



Because of the presence of potentially toxin-producing blue-green algae, sample was submitted to a sub-contract laboratory for analysis of microcystin and anatoxin-A.

Respectfully,

**AQUATIC CONSULTING & TESTING, INC,**

A handwritten signature in black ink that reads "Frederick A. Amalfi." The signature is written in a cursive style with a large initial 'F' and a stylized 'A'.

Frederick A. Amalfi, Ph.D., Laboratory Director

## ALGAE IDENTIFICATION

AC&T Lab No.	CE05918	Date Collected	07/24/22
Client I.D.	White Mtn Lakes	Collected By	Client

Divisions: bac=Bacillariophyta; chl=Chlorophyta; cry=Chrysophyta; cyn=Cyanophyta; eug=Euglenophyta; hap=Haptophyta; pyr=Pyrrhophyta  
Forms: u=unicell; c=colony; f=filament; g= flagellate

Genus	Div.-Form	Rel. Count	Total per mL	Comp.	Genus	Div.-Form	Rel. Count	Total per mL	Comp.
<i>Achnanthes</i>	bac-u				<b><i>Microcystis</i></b>	cyn-c	245	22550	53.85%
<b><i>Anabaena</i></b>	cyn-f	192	17672	42.20%	<i>Microspora</i>	chl-f			
<i>Ankistrodesmus</i>	chl-u				<i>Mougeotia</i>	chl-f			
<i>Aphanocapsa</i>	cyn-c				<i>Navicula</i>	bac-u			
<i>Asterionella</i>	bac-c				<i>Nitzschia</i>	bac-u			
<i>Botryococcus</i>	chl-c				<i>Oocystis</i>	chl-c			
<i>Carteria</i>	chl-ug				<i>Oscillatoria</i>	cyn-f			
<i>Ceratium</i>	pyr-ug				<i>Pandorina</i>	chl-cg			
<i>Chaetoceros</i>	bac-c				<i>Pediastrum</i>	chl-c			
<i>Chlamydomonas</i>	chl-ug				<i>Peridinium</i>	pyr-ug			
<i>Chlorella</i>	chl-u				<i>Phacotus</i>	chl-ug			
<i>Chlorococcum</i>	chl-c				<i>Phacus</i>	chl-ug			
<i>Chroococcus</i>	cyn-c				<i>Pinnularia</i>	bac-u			
<i>Chroomonas</i>	crp-ug				<i>Pithophora</i>	chl-f			
<i>Closterium</i>	chl-u				<i>Prymnesium</i>	hap-ug			
<i>Cocconeis</i>	bac-u				<i>Rhizoclonium</i>	chl-f			
<i>Coelastrum</i>	chl-c				<i>Rhoicosphenia</i>	bac-u			
<i>Cosmarium</i>	chl-u				<i>Rhopalodia</i>	bac-u			
<i>Cosmocladium</i>	chl-c				<i>Scenedesmus</i>	chl-c			
<i>Crucigenia</i>	chl-c				<i>Scytonema</i>	chl-f			
<i>Cryptomonas</i>	crp-ug				<i>Selanastrum</i>	chl-u			
<i>Cyclotella</i>	bac-u				<i>Sphaerocystis</i>	chl-c			
<i>Cylindrospermopsis</i>	cyn-f				<i>Spondylumorum</i>	chl-c			
<i>Denticula</i>	bac-u				<i>Spirogyra</i>	chl-f			
<i>Dinobryon</i>	cry-c				<i>Spirotaenia</i>	chl-u			
<i>Dunaliella</i>	chl-ug				<i>Stephanodiscus</i>	bac-u			
<b><i>Eudorina</i></b>	chl-c	11	1012	2.42%	<i>Stigeoclonium</i>	chl-f			
<i>Euglena</i>	eug-ug				<i>Surirella</i>	bac-u			
<i>Fragilaria</i>	bac-u				<i>Synechococcus</i>	cyn-u			
<i>Frustulia</i>	bac-u				<i>Synechocystis</i>	cyn-c			
<i>Glenodinium</i>	pyr-ug				<i>Synedra</i>	bac-u			
<i>Golenkinia</i>	chl-c				<i>Synura</i>	cry-cg			
<i>Gomphonema</i>	bac-u				<i>Tetraedron</i>	chl-u			
<i>Gonium</i>	chl-cg				<i>Tetrastrum</i>	chl-c			
<i>Gyrosigma</i>	bac-u				<b><i>Trachelomonas</i></b>	eug-ug	7	644	1.54%
<i>Holopedium</i>	cyn-c				<i>Vaucheria</i>	chl-f			
<i>Hydrodictyon</i>	chl-c				<i>Volvox</i>	chl-cg			
<i>Lyngbya</i>	cyn-f				<i>Zygnema</i>	chl-f			
<i>Mastigloia</i>	bac-u								
<i>Meridion</i>	bac-u								
<i>Merismopedia</i>	cyn-c								

