NM WRRI Student Water Research Grant Final Report Form

Progress Report due October 1, 2020
Draft Final Report due April 30, 2021
Final Report due May 31, 2021

1. Student Researcher: **Sergei Shalygin**
   Faculty Advisor: **F. Omar Holguin**

2. Project title: **Assessment of the cyanobacterial Harmful Algal Blooms (cHABs) and toxins in the blooming water bodies of New Mexico**

3. Description of research problem and research objectives.

Scientific questions related to water quality and drinking waters has become more and more urgent in the age of expensive industry and agriculture, worldwide. Nutrient load from the fields and waste waters from industry along with Climate change are sculpturing natural equilibrium in the water ecosystem. This global trend has become vivid in the State of New Mexico with past summer mysterious shut down of several recreational lakes. Cyanobacterial Harmful Blooms (cHABs) is a recent ecological problem in the lakes and rivers of the USA (Meyer et al. 2017, Kramer et al. 2018) and globally (Sciuto et al. 2015, Sendall and McGregor 2018). Toxins produced during cHABs (and subsequently released to the water column) causes human death (Blaha et al. 2009, Svirčev et al. 2019) and animal mortality (Trevino-Garrison et al. 2015) . Potent cyanobacterial toxin - microcystin causes fatal liver damage in humans (Carmichael 1994). Recently described cyanobacterial genus Aetokthonos is responsible for the death of Bald Eagles, due to toxin production (Wilde et al. 2014). In the water bodies of the State of New Mexico, several cHABs were detected in summer 2019 (Abiquiu and Cochiti Lakes were shut down because of large coastal Microcystis blooms, https://www.youtube.com/watch?v=TcnnvniF5ng), and scientist associated pet's mortality with these events (https://www.ewg.org/key-issues/water/toxicalgae). However, information concerning cHABs in the state of NM is inadequate and in need of development. The State of New Mexico is a center of agriculture and our desert climate requires intensive irrigation, which is largely surface irrigation from water systems like the Rio Grande river and other open water bodies such as lakes and reservoirs. Agricultural nutrient load, together with high summer temperatures, are known factors of cHABs development. These factors suggest a need for extensive monitoring of irrigation and recreational water bodies within the State of New Mexico.
We are proposing the following aims for this research:
Aim 1. Biomonitoring of endogenous cyanobacterial toxic species in the water system of the Rio Grande.
Macroscopic biomass of the cHABs will be collected and analyzed phenotypically under a microscope. At the same time, genomic DNA will be extracted, and it will be sent for Next-generation sequencing of the toxin-producing genes. Biomass and water quality parameters (pH, temp, alkalinity, N, P) will also be quantified.
Aim 2. Detection of the cyanobacterial toxins with mass spectrometry from both water samples and biomass.
It is possible that blooms will not be visible; we will be targeting biomarkers from the water samples for mass spectrometry. Both toxins and bioactive compounds from the blooms and the

water column will be concentrated and extracted and analyzed, utilizing high accuracy mass spectrometry.

4. Description of methodology employed.

Samples of water/biomass were collected in the Rio Grande water system (Caballo reservoir and Elephant butte Lake) and in the following lakes: Abiquiu Lake and Snow Lake; twice – July-August 2020 and October 2020 (Fig. 1). Each lake has 3 sample sites in the shallow parts of the lakes. EPA Cyan app was utilized to localize potential cyanobacterial blooms within the lakes (Fig. 2). Morphological identification of the dominant species of cyanobacteria in the cHABs was carrying out using light microscopy with the DIC contrast within 24 hours after samples were collected. Pictures of the visible colonies and microcolonies of observed taxa was captured with a digital camera and shared in the publicly available database CRIS (http://kpabg.ru/cris/?q=node/16). Some particular blooms was used for the confocal microscopy experiments aiming chlorophyll detecting and localization of pigments in the cells. Toxins/bio-active compounds was extracted with mass spectrometer grade acetonitrile prior screening on the tandem mass spectrometer. MRM (Multiple Reaction Method) method was used for the initial screening of major toxins. MasslynX 4.1. software was used chromatogram visualization. Samples ran on Positive mode according to Waters application mode, together with standard for multiple microcystin variants (Degryse et al. 2017).
DNA was extracted using DNeasy UltraClean Microbial Kit (Cat ID: 12224-50, QIAGEN, Venlo, Netherlands). The 16S rRNA gene PCR primers 27F/1492R with barcode on the forward primer were used in a 35-cycle PCR (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 90 seconds, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products are checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples are pooled together in equal proportions based on their molecular weight and DNA concentrations. The PCR pool is then purified using Ampure PB beads (Pacific Biosciences).
The SMRTbell libraries (Pacific Biosciences) are prepared following the manufacturer's user guide and sequencing performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on the PacBio Sequel following the manufacturer's guidelines. After completion of initial DNA sequencing, each library undergoes a secondary analysis, Circular Consensus Sequencing, using PacBio's CCS algorithm. The CCS algorithm aligns the subreads individually from each template to generate consensus sequences thereby correcting the stochastic errors generated in the initial analysis. Sequence data is then processed using the MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, The CCS sequencing data is depleted of barcodes, oriented 5' to 3', sequences <150bp removed, and sequences with ambiguous base calls removed. Sequences were denoised, OTUs generated and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated database derived from and NCBI (www.ncbi.nlm.nih.gov).

