutaraldehyde. 10x objective and 100 µm flow cell were used for analysis. A- *Cuspidothrix issatschenkoi*, B-*Pseudanabaena limnetica*, C- *Dolichospermum flos-aquae*, D-*Sphaerospermopsis aphanizomenoides*, E-*Cylindrospermopsis raciborskii*, F- *Microcystis aeruginosa.* Scale bar 20 µm.

### 4.4 Identification of toxicity genes

**Detection of microcystin-producing bacteria by PCR and real-time PCR**

Seven samples from the river Ural were filtered for DNA isolation and detection of the genes responsible for algal toxin synthesis via PCR method. Samples from T1 Delta, T3 Ship, T4 Low Peretaska, T6 Peretaska, T9 Geolog, T12 Low Mahambet, and T13 High Mahambet were processed with primers specific for microcystin and cylindrospermopsin. Primers specific for these genes were indicated in Table 1. Detection of cylindrospermopsin (*cyrA*) revealed no distinct band for the presence of these genes in algal samples from the river Ural. The expected PCR product size of 247 base pairs amplified by the primers specific for *mcyE* gene, responsible for *Microcystis*-specific microcystin expression, could be observed as a distinct band at DNA sample from T12 Low Mahambet. The primer design was provided by Vaitomaa and co-authors (2003). According to SYBR Green I Real Time-PCR results, the melting peaks of positive control of microcystin and samples T1 Delta and T12 Low Mahambet coincided, thus revealing the presence of microcystin in the algae from these sampling sites. Quantification was set by normalising abundance data through total DNA concentrations and showed that a 2.05-fold difference of *mcyE* gene copies/ng of DNA in the sample T1 Delta rather than in the sample T12 Low Mahambet.

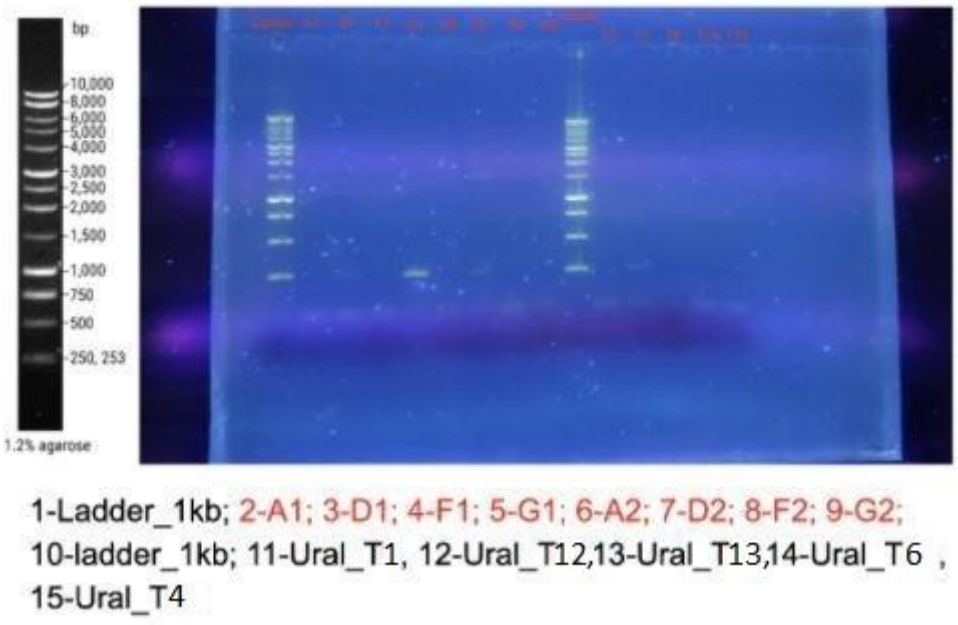


Figure 8. Representation of gel electrophoresis using samples from Ural river and primers specific for Microcystis spp. specific mcyE primers. The image represents positive control from the G1 tank of LMWE-mesocosm, University of Aarhus. The band for T12 Low Mahambet is visible on the left side of the gel at 250 bp matching the positive control. The part was conducted in collaboration with Y. Mirasbekov.

**Sanger sequencing**

The PCR products of the samples T1 Delta, T4 Low Peretaska, T6 Peretaska, T12 Low Mahambet and T13 High Mahambet were purified and sent for Sanger sequencing to Evrogen JSC, Russia (this part done in collaboration with Y. Mirasbekov). The sequencing was performed using *mcyE* forward primer. The PCR products of T4 Low Peretaska, T6 Peretaska and T13 High Mahambet supported RT-PCR results and did not reveal any microcystin-related gene sequences. Sequences of phytoplankton samples from T1 Delta and T12 Low Mahambet, which showed a positive result in PCR/RT-PCR, were indicated in Table 3.

**Table 3. Sanger sequencing data for genus-specific microcystin synthetase (*mcyE*) gene presence in phytoplankton samples from Ural River.**

| Sample Name | Sequence |
|---|---|
| T1 | GCCCGAGCAACGAATACTCATAGACTTAAGATCGAAACTTCTATCCTAGCTTTC TTGATGACAAAAAAGACAATGGAACCGGCGATTTAGGCAAGCAAACTGCTCC CGTGTATCATTGAGTTTATGGGACGAAAAGATAATCAAGTTAAGGTCAATGGT TATCGAATTGACCCCGGAGAAATTCAAACACGACCGTTGCTCCTTGA |
| T12 | CCTCAATCACTAGCCAACCCTGAAATGACTCAGGAAATGTTAAACCTAGCTTT CTTGAGTGAGACAAAAACTCTCTTTAGAACCGGCGATTTAGGCAAGCAAACT GCTCCGGGTATCATTGAGTTTATGGGACGAAAAGATAATCAAGTTAAGGTCAA TGGTTATCGAATTGACCCCGGAGAAATTGAATATCAATTGACTCGTTATGCTCC CATTGA |

The sequences were further analysed using the BLAST (Basic Local Alignment Search Tool) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare the nucleotide sequences from PCR-products obtained from Ural river algal samples to the available *Microcystis*-specific *mcyE* gene sequences in the NCBI database. The program produced the best matches, which were later examined for the homology of sequences with Ural samples in the NCBI sequence database. Our results demonstrated 88% and 96% homology of PCR products with the *Microcystis*-specific *mcyE* gene. The data is shown in Table 4 below.

