# Potentially Toxigenic (PTOX) Cyanobacteria Screen

**Spicer Group**

Prepared: August 31, 2016
Prepared By: GreenWater Laboratories
Received: 8/31/16

| Sample ID | Site | Collection Date |
|---|---|---|
| AG 1 | P.P. Lake | 8/30/16 |
| AG 2 | P.P. Lake | 8/30/16 |
| AG 3 | P.P. Lake | 8/30/16 |
| AG 4 | P.P. Lake | 8/30/16 |
| AG 5 | P.P. Lake | 8/30/16 |

## Method

One mL aliquots of the samples were prepared using Sedgewick Rafter cells. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TS100 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

## Results

**AG 1**

The dominant PTOX cyanobacteria observed were *Dolichospermum* spp., cyanobacteria unicells resembling *Microcystis* sp. and *Aphanizomenon* sp.

**AG 2**

The dominant PTOX cyanobacteria observed were *Dolichospermum* spp., cyanobacteria unicells resembling *Microcystis* sp. and *Aphanizomenon* sp. The PTOX cyanobacterium *Woronichinia naegeliana* was also observed.

**AG 3**

This sample appeared to be the most filamentous. Macroscopically, a denser film was observed at the surface of the sample. The dominant PTOX cyanobacteria observed were *Dolichospermum* spp., cyanobacteria unicells resembling *Microcystis* sp. and *Aphanizomenon* sp. The PTOX cyanobacterium *Woronichinia naegeliana* was also observed.

**AG 4**

The dominant PTOX cyanobacteria observed were *Dolichospermum* spp., cyanobacteria unicells resembling *Microcystis* sp. and *Aphanizomenon* sp. The PTOX cyanobacterium *Woronichinia naegeliana* was also observed.

**AG 5**

The dominant PTOX cyanobacteria observed were *Dolichospermum* spp., cyanobacteria unicells resembling *Microcystis* sp. and *Aphanizomenon* sp. Other PTOX cyanobacteria observed were *Woronichinia naegeliana* and colonies of *Microcystis* sp. This sample appeared to have the greatest abundance of *Woronichinia naegeliana*.

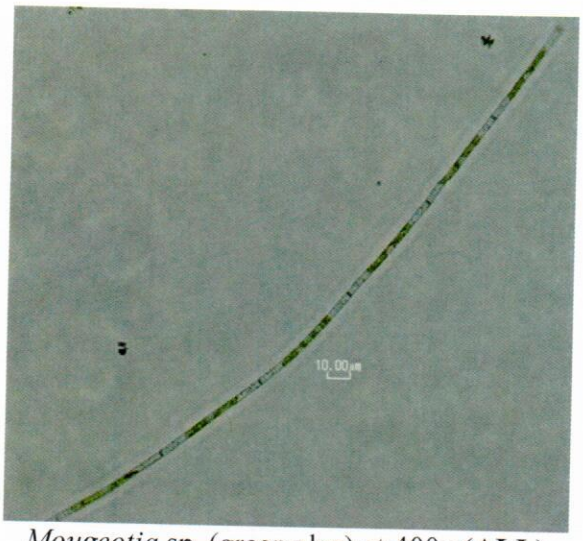
## Observations and Recommendations

Every sample was dominated by non-toxin producing filamentous green algae, *Mougeotia* sp. The cyanobacterium *Limnoraphis* sp. was also observed in every sample, but is not currently a known toxin producer.