5. Description of results; include findings, conclusions, and recommendations for further research.

Based on the morphological observation potentially toxic cyanobacteria such as *Microcystis* spp. (Fig. 3, 4), *Aphanizomenon* spp. (Fig.5), and *Dolichospermum* spp, were found in all samples collected from different reservoirs (for more details, see Table 1). Exception was Elephant Butte Lake, which was a test object and eventually was removed from analysis (Because of lack of the visible cyanobacterial bloom). Next-Generation molecular analysis (PacBio) detected potentially toxic cyanobacterial species in Elephant Butte Lake in contrast to morphological observation. Overall, morphological analysis was accurate and similar to PacBio analysis; however, PacBio revealed more species, being more sensitive (Table 2). PacBio analysis showed general distribution of major bacterial phyla in the samples (Fig.6). Highest relative abundance of the total cyanobacteria have been found in Snow Lake [Sample 1] and in Abiquiu Lake in both seasons (Summer/Fall). In the rest of the samples (Snow Lake 2, 3 and Caballo Lake), cyanobacteria were more frequent in the fall; however species composition dramatically shifted from *Aphanizomenon*/*Dolichospermum* prevalence to *Microcystis* spp. domination. Exception was Caballo Lake where *Microcystis* spp. dominating community changed to community with *Limnothrix* and *Pseudanabaena*. It is quite surprising that in about 2 months, bacterial communities changed significantly. Even though *Aphanizomenon* spp. could potentially produce microcystins (detected toxins in this study), communities with the presence of *Microcystis* spp. were actually producing the microcystins. Following lakes were found to contain different microcystin derivatives: Caballo Lake (both water and biomass), Abiquiu Lake (biomass), and Snow lake (mostly biomass). Important to notice that only in fall season Snow Lake showed presence of the toxins. The fact that toxins were not realized into the water may be interpreted as follows: since there are not many predators are alive during cold season, cyanobacterial communities were synthesizing toxins to release them in future to compete with more developed predators (in the spring and summer). Nutrient analysis showed low Nitrogen and low N/P ratios with are typical for cyanobacterial communities (Figure 7, 8). Low nutrient levels are not preventing heterocytous cyanobacteria (they may fix atmospheric nitrogen) such as *Aphanizomenon* spp., and *Dolichospermum* spp. to develop. Question is why *Microsystis* spp. are well developed in oligotrophic ecosystems? More research needed to answer that question. Nutrient analysis did not allow separating major driver of the cHAB. As a conclusion, following New Mexican water bodies: Caballo Lake, Snow Lake, and Abequiu Lake sustained toxic blooms in summer/fall 2020. Several derivatives of microcystin was detected using mass spectrometry: MC-LR, MC-RR, MC-YR, MC-LY (Fig.9). Estimation of the biomass and quantification of the toxin's concentration still needs to be accomplished. After that materials obtained in this research will be published in recognized scientific journal.

6. Provide a paragraph on who will benefit from your research results. Include any water agency that could use your results.

EPA and New Mexico Environment Department will surely use this information if published.

7. Describe how you have spent your grant funds. Also provide your budget balance and how you will use any remaining funds. If you anticipate any funds remaining after May 31, 2021, please contact Carolina Mijares immediately. (575-646-7991; mijares@nmsu.edu)

Purchased items:
MC-LR (Toxin standard) SIGMA ALDRICH US 2O2OO7O9, Chl A (Chlorophyll standard) SIGMA ALDRICH US 2O2OO7O8 and other standards – $ **1652.39**
Lamp for cyanobacterial isolation – $ **12.35**
DNA analysis – $ **4025.00**
Domestic travel –$ **1283.52**
Freight – $ **50.88**
TOTAL SPENT $ **7024.14**

8. List presentations you have made related to the project.

65th Annual NM Water Conference

9. List publications or reports, if any, that you are preparing. For all publications/reports and posters resulting from this award, please attribute the funding to NM WRRI and the New Mexico State Legislature by including the account number: NMWRRI-SG-2020.

Progress report

10. List any other students or faculty members who have assisted you with your project.

Dr. Jackie Jarvis helped with UPLC/MS. Dr. Peter Cooke helped with confocal microscopy.

Barbara Hunter performed nutrient analysis.

11. Provide special recognition awards or notable achievements as a result of the research including any publicity such as newspaper articles, or similar.

N/A

12. Provide information on degree completion and future career plans. Funding for student grants comes from the New Mexico Legislature and legislators are interested in whether recipients of these grants go on to complete academic degrees and work in a water-related field in New Mexico or elsewhere.

About 90% of project is accomplished. I am going to pursue carrier in academia on projects related to water quality.

Figure 1. Map of the sample sites

Figure 2. Screenshot of the EPA CYAN application, showing potential cHAB in the Caballo Lake, bottom right side (green color)