**Table 4. Homology of Ural samples with *Microcystis* genus-specific *mcyE* gene.**

| Sample PCR product | Size (b.p.) | Description of the best match | Max Score | E value | Percent. identity |
|---|---|---|---|---|---|
| *mcyE* -T1 | 206 | *Microcystis viridis* NIES-102 DNA, complete genome | 205 | 1e-48 | 88.02% |

| | | | | | |
|---|---|---|---|---|---|
| *mcyE*-T12 | 218 | *Microcystis* sp. GL280646 microcystin synthetase E (mcyE) gene, partial cds | 336 | 2e-88 | 96.26% |

### 4.5 Potentially toxic algae in lakes and rivers around Kazakhstan

#### Description of sampling sites at the lakes and rivers around Kazakhstan

After validating the method of combined imaging flow cytometry with molecular biology analysis on Ural river samples, the area of research has to be extended to a larger number of water bodies. The initial step includes an identification of water bodies potentially prone to HAB blooms. Different Kazakhstani lakes and rivers with a known history of pollution and massive fish kills were selected to collect the samples of water and phytoplankton (Fig. 2 ). Samples were taken from the river Ishim in Akmola province, Fedorov reservoir in Karagandy province, river Irtysh in East Kazakhstan province, lake Parchach and river Karatal in Almaty province, river Syr-Darya in Kyzylorda province, river Jigirgen in Turkestan province, river Badam in Southern Kazakhstan province, river Ural in Atyrau province, Aktobe reservoir in Aktobe province, river Chagan in Western Kazakhstan province, and lake Balkyldak in Pavlodar province. River Ishim passes through the city of Nur-Sultan and therefore accumulates the pollutants produced by the urban population. The water authorities acknowledge the river is susceptible to dense growth of algae in the warm season (lenta.inform.kz). Fedorov reservoir in Karagandy province is an artificially created water body that was aimed at distributing water for the agricultural entities and farms nearby. River Irtysh, a sample taken in Semey city, encountered both heavy pollution from industrial activities in the region as well as a case of fish kill in the past. The fish kill, associated with huge spots of brown-red colour, took place in May 2016. The fish kill in the lake Parchach occurred in August 2020. River Syr-Darya in Kyzylorda region is considered to be one of the sources of water which supports agricultural activity in the region. River Jigirgen in Turkestan province is a small river passing through the villages in the Turkestan province with no certain history of pollution. The fish kill took place recently in March 2021 in the river Badam passing through Shymkent city. River Karatal flows through Taldykorgan city, where heavy metals processing plants are located. River Ural in the Atyrau region encountered a prominent case of fish die-off along with less extensive cases starting from December 2018. Aktobe reservoir in Aktobe province also possessed a history of one case of fish kill which happened several years ago, specifically, in July 2018. Finally, river Chagan

passes through Uralsk city while lake Balkyldak in Pavlodar province, infamous for mutating fish, accumulates the wastes of mercury from the nearby plant. It can be observed that the water resources are an integral part of human activities and therefore they are affected in every region of Kazakhstan.

**Detection of potentially toxic species of algae in Kazakhstani lakes and rivers with IFC**

Using FlowCAM imaging flow cytometer, the dominant genera of toxic cyanobacteria detected in the Kazakhstani lakes and rivers were *Pseudanabaena limnetica, Cuspidothrix* spp., and *Microcystis aeruginosa*. Minute quantities of *Cylindrospermopsis raciborskii* and *Dolichospermum* spp. were visualised from the samples collected from lakes and rivers around Kazakhstan in autumn 2020. Thus, samples from S1 river Ishim (near Nur-Sultan) were collected in October 2020 at a correspondingly low water temperature at 9-11$^{o}$C, but contained moderate densities of *Dolichospermum* sp. (11 particles/ml) as well as smaller quantities of *Microcystis aeruginosa*, *Cuspidothrix* sp., and *Pseudanabaena limnetica*. Although the temperature of the river Irtysh (S3) dropped to 7$^{o}$C in October 2020, the density of *Cuspidothrix* spp. of 9.27 particles/ml was detected. Lake Parchach (S4), a small lake in the center of Almaty city, fed by the ground waters, was populated by relatively high density (39-77 particles/ml) of colonies of *Microcystis aeruginosa* and low quantities of *Pseudanabaena limnetica*. The highest concentration of 4096 particles of *Pseudanabaena limnetica* and 101 particles of *Cuspidothrix* spp. per ml of water was found in S5 sampling site (river Syr-Darya near Kyzyl-Orda) at a temperature of 17-18$^{o}$C. Other cyanobacterial species such as *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* were also found in the samples from the river. Samples repeated from the river Ural in October 2020 demonstrated the same composition of species *Pseudanabaena limnetica*, *Dolichospermum* spp., *Cuspidothrix* spp., and *Cylindrospermopsis* spp., however, the abundances lowered significantly. The highest density of 168 particles/ml of *Pseudanabaena limnetica*, 16 particles/ml of *Cuspidothrix* spp. and 12 particles/ml of *Dolichospermum* spp. were detected in the Ural. Similar quantities of these species were detected at the artificial channel Peretaska, coming from the Ural river. The tributary of Ural river, river Chagan (S11), contained insignificant amounts of *Microcystis aeruginosa, Pseudanabaena limnetica* and *Dolichospermum* spp. Lake Balkyldak (S12) was inhabited by *Pseudanabaena* spp. in a modest quantity of 16 particles/ml.