check 100.00%

Aquatic Consulting & Testing, Inc.  
1525 W. University Dr., Suite 106  
Tempe, Arizona 85281

Count (cells/mL) 4.19E+04

Client Name: WMLCRID  
 Address: 2015 Silver Creek Dr  
Show Low AZ 85901  
 City, State, Zip  
 Phone: 928-278-8035  
 Fax: MARK.NREC@gmail.com  
 Contact: Mark Honberger  
 Sampler Signature: [Signature]

SAMPLE ID	SAMPLE Date	SAMPLE Time	SAMPLE TYPE
White mtn lakes	07/24	12:00	SW



**CHAIN OF CUSTODY**

PWS ID # \_\_\_\_\_

PAGE \_\_\_\_\_ OF \_\_\_\_\_

Chemistry		Biology		Biomon		PO#	
<input type="checkbox"/> Metals (See Below)	<input type="checkbox"/> TDS <input type="checkbox"/> TSS <input type="checkbox"/> TS <input type="checkbox"/> SETT <input type="checkbox"/> TVS <input type="checkbox"/> VSS	<input type="checkbox"/> Total Coliform <input type="checkbox"/> P/A <input type="checkbox"/> Coliform <input type="checkbox"/> MPN	<input type="checkbox"/> E. Coli <input type="checkbox"/> Fecal Strept	<input type="checkbox"/> Acute <input type="checkbox"/> Chronic	<input type="checkbox"/> AWET (SWRO)	Project	PO#
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<input type="checkbox"/> O-6 <input type="checkbox"/> TPHC <input type="checkbox"/> MBAS <input type="checkbox"/> CN <input type="checkbox"/> Sulfide	<input type="checkbox"/> BOD <input type="checkbox"/> COD <input type="checkbox"/> New Source	<input type="checkbox"/> Total Coliform <input type="checkbox"/> P/A <input type="checkbox"/> Coliform <input type="checkbox"/> MPN	<input type="checkbox"/> Fecal Coliform <input type="checkbox"/> MPN <input type="checkbox"/> MF	<input type="checkbox"/> Plate Count <input type="checkbox"/> BIOLOG	<input type="checkbox"/> Algae ID + Count	No. of Containers	Laboratory Number
<input type="checkbox"/> TDS <input type="checkbox"/> TSS <input type="checkbox"/> TS <input type="checkbox"/> SETT <input type="checkbox"/> TVS <input type="checkbox"/> VSS	<input type="checkbox"/> O+G <input type="checkbox"/> TPHC <input type="checkbox"/> MBAS <input type="checkbox"/> CN <input type="checkbox"/> Sulfide	<input type="checkbox"/> TAP <input type="checkbox"/> O-Pg	<input type="checkbox"/> Nitrate + Nitrite <input type="checkbox"/> Nitrite	<input type="checkbox"/> TKN <input type="checkbox"/> Ammonia	<input type="checkbox"/> THMs <input type="checkbox"/> HAAS	<input type="checkbox"/> NaOH	<b>CE-05918</b>
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<input type="checkbox"/> 8260 <input type="checkbox"/> 624 <input type="checkbox"/> BTEX	<input type="checkbox"/> 8260 <input type="checkbox"/> 624 <input type="checkbox"/> BTEX	<input type="checkbox"/> Perchlorate <input type="checkbox"/> Radio <input type="checkbox"/> Asbestos					

Metals:  Al  Sb  As  Ba  Be  B  Cd  Ca  Cr  Co  Cu  Fe  Pb  Mg  Mn  Hg  Mo  Ni  Se  Ag  Na