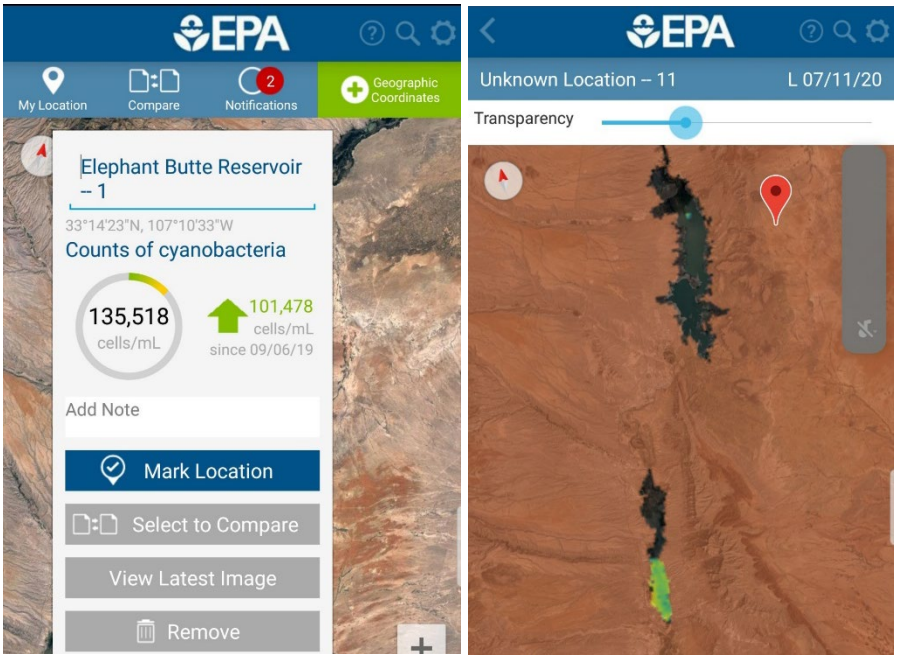


Figure 3. Macroscopic bloom (toxic) with one of the dominant species *Microcystis aeruginosa* found in Caballo Lake.

Figure 4. Microscopic photograph of potentially toxic cyanobacterium *Microcystis aeruginosa*.

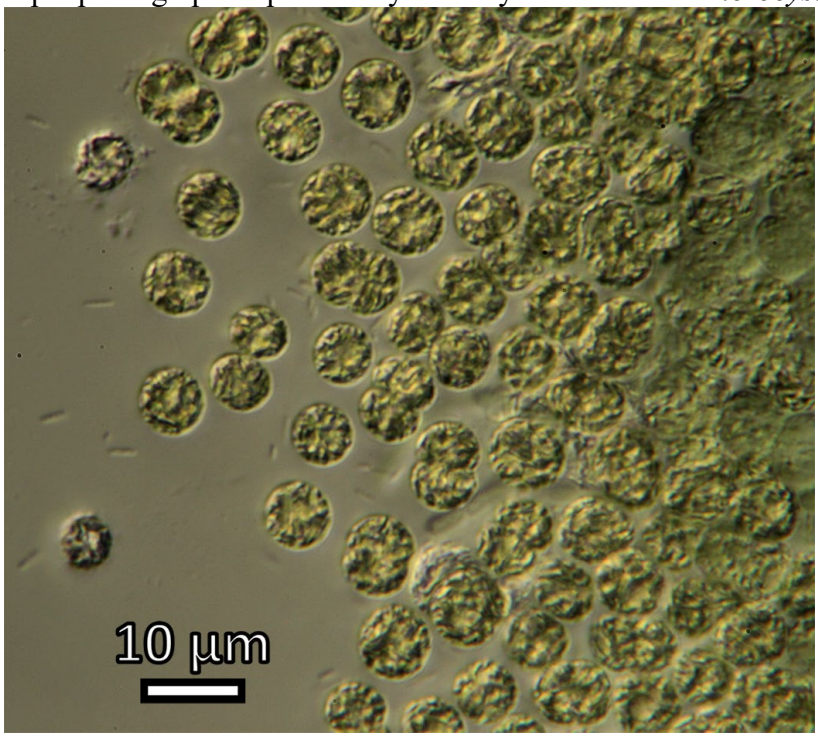


Figure 5. Confocal image of the filaments of the *Aphanizomenon flos-aquae* from Snow Lake, chlorophyll A and phycobilins is depicted with red color.