Among 12 sampling sites across the territory of Kazakhstan, four samples Fedorov reservoir, River Jigirgen, River Badam and Aktobe reservoir did not contain any traces of potentially

toxic cyanobacteria. The temperature during the time of collection dropped to 10-12ºC in most of the regions, excluding the southern region, where the temperatures reached 15-18ºC. Therefore, the abundance of cyanobacteria was modest compared to the summer season.

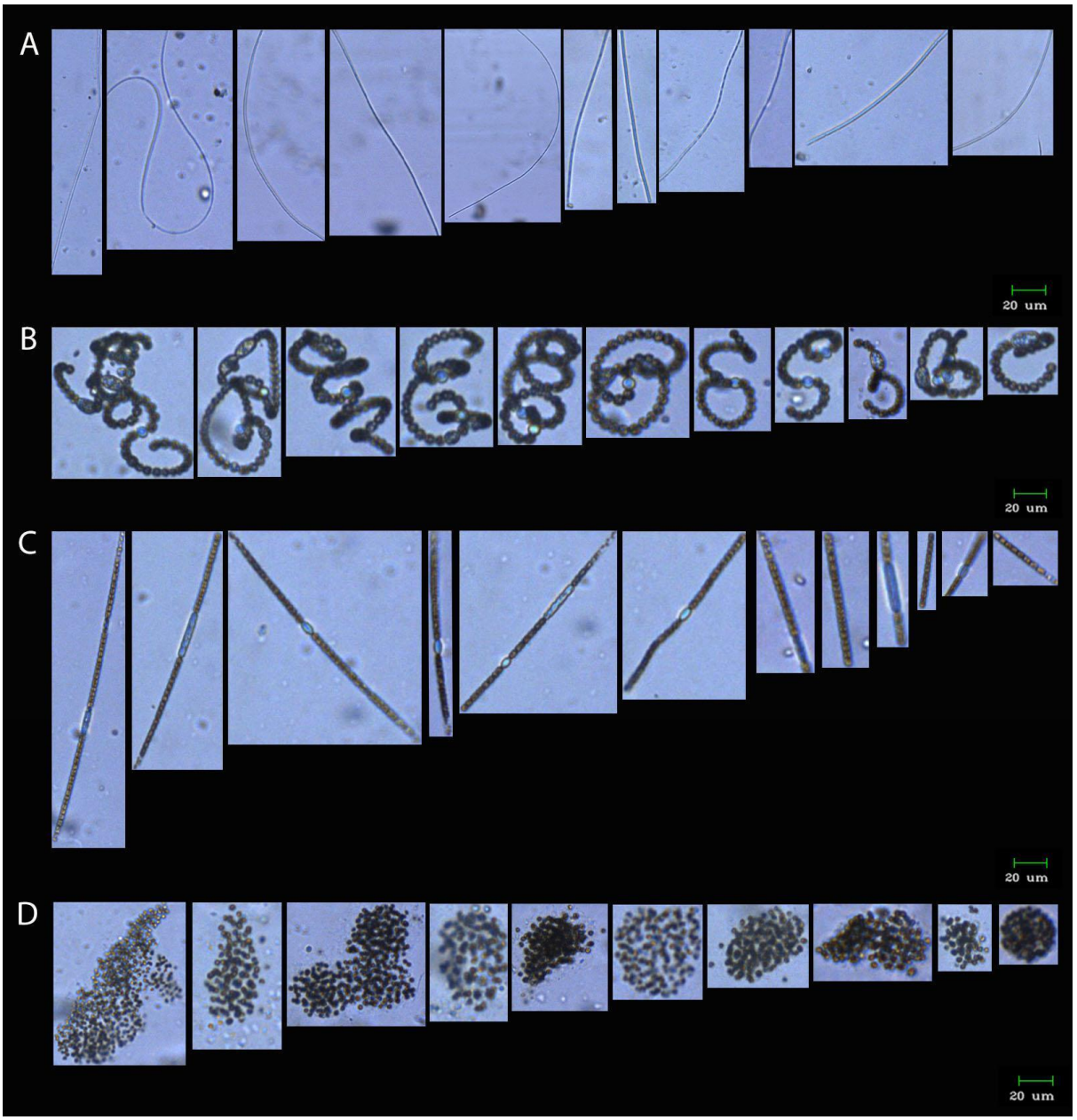


Figure 9. Examples of potentially toxic cyanobacterial species found in different Kazakhstani lakes and rivers recorded by FlowCAM. Fixation with 0.5% paraformaldehyde. Objective 10x and 100 µm flow cell. A - *Pseudanabaena limnetica*, B - *Dolichospermum flos-aquae*, C - *Cuspidothrix issatschenkoi*, D - *Microcystis aeruginosa*. Scale bar 20 µm.