Sample Types: DW, GW, SW, WW, AQ, Soil, Sludge or Solid

1. Relinquished By: [Signature] Date: 7/25/22 Time: \_\_\_\_\_

2. Relinquished By: UPS Date: \_\_\_\_\_ Time: \_\_\_\_\_

3. Relinquished By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_

1. Received By: UPS Date: \_\_\_\_\_ Time: \_\_\_\_\_

2. Received By: M Date: 07/26/22 Time: 800

3. Received By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_

Sample Receiving: Intact:  Yes  No

Temp: 23°C Auth Init: \_\_\_\_\_

Pres:  Yes  No/Lab

Sterile:  Yes  No

Total # containers: 1

**Attn: Your signature on this document authorizes analysis regardless of sample condition at time of submittal**

## Anatoxin-a & Microcystins/Nodularins Report

*Project: Aquatic Consulting and Testing Inc.*

Submitted to: Fredrick Amalfi  
 Organization: Aquatic Consulting and Testing Inc.  
 Address: 1525 W University Dr Ste 106 Tempe AZ 85281  
 Email: [ramalfi@aquaticconsulting.com](mailto:ramalfi@aquaticconsulting.com)  
 Sample Receipt Date: 27 July 2022  
 Sample Condition: 13.0 °C upon arrival  
 Report# 220724\_Aquatic\_Consulting\_Testing  
 Date Prepared: 29 July 2022  
 Prepared by: Amanda Foss

Table 1: Samples analyzed

<u>Sample ID</u>	<u>Collection Date</u>
CE05918	24 July 2022

**Analytes:** Anatoxin-a (ATX), Microcystins/Nodularins (MCs/NODs)

Abbreviations	
NA	Not Applicable
MDL	Method Detection Limit
MQL	Method Quantification Limit
ND	Not Detected above the MDL
Blank	Regent Water free from interferences
LFB	Lab Fortified Blank
CCC	Continued Calibration Check
LFSM	Lab Fortified Sample Matrix
LFSMD	Lab Fortified Sample Matrix Duplicate
LD	Lab Duplicate
IS	Internal Standard
—	Not Analyzed
MRL	Method Reporting Limit
CV	Low-range calibration verification

## Sample Preparation

### *Water Sample Freeze-Thaw*

The sample was inverted for 60 seconds to mix. A subset was transferred to a 15 mL vial. Three freeze-thaw cycles were employed prior to additional sample preparation and subsequent analysis.

## Analytical Techniques

### *Enzyme-Linked Immunosorbent Assay (ELISA)*

#### *MCs/NODs*

A microcystins/nodularins Adda ELISA (Abraxis) was utilized for the quantitative and sensitive congener-independent detection of MCs/NODs (US EPA Method 546 & Ohio EPA DES 701.0). The current method reporting limit is 0.30 ng/mL (ppb) based on kit sensitivity, dilution factors, and initial demonstration of capability.

### *Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)*

#### *ATX*

High performance chromatography coupled with tandem mass spectrometry was used for a targeted anatoxin-a analysis. The  $[M+H]^+$  ion for ATX ( $m/z$  166) was fragmented and the product ions ( $m/z$  91, 131, 149) were monitored. The  $[M+H]^+$  ion for the internal standard  $[^{13}C_4]ATX$  ( $m/z$  171) was fragmented and the product ion ( $m/z$  153) was monitored. The internal standard method was utilized for quantification.

Qualifier	Flag
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CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

## Quality Control

Table 2: Quality Assurance/Quality Control (QA/QC) samples (IS and LFSM) prepared for analyses pre-extraction. Additional QA/QC checks included LFBs, continued calibration checks and external curves.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
ATX	0.1	CE05918	LFSM	107%
[ <sup>13</sup> C <sub>4</sub> ]ATX	1.0	all aliquots	IS	90 ± 3%

\*Control limits: water LFSM ± 30%; complicated matrix LFSM and when LFSM within 2x MDL ±50%; IS ± 50%

Table 3: Adda MCs/NODs ELISA Quality Control Value Table

Date Analyzed:	29-Jul-22	Requirement	Pass/Fail
R <sup>2</sup> value:	1.000	≥0.98	PASS
%CV 0.00STDs:	2.2 - 8.4%	≤15%	PASS
LFB (1 ppb) recovery:	108%	±40% True Value	PASS
%CV 0.00LFB:	4.2%	≤20%	PASS
Low CCC (0.15 ppb) recovery:	132%	±50% True Value	PASS
LRB	<0.08	<0.08	PASS

## Results

Table 5: Raw ELISA Data including the sample identification, analyte, date analyzed, interpolated values 1 and 2, the dilution factor, %CV of the absorbance values, and average final concentration (ng/mL; ppb).