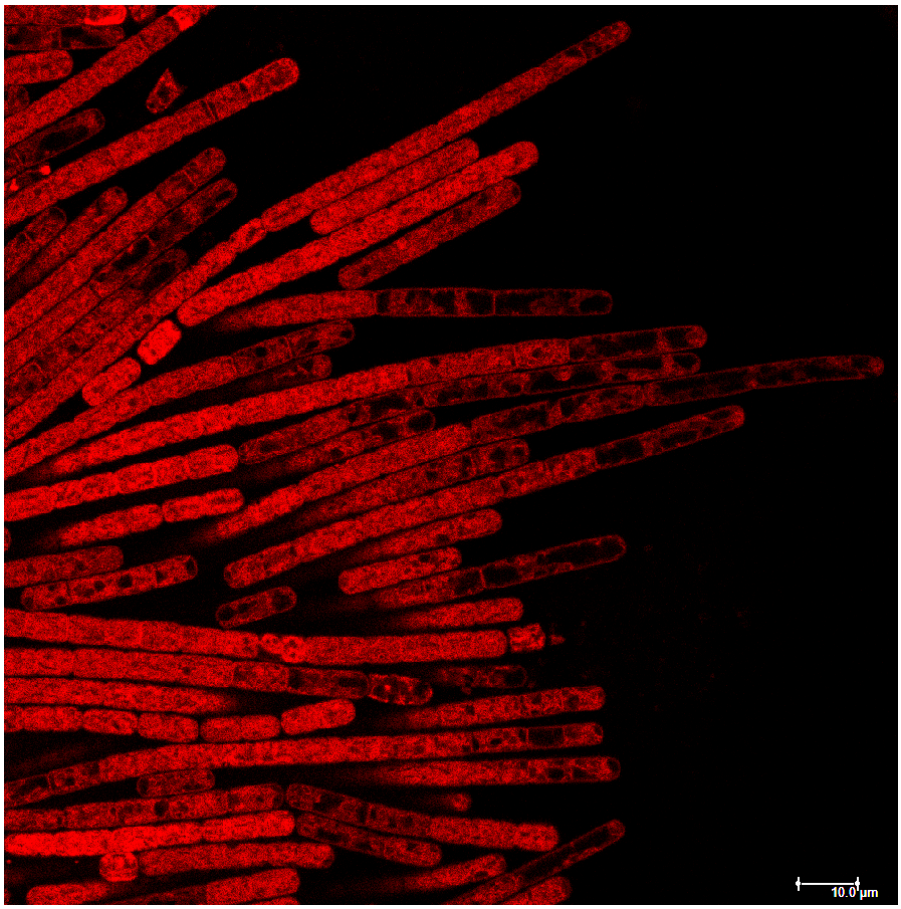


Figure 6. Bar plot of the relative abundance of major bacterial orders. Taxonomic groups that were outside of the scope of research were grouped under the "others" category. The 5 most abundant orders across all samples are displayed in the legend. Note that A: stands for