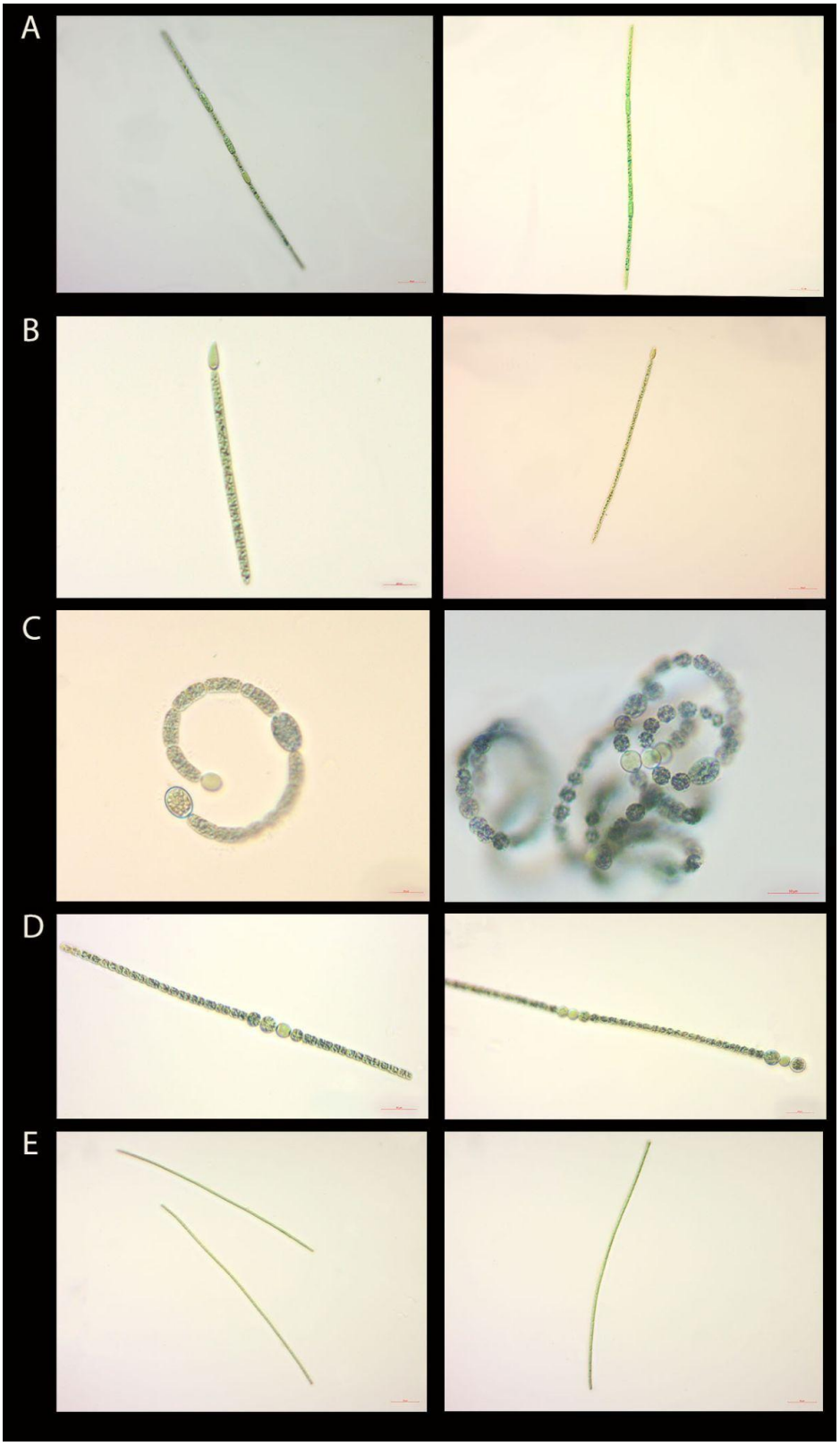


Figure 10. Microscopic images of the identified potentially toxic cyanobacteria in river Ural. Objective 40x. Cells fixed with 1% glutaraldehyde solution. A - *Cuspidothrix issatschenkoi*, B - *Cylindrospermopsis raciborskii*, C - *Dolichospermum flos-aquae*, D - *Sphaerospermopsis aphanizomenoids*, E - *Pseudanabaena limnetica*. Identification to the species level using microscopy. Scale bar 50 µm.

# 5. DISCUSSION

## 5.1 Detection of potentially toxic algae by IFC

In this study, we developed a combined methodology of IFC and PCR-based analysis to detect and monitor the toxic algal blooms, and validated this methodology on the field samples from lakes and rivers of Kazakhstan. In the available literature, there were no published works on detecting and monitoring toxic algal blooms conducted in Kazakhstan. Moreover, publications on the activity of toxic species of algae in Kazakhstani lakes and rivers are also absent. Therefore, the cases of fish death and red tides taking place in national water bodies were not linked to the toxic algae. The only mention of a highly invasive species of cyanobacterium *Cylindrospermopsis raciborskii* in the northern part of the Caspian Sea and the lake Akkul in southern Kazakhstan was reported by Padisak (1997). As it is known, *Cylindrospermopsis raciborskii* produces a hepatotoxin cylindrospermopsin, which was responsible for an epidemic of hepatoenteritis on the Palm Islands in Australia in 1979 (Griffiths & Saker, 2003). Besides Padisak's review, phytoplankton composition in different Kazakhstani lakes was investigated by Israeli scientist Barinova and co-authors (2011, 2018). Water blooming of *Anabaena flos-aquae, Anabaena constricta, Microcystis aeruginosa* was reported only in lake Chebachiye, Burabai National Park by Nurashov & Sametova, 2015.. Thus, the present work is the first study on the detection and monitoring of potentially toxic algae in relation to fish kills in Kazakhstan. The development of methodology to detect and identify toxic algae could foster implementation of effective measures for mitigation of the negative consequences of HABs in Kazakhstan. Worldwide, the practice of HAB detection takes place using traditional light microscopy as an imaging and quantitation method (Haellegraf et al., 2004) Taking into account how laborious the counting using microscopy is, scientists were looking for high-throughput methods like cytometry. Thus, with the premises of using flow cytometry for phytoplankton research in 1980-1990s, automated imaging flow cytometry was firstly used for recognition of natural communities of red tide dinoflagellate *Karenia brevis* in the beginning of 2000s (Legendre et al., 2001; Buskey & Hyatt, 2006). 5-70% of unspecific cells were processed as target cells of natural communities of *Karenia brevis*. This showed that automated algorithms of FlowCAM often should be re-checked manually as was implemented in the present study. Then, in 2010, the first harmful *Dinophysis* bloom (dinoflagellate) was detected by imaging flow cytometer FlowCytobot. It is notable that the instrument, aimed at monitoring a bloom of *Karenia brevis* in the Texas