Sample ID	Sample Type	Analyte	Date analyzed	Value 1 (ng/mL)	Value 2 (ng/mL)	Dilution Factor	%CV	Average (ng/mL)
CE05918	FS	MCs/NODs	7/29/2022	0.21	0.23	1000	0.9	220
CE05918	FS	MCs/NODs	7/29/2022	2.94	3.02	100	0.8	298

**Summary of Results**

Table 4: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the ELISA method and anatoxin-a (ATX). All data are reported as ng/mL (ppb) with the standard deviation where applicable.


Sample ID	MCs/NODs	ATX
CE05918	259	ND
<i>MRL (ng/mL):</i>	<i>0.30</i>	<i>0.05</i>
<i>Analyst Initials:</i>	<i>AF</i>	<i>MA</i>
<i>Date Analyzed:</i>	<i>7/29/22</i>	<i>7/29/22</i>

**Interpretations:**

High levels of microcystins/nodularins were determined to be present in the sample. The level of MCs/NODs detected in the submitted sample currently *exceeds* the current 'Draft EPA Recommended Value for Recreational Criteria and Swimming Advisory', which is currently 8 ng/mL (ppb) total microcystins. The WHO recreational guidance value for microcystin is currently 24 ng/mL (ppb) (World Health Organization (WHO), 2020a).

Anatoxin-a was not detected above the method reporting limits.

World Health Organization (WHO), 2020a. Cyanobacterial toxins: microcystins. Guidel. Drink. Qual. Guidel. Safe Recreat. Water Environ. 63.

Submitted by:   
Mark T. Aubel, Ph.D.  
Lab Director  
Date: July 29, 2022

*The results in this report relate only to the samples listed above.  
This report shall not be reproduced except in full without written approval of the laboratory.*



Client Name: <u>Aquatic Consulting</u> Address: _____ Street City, State, Zip _____ Phone: _____ Fax: _____ Contact: _____ Sampler Signature: <u>[Signature]</u>		PO# _____ Project _____ Remarks: <u>SUB TO Water Lab's Green</u> Laboratory Number _____		
SAMPLE ID <u>CE05918</u>	SAMPLE Date <u>07/25/12</u>	SAMPLE TYPE <u>AA</u>	No. of Containers HCl _____ Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> _____ H <sub>2</sub> SO <sub>4</sub> _____ HNO <sub>3</sub> _____ NONE _____ NaOH _____ NaOH/ZnAc _____	
			Metals (See Below) TDS □ TSS □ TS □ SETT □ TVS □ VSS HEM □ TPH □ MBAS □ CN □ Sulfide BOD □ COD □ New Source Total P, P-a, P-b Nitrate + Nitrite □ Nitrite THM's □ HAAS 525 □ 625 □ 8270 524 □ 624 □ 8260 Perchlorate □ Radio □ Asbestos	
Chemistry Total P, P-a, P-b <u>X</u> Nitrate + Nitrite □ Nitrite <u>X</u> THM's □ HAAS 525 □ 625 □ 8270 524 □ 624 □ 8260 Perchlorate □ Radio □ Asbestos		Biology Total Coliform □ Presence/Absence □ MPN E. Coli □ Fecal Strep Fecal Coliform □ MF MICRO SCOPE ID Plate Count Algae ID/Count Acute □ Chronic		
WET		Laboratory Number		

Metals:  Al  Sb  As  Ba  Be  B  Cd  Ca  Co  Cr  Cu  Fe  Hg  Mn  Mo  Ni  Pb  Se  Sn  Ti  Tl  V  Zn  
 Sample Types: DW, GW, SW, WW, AQ, S, Sludge or Solid

Sample Receiving:  YES  NO  
 Intact:  Yes  No  
 Temp: \_\_\_\_\_ Auth Init: \_\_\_\_\_  
 Pres: \_\_\_\_\_ Yes/No  No/Lab  
 Sterile: \_\_\_\_\_ Yes  No  
 Total # containers: 1

1. Relinquished By: [Signature] Date: 07/25/12 Time: 1:30  
 2. Relinquished By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_  
 3. Relinquished By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_

1. Received By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_  
 2. Received By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_  
 3. Received By: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_

Attn: Your signature on this document authorizes analysis regardless of sample condition at time of submittal