Abiquiu Lake and SL stands for Snow Lake, SUM means summer.

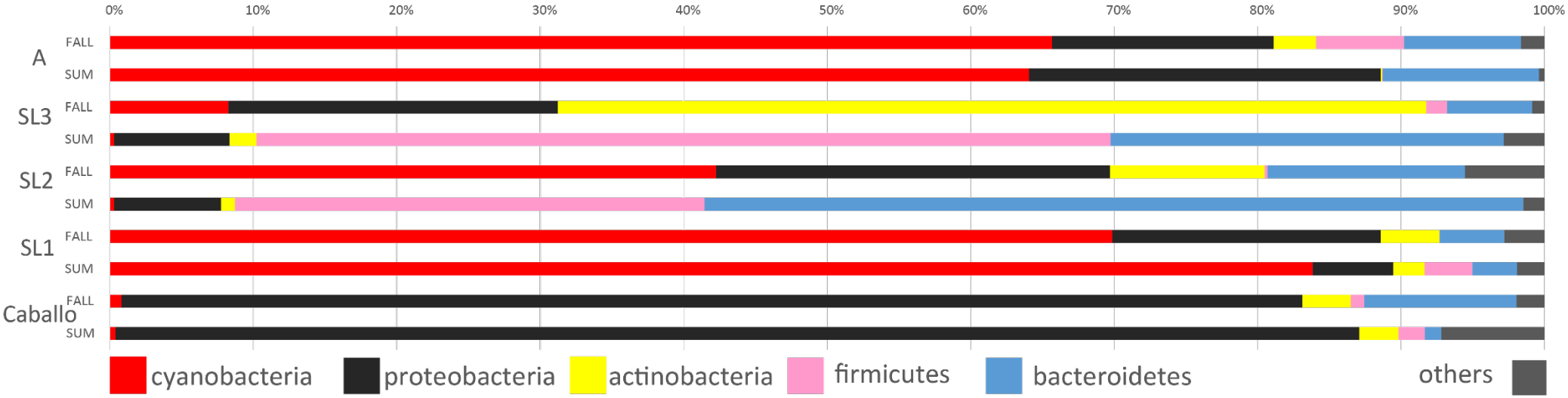


Figure 7. Nutrient analysis of selected reservoir (Caballo Lake) including Phosphorous

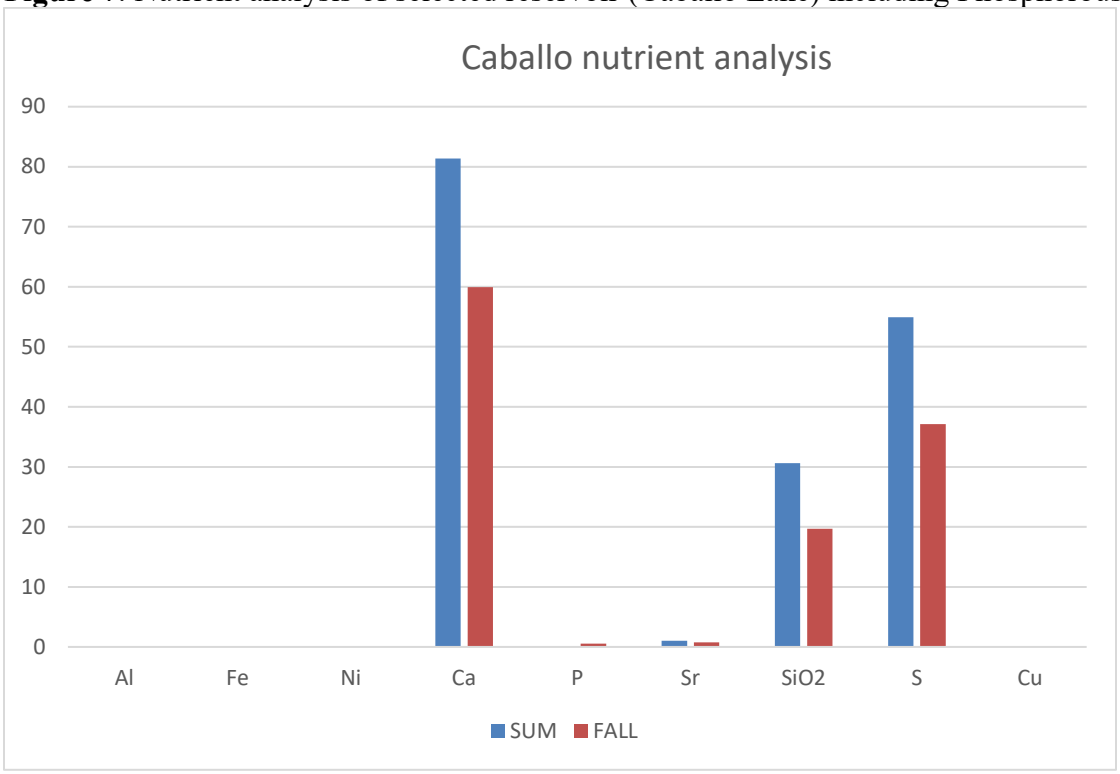


Figure 8. Concentration of different forms of Nitrogen in the waterbodies of New Mexico