coastal waters, helped to detect a new-forming bloom of *Dinophysis* cf. *ovum* (Campbell et al., 2013). Additionally, the source of blooming, cell life cycle and grazing frequencies and other biological factors that affect the run of the blooming could be observed by imaging flow cytometer (Campbell et al., 2010). In the present study, the cell abundance data indicated the potential sources of the blooming as the peaks in cell density were visible among sampling sites. Looking at the advantages the imaging flow cytometers provide over microscopy, the technology is still not widespread in the environmental research laboratories around the world. It was believed a decade ago that IFC does not provide adequate imaging which allows to recognize the organisms to the genus and species level. However, Olson & Sosik (2007) demonstrated that quality of the images combined with the fluorescence and light scattering data, provided by IFC, substantially promotes taxonomic resolution. Moreover, new generation of IFC instruments increased their magnification to the one close to microscopy (Yokogawa Fluid Imaging Technologies). Equipped with 10x and 20x objective, imagery of FlowCAM in this work visualised filaments with a sufficient resolution to recognize the morphological features and allow partial identification of species. Complete identification of species could be confirmed by microscopy. In summary, IFC provided fast and high-content analysis of phytoplankton species living in the Ural river and other Kazakhstani lakes and rivers, which can be used for detection and identification of algal bloom togther with PCR-based and traditional technologies

**5.2 Blooming of potentially toxic cyanobacteria in Ural river**

Approximately 4 tonnes of natural fish and more than 100 tonnes of farmed fish died in the massive fish kill in December 2018 in Ural river (informburo.kz) The first case of fish kill was encountered in the artificial shallow channel Peretaska. We undertook an expedition in 2019 six months later after March 2019 fish kill at Ural river. However, in September 2019 not only the visual appearance of water in Peretaska showed intensive growth of algae, but also abundances of all species of potentially toxic cyanobacteria identified in the river Ural were visibly increased comparing with river Ural mainstream. According to Figure 5, the background level of abundance of *Dolichospermum* spp., *Cuspidothrix* spp., and *Pseudanabaena limnetica* at the sampling sites T1-T5 and T7-T9 were under 50 filaments/ml whereas cyanobacterial abundance at T6 Peretaska was 3-folds higher. Accordingly, the temperature in the channel remained similarly high in summer and winter seasons (26$^{o}$C). Possibly, the temperature anomaly was linked to the thermal discharge of the plants that

withdraw water for cooling systems from the channel as illustrated on Figure 10. It is known that power plant discharges influence the phytoplankton mortalities near the discharge places and within the cooling system tubes (Briand, 1975). However, the rising temperature near the discharge places can also have a short-lived effect of excessive growth of phytoplankton (Ye et al., 2019).

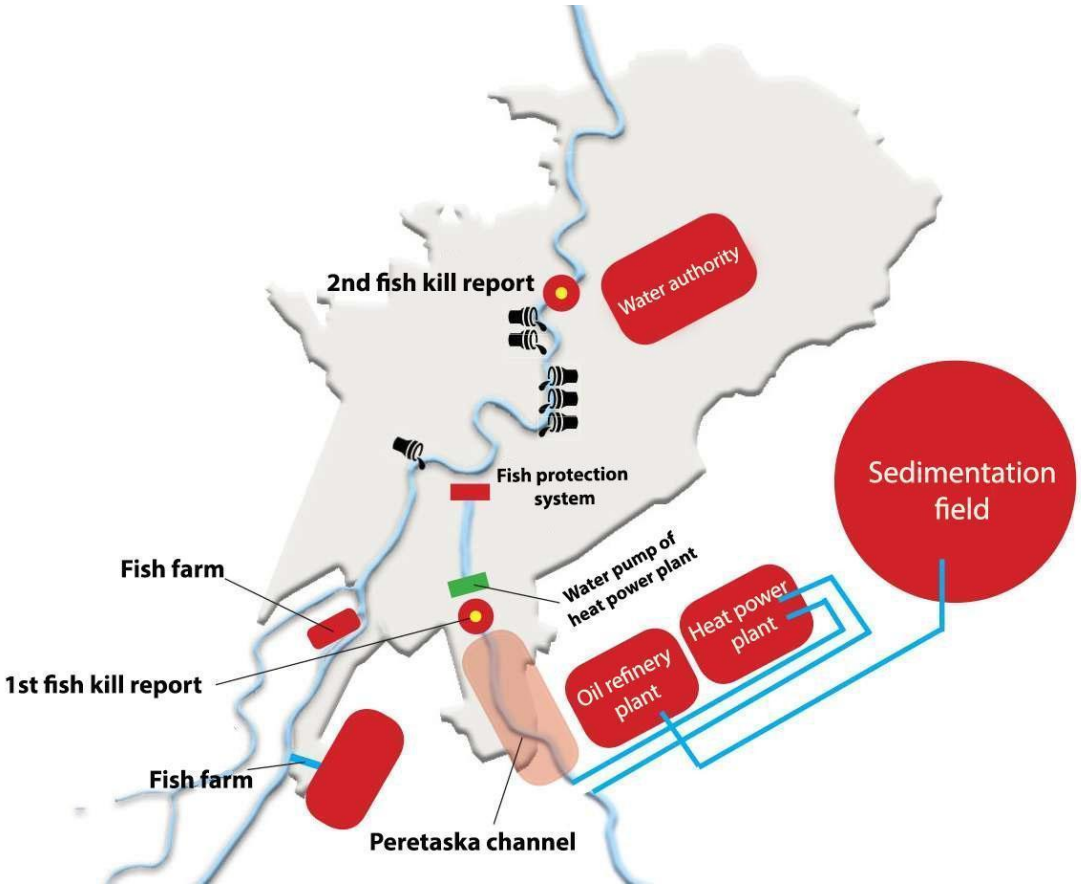


Figure 11. Schematic diagram of the Peretaska channel, stemming from the Ural river. The first fish kill report was announced in the beginning of the Peretaska channel. Next, fish kill was reported in the Ural river. The diagram demonstrates locations of nearby buildings and entities.