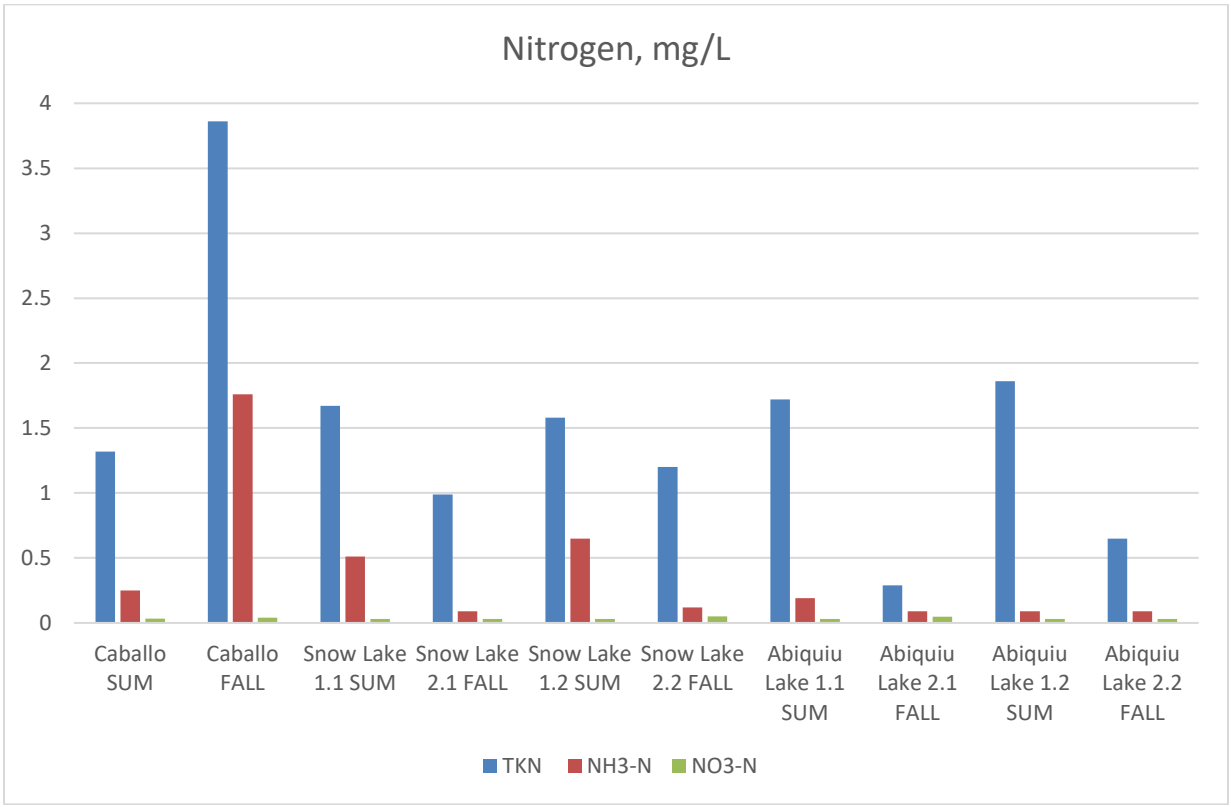
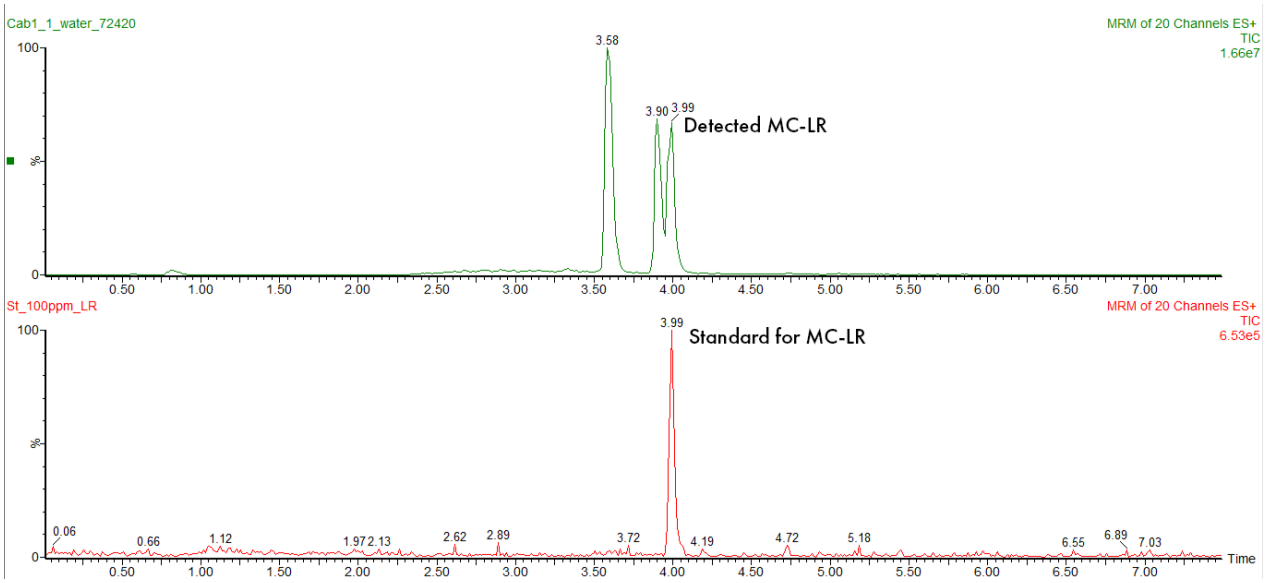
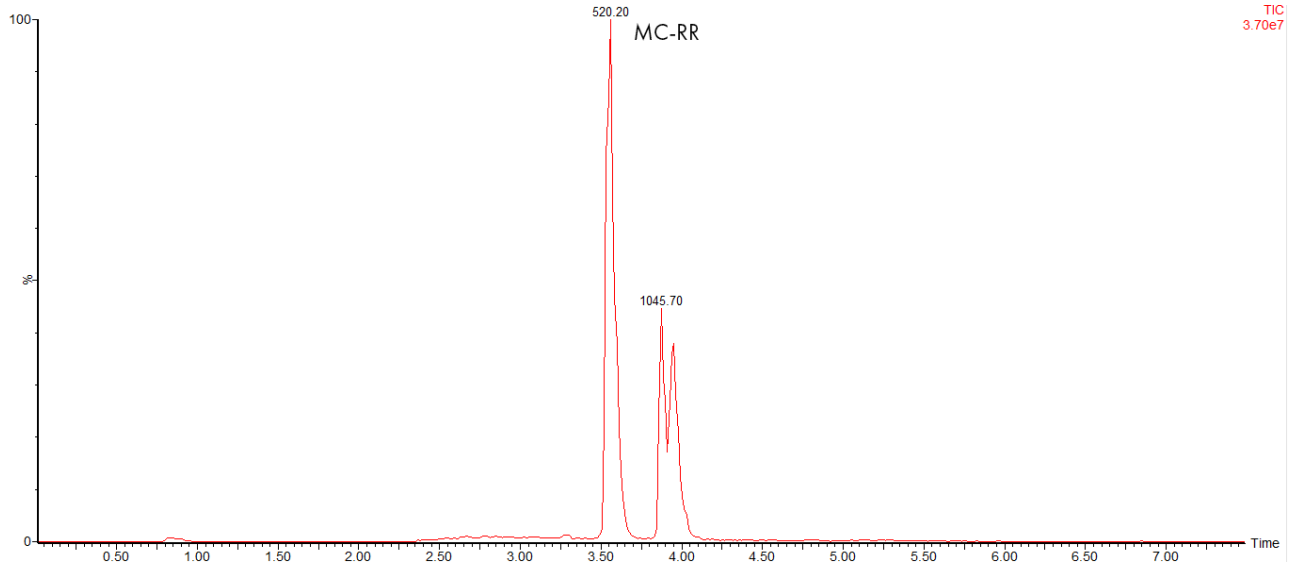


Figure 9. Chromatograms of the detected toxins. **A**: MC-LR, **B**: MC-RR, **C**:MC-YR, **D**: MC-LY

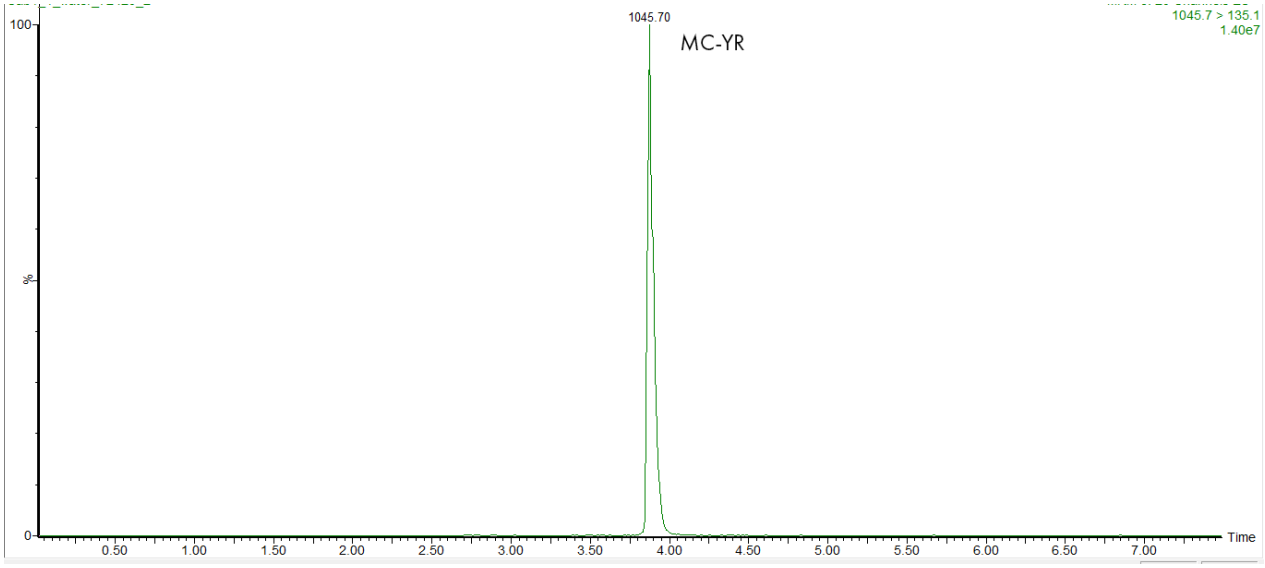
A



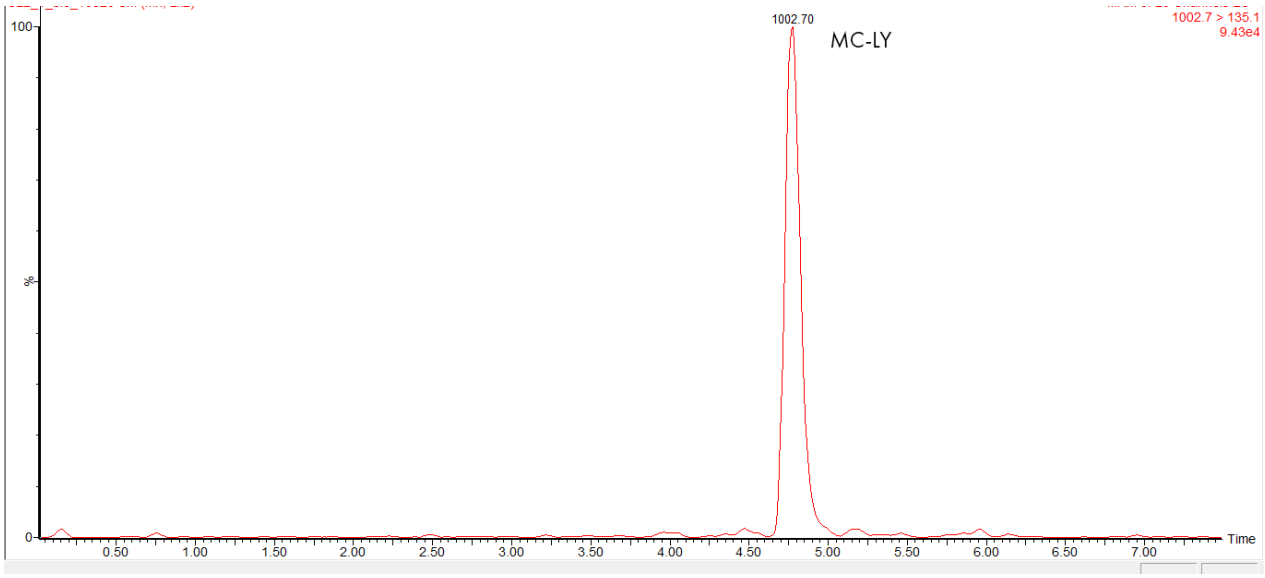
B



C


1045.70
MC-YR
1045.7 > 135.1
1.40e7
100
%
0
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
5.00
5.50
6.00
6.50
7.00
Time

D


1002.70
MC-LY
1002.7 > 135.1
9.43e4
100
%
0
0.50
1.00
1.50
2.00
2.50
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4.00
4.50
5.00
5.50
6.00
6.50
7.00
Time

Table 1. Morphological characterization of the cHABs collected from the different waterbodies of the New Mexico

	JULY-AUGUST 2020						
		Snow Lake			Abiquiu		
Lakes	Caballo1	1	2	3	1	2	EB
	Microcystis aeruginosa	*Aphanizomenon flos-aquae*	*Aphanizomenon flos-aquae*	*Aphanizomenon flos-aquae*	*Aphanizomenon flos-aquae*	*Aphanizomenon flos-aquae*	
	Pseudanabaena mucicola	*Microcystis smithii*	*Dolichospermum spiroides*	*Dolichospermum sp.*	*Pseudanabaena limnetica*	*Pseudanabaena limnetica*	
	Dolichospermum spiroides	*Dolichospermum sp.*	*Dolichospermum sp.*				
LM	*Dolichospermum* sp.						
Other algae	Desmids, Green algae and Diatoms: *Ulnaria* sp., *Cymbella* sp.	Diatoms: *Stephanodiscus* sp., *Cymbella* sp., *Rhopalodia* sp., *Navicula* sp. Euglenoids: *Trachelomonas* sp. Dinoflagellates: *Ceratium* sp.	Diatoms: *Puncticulata* sp., *Navicula* sp. *Rhopalodia* sp., *Hantzschia* sp.	Some centric diatoms	Some Dinobryaceae occurs, minor Diatoms *(Navicula)*		
Notes:	Bloom was heavily dominated by cyanobacteria (*Dolichospermum* sp. *Microcystis aeruginosa*) with trace amounts of Desmids, Green algae and Diatoms. Some *Microcystis* colonies	Bloom former in the shape of elongated half-moon clusters. Bloom looked like pea-soup, or green paint with visible structure differ from *Microcyscis/Dolichospermum* blooms. Clusters were several millimeters long. Microscopically filaments	similar to SL1	similar to SL1	Bloom dominated ny *Aphanizomenon*	similar to A1	no visible bloom

	had a core (nucleus) with the cells without aerotopes (about 20 cells), in the outside layer aerotopes was present.	were clustering together in the typical Aphanizomenon-like way. Apical cells within the cluster were containing less aerotopes and were very elongated to compare with regular vegetative cells, length of apical cells: up to 35 micrometers. Apical cell appeared to be narrowed than regular cells, 5 micrometers. Intercalary heterocytes were observed, brownish by color with clear pores, L:10-12 micrometers, W:6.4-7.6 micrometers. Also, elongated akinetes observed with granular texture and slightly different color from regular cells, L:26-70 micrometers, W: 6-7 micrometers. Vegetative cell slightly constricted with bunch of aerotopes, W:6-6.4 micrometers, L:same as L, slightly longer than wide.					