The level of nitrates and phosphates were higher in the channel Peretaska than in the river, though a substantial surpass in the concentration was not detected. The level of oxygen was significantly reduced in the channel, reaching 3.7 mg/L. As it is known, long-lasting blooms can be harmful to aquaculture due to depleted dissolved oxygen level, which is vital for fish and other biota. The aquatic system of the channel is likely to be influenced by multiple abiotic factors which affect the growth of algae, therefore, strict control of the water quality in the channel should be advocated for the prevention of the negative effects of excessive growth of algae.

A highly invasive species *Cylindrospermopsis raciborskii* was reported by Ussachev (1938, cited by Padisak, 1997) in the northern part of Caspian Sea. Present study revealed distribution of this species along the entire study area in the river Ural. However, cylindrospermopsin encoding genes using primers indicated in Table 1 were not detected in the samples.

Blooming of potentially toxic cyanobacteria was also visible upstream the river at the sampling sites T10-T14. The highest abundances of potentially toxic species were found at the sampling sites T10 Almaly, T11 Saraishyk and T14 100 km ranged from as low as 123 particles/ml to as high as 885 particles/ml while background level of abundance was near 50 particles/ml. Chlorophyll-a measurements ranged from 36 to 53 $\mu g/L$. It was established by WHO (2003) that the concentration of 20 000-100 000 cells/ml (10-50 μg/L chlorophyll-a) is considered as a HAB which can bring from mild to moderate health risks. The concentrations of potentially toxic cyanobacteria in the river Ural did not achieve those values due to a fall-winter season, and we can only hypothesize a possible link to fish kills in the area in 2018-2019. Still, it is argued that fish mortalities can be caused by exceedingly toxic microalgae at low concentrations (Berdalet et al., 2015).

### 5.3 PCR-based identification of toxin-producing genotype strains in Ural river

We encountered *Microcystis aeruginosa* at the sampling site T12 Low Mahambet. The presence of the toxin-encoding genes *mcyE*, responsible for *Microcystis*-specific microcystin synthetase expression, was found at the sampling sites T1 Delta and T12 Low Mahambet. The comparative analysis among these two sampling sites indicated higher abundance in the T1 Delta by IFC. Comparatively, the biomass parameter (chlorophyll-a) was measured to be exceptionally high at T12 Low Mahambet (172 $\mu g/L$) and 10 times lower at T1 Delta (17 $\mu g/L$). The toxin gene concentration can be reciprocal with biomass, its relationships can be ambiguous in the environmental sample, which are usually dominated by non-toxic species of organisms (Ngwa et al., 2014). Still, no *Microcystis* spp. was encountered in the estuary of the river Ural. Sanger sequencing data was analysed through BLAST and matched for homology sequences in other organisms. The data on microcystin encoding gene presence was supported by Sanger sequencing results (Table 3).

From the results of RT-PCR, possible toxicity of the identified cyanobacteria in the river Ural can be assumed. Combination of PCR and RT-PCR provided a qualitative and partially

quantitative assessment of the toxicity of potentially toxic cyanobacterial species. The methodology was comparatively unchallenging and inexpensive if juxtaposed with widely used methods for toxin identification like HPLC (High Performance Liquid Chromatography), Mass Spectrometry (MS) and ELISA (enzyme-linked immunosorbent assay) (Zhang & Zhang, 2014). For a majority of the laboratories around the world, supply of these expensive instruments and reagents is not available. Therefore, RT-PCR analysis is a convenient alternative for detection of the toxin-producing species of algae in the blooms.

### 5.4 Potentially toxic cyanobacteria in Kazakhstani lakes and rivers

Freshwater resources are exceptionally vital in Kazakhstan since the climatic features are prone to aridity, especially in the southern regions, with no access to the ocean. Some of the research areas such as River Irtysh, River Ural, River Syr-Darya, and River Ishim play essential roles in maintaining drinking water, irrigation for agriculture, farming and fish farming, industrial treatment, energy generation, and other purposes. Therefore, protection and monitoring of water quality in Kazakhstan is a prioritized responsibility. During the last year, 14 cases of fish kills could be encountered on the media sources (24.kz). The cases are mostly explained by oxygen depletion. However, possible causation of oxygen depletion by excessive growth of algae and toxic cyanobacteria is usually disregarded, therefore, monitoring of the phytoplankton communities is absent. Development of methodologies for monitoring and successful detection of HABs in Kazakhstan may significantly foster ecological protection of freshwater resources and potentially enhance water quality.

In the present study, when the methodology was transferred to a larger number of lakes and rivers, we could detect similar species of potentially toxic cyanobacteria in the lakes Parchach, Balkyldak and rivers Irtysh, Syr-Darya, Ishim, Ural and its tributary Chagan. Since study areas included freshwater sources, cyanobacterial species were tolerant to such habitats. Cyanobacterial blooms, expanding around the world, put a great threat to the diversity and integrity of the aquatic system of freshwater reservoirs. Unfortunately, world examples of resource rich water reservoirs which were invaded by cyanoHABs like Lake Michigan and Lake Erie on the border of Canada and USA, Baltic Sea in northern Europe, Lake Victoria and River Nile in Africa, Barwon-Darling river in Australia are numerous (Paerl & Otten, 2013). That is why it is not remarkable that the most common potentially toxic species *Pseudanabaena limnetica* was detected in each sample from Kazakhstani lakes and rivers in different concentrations. Specifically, the organism was the most abundant in the Syr-Darya

river (4000 cells/ml) and scarce in river Irtysh (3 cell/ml). It is commonly considered that Pseudanabaena spp. are rarely recognized as a dominant bloom-forming genus (Acinas et al., 2008). However, Gao and co-authors (2018) reported superior tolerance of *Pseudanabaena* spp. to low light, disturbance and phosphorus deficiency along with preferences for warm temperature and nitrogen supply, which may be a reason for its contribution and even dominance in the cyanobacterial blooms. It can be observed that the rivers passing through the main cities (Irtysh, Ishim, Ural, Syr-Darya) are more influenced by urban, agricultural and industrial run-offs and consequently are more populated by potentially toxic cyanobacteria. Smaller rivers like River Jigirgen and River Badam did not contain the traces of potentially toxic cyanobacteria, except River Chagan which is a tributary of river Ural and may obtain a small fraction of potentially toxic species with the river flow. The artificial reservoirs (Fedorov reservoir, Aktobe reservoir) were not affected by potentially toxic cyanobacteria. Lakes Parchach and Lake Balkyldak are tiny, low-water lakes with modest fish resources, which encountered a moderate density of potentially toxic cyanobacteria. Lake Parchach appeared to be abundant in *Microcystis aeruginosa* colonies, which could be possibly associated with a recent fish kill in August 2020. It is reported that lake Balkyldak is highly polluted with industrial wastes from the heavy industry plants of Pavlodar region, which induced mutations and lesions in fish (inform.kz). Cyanobacterium *Pseudanabaena limnetica* could adapt to these conditions.

### 5.5 Statistical analysis

The number of samples used for statistical analysis in river Ural was 14. Due to a small sample size, no statistically significant relationships could be identified between biological and environmental data obtained in the Ural river. The interpretations demonstrated that abundance was not affected by any of the environmental parameters.

The number of samples for statistical analysis in Kazakhstani lakes and rivers was ranging from two to four which matched the number of weeks of sample collections conducted at the sampling sites. The parameters temperature and pH (Litmus paper strips) were measured for 11 sampling sites. No statistically significant correlation could be observed from the data available.

Ideally, an expedition should be planned during a seasonal bloom of cyanobacteria: July-August. However, the current sampling is overlapping in time with a winter fish kill on the Ural river (December 2018-March 2018). Ideally, to develop mitigation measures against

possible toxic blooms, it is a future direction for this research to conduct an expedition during a seasonal bloom (July-August) and collecting environmental parameters which potentially correlate with the growth of potentially toxic cyanobacteria.

The Aims of the Thesis did not include finding correlations between environmental factors and cyanobacteria diversity and abundances.

### 5.6 Limitations of the study

The limitations of this study are partly explained by issues related to COVID-2019 epidemics. Our initial plan was to repeat field expeditions in Spring-Summer 2021. It was impossible, though another small fish-kill happened in April 2021 on the other channel of River Ural.

We were able to restart field work only in September 2020, and have to use volunteer help. Moreover, sampling of water during the initial expedition to river Ural happened six months later than the incidence of a fish kill (December 2018-March 2019). Therefore, we can not correlate a fish kill in 2018-2019 to the presence of potentially toxic species, though a cyclic nature of fish kills on river Ural (2018 December-2019 March, April 2020) and coincidence with places of fish kills and detection of gene-toxic cyanobacteria suggest this relationship. Secondly, although we could identify potentially toxic cyanobacteria, and even detect microcystin-producing genes in algal samples from the river Ural, we could not utilize the methods for full quantification of the genes and species. RT-PCR-based analysis is helpful in identification of species and enumeration of organisms responsible for toxic gene expression. However, lack of control of a known number of copies of microcystin-encoding genes was a limitation in this study making real-time PCR results semi-quantitative (no absolute gene copies numbers but possibility to compare different samples).

In addition, a limited number of the samples and decreasing temperatures in the fall did not allow us to observe the dynamics of cyanobacterial blooms. It could be observed that the abundance of the samples collected in fall were lowering.

To sum it up, using IFC as methodology for detection of toxic cyanobacteria, we identified five species of potentially toxic species of cyanobacteria. Combining the method with RT-PCR molecular analysis, we confirmed the presence of potentially toxic cyanobacteria in the river Ural. Toxicity of cyanobacteria species detected in other lakes and rivers around Kazakhstan has to be validated.

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# 7. APPENDICES

Appendix 1. Abundance of potentially toxic species of cyanobacteria in the river Ural detected in August 2019 (phytoplankton live samples).

| Sampling site | Dolichospermum (particles/ml) | Cylindrospermopsis (particles/ml) | Sphaerospermopsis (particles/ml) | Cuspidothrix (particles/ml) | Pseudanabaena (particles/ml) | Microcystis (particles/ml) |
|---|---|---|---|---|---|---|
| T1 Delta | 2.81 | 6.96 | 0.62 | 2.39 | 2.27 | 0 |
| T2 Channel | 36 | 6.03 | 0.2 | 18 | 50 | 0 |
| T3 Korabl | 6.08 | 2.84 | 1.32 | 21 | 15 | 0 |
| T4 Low Peretaska | 17 | 2.11 | 0.7 | 14 | 7.53 | 0 |
| T5 Balykshy | 6.96 | 0.21 | 1.04 | 14 | 5.09 | 0 |
| T6 Peretaska | 79 | 15 | 6.62 | 198 | 199 | 0 |
| T7 Oil Refinery Water Intake | 8.32 | 3.71 | 2.31 | 61 | 143 | 0 |
| T8 Water authority | 0.4 | 0.8 | 0.1 | 10 | 14 | 0 |
| T9 Geolog | 16 | 6.82 | 2.1 | 123 | 79 | 0 |
| T10 Almaly | 134 | 47 | 12 | 634 | 390 | 0 |
| T11 Saraishyk | 106 | 32 | 20 | 885 | 217 | 0 |
| T12 Low Makhambet | 1.31 | 3.43 | 0.71 | 204 | 27 | 11 |
| T13 High Makhambet | 36 | 14 | 17 | 112 | 31 | 0 |
| T14 100 km | 90 | 17 | 14 | 510 | 127 | 0 |

Appendix 2. Abundance of potentially toxic species of cyanobacteria in the river Ural detected in August 2019 (fixed with 0.5% PF phytoplankton samples).