Continued

OCTOBER 2020						
	Snow Lake 2			Abiquiu2		
Caballo2	1	2	3	1	2	EB
Limnothrix sp. .	*Microcystis flos-aquae*	*Woronichinia naegeliana*	*Microcystis flos-aquae*	*Microcystis aeruginosa*	*Microcystis aeruginosa*	*Dolichospermum sp.*
Pseudanabaena sp	*Microcystis viridis*	*Microcystis flos-aquae*	*Aphanizomenon flos-aquae*	*Pseudanabaena mucicola*	*Pseudanabaena mucicola*	
Lyngbya sp.	*Aphanizomenon flos-aquae*	*Aphanizomenon flos-aquae*	*Dolichospermum sp.*		*Microcystis viridis*	
Phacus sp., *Trachelomonas* sp., *Ceratium* sp.	Green algae		lots of amoebas, flagellates and bacteria	Diatoms *(Navicula),* green algae	Diatoms, Green algae	desmids, diatoms
Bloom was dominated by filamentous green algae, diatoms euglinoids and desmids.	Bloom dominated by *Microcystis* spp., Some *Microcystis* colonies had a core (nucleus) with the cells without aerotopes (about 20 cells), in the outside layer aerotopes was present.	Bloom was dominated by *Microcystis, Aphanizomenon* was minor				

Abbreviations: LM –light microscopy, EB – Elephant Butte Lake

Table 2. Cyanobacterial species composition based on the molecular PacBio method

	JULY-AUGUST 2020					
		Snow Lake			Abiquiu	
Lakes	Caballo1	1	2	3	1	EB
PacBio	*Synechococcus sp.*	*Aphanizomenon flos-aquae*	*Synechococcus sp.*	*Synechococcus sp.*	*Aphanizomenon flos-aquae*	*Microcystis aeruginosa*
	Aphanizomenon flos-aquae	*Anabaena sp.*	*Cyanobium sp.*	*Cyanobium synechococcus sp.*	*Pseudanabaena sp.*	*Synechococcus sp.*
	Cyanobium sp.	*Synechococcus sp.*	*Cyanobium synechococcus sp.*	*Aphanizomenon flos_aquae*	*Gloeobacter spp.*	*Cyanobium sp.*
	Chlorogloea microcystoides	*Microcystis aeruginosa*	*Microcystis aeruginosa*	*Cyanobium sp.*	*Microcystis aeruginosa*	*Microcystis ichthyoblabe*
	Microcystis aeruginosa	*Cyanobium sp.*	*Aphanizomenon flos-aquae*		*Cyanobacterium spp.*	*Oscillatoria sp.*
	Chroococcidiopsis spp.	*Gloeobacter spp.*			*Synechococcus sp.*	*Cyanobacterium spp.*
	Microcystis ichthyoblabe	*Microcystis ichthyoblabe*			*Dolichospermum anabaena flos-aquae*	*Aphanizomenon flos-aquae*
	Pseudanabaena sp.	*Cyanobacterium spp.*				*Chroococcidiopsis spp.*
	Calothrix sp.	*Cyanobium synechococcus sp.*				*Pseudanabaena sp.*
		Dolichospermum anabaena flos-aquae				*Pseudanabaena catenata*
						Prochlorococcus spp.
						Nostoc sp.

Continued

OCTOBER 2020						
	Snow Lake 2			Abiquiu2		
Caballo2	1	2	3	1	2	EB
Chroococcidiopsis spp.	*Microcystis aeruginosa*	*Microcystis aeruginosa*	*Microcystis aeruginosa*	*Microcystis aeruginosa*	*Microcystis aeruginosa*	*Synechococcus sp.*
Cyanobium sp.	*Microcystis ichthyoblabe*	*Microcystis ichthyoblabe*	*Microcystis ichthyoblabe*	*Pseudanabaena sp.*	*Aphanizomenon flos-aquae*	*Aphanizomenon flos-aquae*
Synechococcus sp.	*Woronichinia naegeliana*	*Aphanizomenon flos_aquae*	*Cyanobacterium spp.*	*Aphanizomenon flos-aquae*	*Microcystis ichthyoblabe*	*Cuspidothrix aphanizomenon issatschenkoi*
Leptolyngbya sp.	*Aphanizomenon flos-aquae*	*Woronichinia naegeliana*	*Aphanizomenon flos-aquae*	*Microcystis ichthyoblabe*	*Stigonema mamillosum*	*Anabaenopsis nadsonii*
Aphanizomenon flos-aquae	*Gloeobacter spp.*	*Anabaena sp.*	*Cyanobium sp.*	*Chroococcus sp.*		*Cyanobium sp.*
	Microcystis sp.	*Prochlorococcus spp.*	*Dolichospermum planctonicum*	*Cyanobacterium spp.*		*Pseudanabaena catenata*
	Synechococcus sp.	*Cyanobacterium spp.*				*Chondrocystis sp.*
	Prochlorococcus spp.	*Anabaenopsis nadsonii*				*Cyanobacterium spp.*
		Synechococcus sp.				*Chroococcidiopsis spp.*
		Microcystis sp.				
		Leptolyngbya sp.				