| Sampling site | Dolichospermum (particles/ml) | Cylindrospermopsis (particles/ml) | Sphaerospermopsis (particles/ml) | Cuspidothrix (particles/ml) | Pseudanabaena (particles/ml) | Microcystis (particles/ml) |
|---|---|---|---|---|---|---|
| T1 Delta | 0 | 0 | 0 | 0 | 0 | 0 |
| T2 Channel | 7.45 | 0 | 0 | 1.41 | 0.7 | 0 |
| T3 Korabl | 101 | 0.2 | 1.4 | 7.33 | 12 | 0 |
| T4 Low Peretaska | 9.54 | 0 | 0.4 | 2.41 | 1.2 | 0 |
| T5 Balykshy | 188 | 0.6 | 4.52 | 66 | 44 | 0 |
| T6 Peretaska | 117 | 0.71 | 2.53 | 32 | 17 | 2.84 |
| T7 Oil Ferinery Water Intake | 95 | 2.11 | 0.9 | 32 | 38 | 0 |
| T8 Water authority | 95 | 1.4 | 2.21 | 52 | 56 | 0 |
| T9 Geolog | 109 | 2.1 | 3.03 | 65 | 57 | 0 |
| T10 Almaly | 102 | 0.5 | 2.72 | 103 | 27 | 0 |
| T11 Saraishyk | 44 | 0.5 | 2.71 | 39 | 13 | 0 |
| T12 Low Makhambet | 11 | 4 | 0.2 | 167 | 130 | 2.61 |
| T13 High Makhambet | 83 | 3 | 10 | 120 | 0.1 | |
| T14 100 km | 93 | 4.41 | 7.92 | 105 | 17 | 0 |

Appendix 3. Abundance of potentially toxic species of cyanobacteria in the lake Parchach, Almaty detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Microcystis(particles/ml) | Pseudanabaena(Particles/ml) |
|---|---|---|
| Almaty Parchach 26.09.2020 | 61 | 9.25 |
| Almaty Parchach 01.10.2020 | 39 | 9.8 |
| Almaty Parchach 04.10.2020 | 77 | 3.9 |
| Almaty Parchach 08.10.2020 | 42 | 1.32 |

Appendix 4. Abundance of potentially toxic species of cyanobacteria in the river Ishim, Nur-Sultan detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Microcystis(particles/ml) | Pseudanabaena(Particles/ml) | Aphanizomenon(part/ml) | Dolichospermum (part/ml) |
|---|---|---|---|---|
| Astana Ishim 03.10.2020 | 0 | 5.29 | 0 | 7.92 |
| Astana Ishim 10.10.2020 | 0 | 9.27 | 0 | 2.65 |
| Astana Ishim 19.10.2020 | 0 | 3.97 | 5.3 | 11 |
| Astana Ishim 26.10.2020 | 4.02 | 6.71 | 0 | 2.68 |

Appendix 5. Abundance of potentially toxic species of cyanobacteria in the river Irtysh, East Kazakhstan detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Microcystis(particles/ml) | Pseudanabaena (particles/ml) | Aphanizomenon (particles/ml) | Dolichospermum (particles/ml) |
|---|---|---|---|---|
| Semey Irtysh 26.09.2020 | 2.65 | 3.98 | 3.9 | 0 |
| Semey Irtysh 05.10.2020 | 0 | 0 | 9.27 | 0 |

Appendix 6. Abundance of potentially toxic species of cyanobacteria in the river Ural, Atyrau detected in Fall 2020 (fixed with 0.5% PF communities)

| Sample Name | Pseudanabaena (particles/ml) | Aphanizomenon (particles/ml) ▲ | Dolichospermum (particles/ml) | Cylindrospermopsis |
|---|---|---|---|---|
| Atyrau Ural 02.10.2020 | 5.3 | 0 | 0 | 0 |
| Atyrau Ural 11.10.2020 | 36 | 0 | 0 | 0 |
| Atyrau Ural 25.09.2020 | 26 | 0 | 0 | 0 |
| Atyrau Ural 18.09.2020 | 168 | 16 | 12 | 2.64 |

Appendix 7. Abundance of potentially toxic species of cyanobacteria in the Peretaska channel coming out of Ural river detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Microcystis (particles/ml) ▼ | Pseudanabaena (particles/ml) | Aphanizomenon (particles/ml) | Dolichospermum (particles/ml) |
|---|---|---|---|---|
| Atyrau Peretaska 11.10.2020 | 23 | 0 | 0 | 0 |
| Atyrau Peretaska 18.09.2020 | 15.6 | 1.7 | 8.8 | 0.5 |
| Atyrau Peretaska 02.10.2020 | 0 | 0 | 0 | 0 |
| Atyrau Peretaska 25.09.2020 | 0 | 0 | 0 | 0 |

Appendix 8. Abundance of potentially toxic species of cyanobacteria in the lake Bakpyldak, Pavlodar detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Pseudanabaena (particles/ml) |
|---|---|
| Pavlodar Bakpyldak 10.10.2020 | 12 |
| Pavlodar Bakpyldak 17.10.2020 | 17 |

Appendix 9. Abundance of potentially toxic species of cyanobacteria in the river Chagan, Uralsk detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Microcystis(particles/ml) | Pseudanabaena(Particles/ml) | Dolichospermum (part/ml) |
|---|---|---|---|
| Uralsk Chagan 03.10.2020 | 0 | 15 | 0 |
| Uralsk Chagan 11.10.2020 | 0 | 20 | 0 |
| Uralsk Chagan 26.09.2020 | 0 | 0 | 0 |
| Uralsk Chagan 19.10.2020 | 0.1 | 2.51 | 0.3 |

Appendix 10. Abundance of potentially toxic species of cyanobacteria in the river Syr-Darya detected in Fall 2020 (fixed with 0.5% PF samples)

| Sample Name | Microcystis(particles/ml) | Pseudanabaena (particles/ml) | Aphanizomenon (particles/ml) |
|---|---|---|---|
| Kyzylorda Syr-Darya 03.10.2020 | 5.28 | 562 | 101 |
| Kyzylorda Syr-Darya 03.10.2020 | 2.64 | 202 | 56 |
| Kyzylorda Syr-Darya 18.10.2020 | 3.96 | 4096 | 66 |

Appendix 11. Ionic composition of water in the river Ural in August 2019.