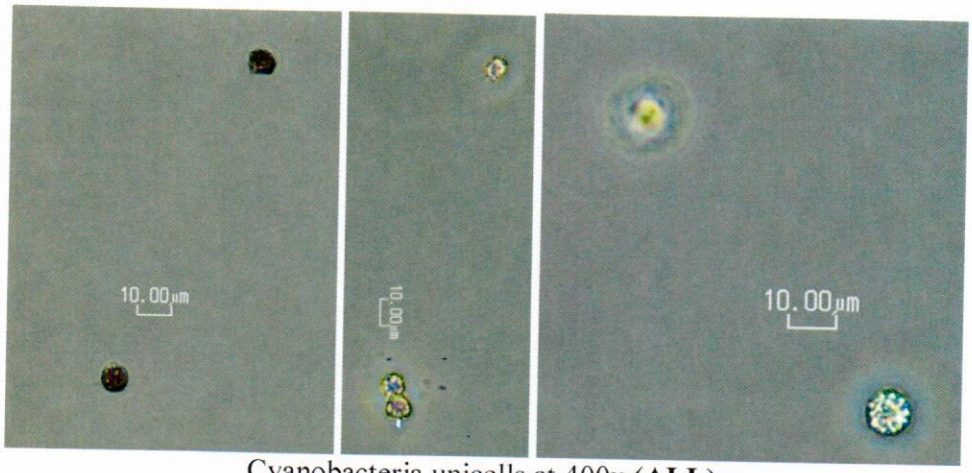
Of potentially toxigenic cyanobacteria, *Dolichospermum* spp. and free-floating cells resembling *Microcystis* sp. were observed in every sample. Based on these observations, toxin analysis for microcystins, anatoxin-a, cylindrospermopsin, and saxitoxin is currently recommended on all samples.

These observations and relative sample comparisons are qualitative. The Total Algae ID/E service requested will provide the quantitative information required to be able to identify significant differences between these samples.

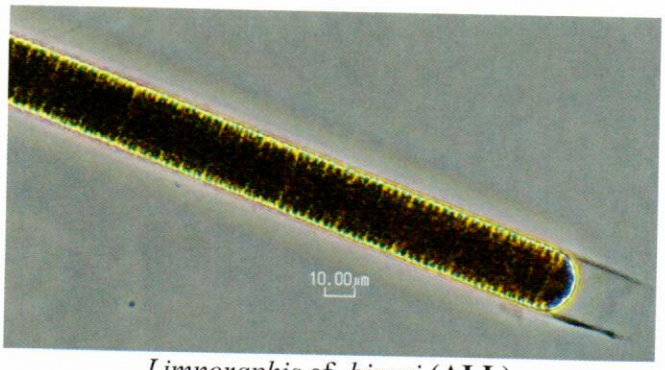
## Micrographs



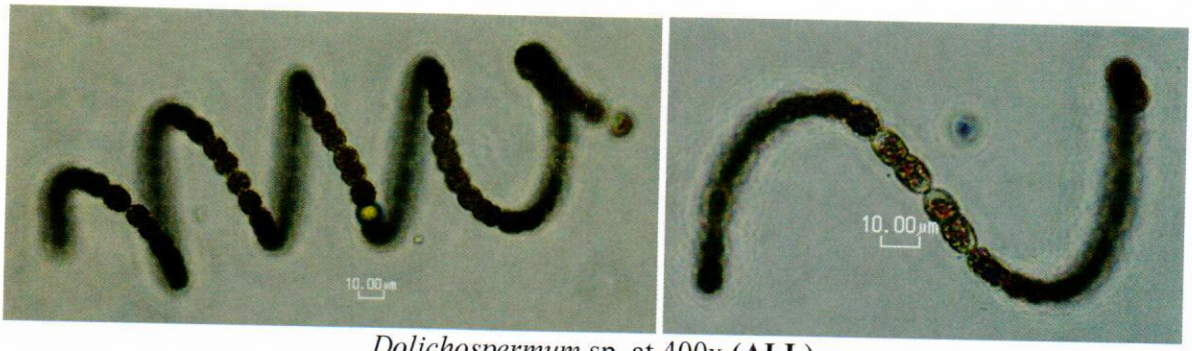

*Mougeotia* sp. (green alga) at 400x **(ALL)**




Cyanobacteria unicells at 400x (**ALL**)




*Limnoraphis* cf. *birgei* (**ALL**)




*Dolichospermum* sp. at 400x (**ALL**)

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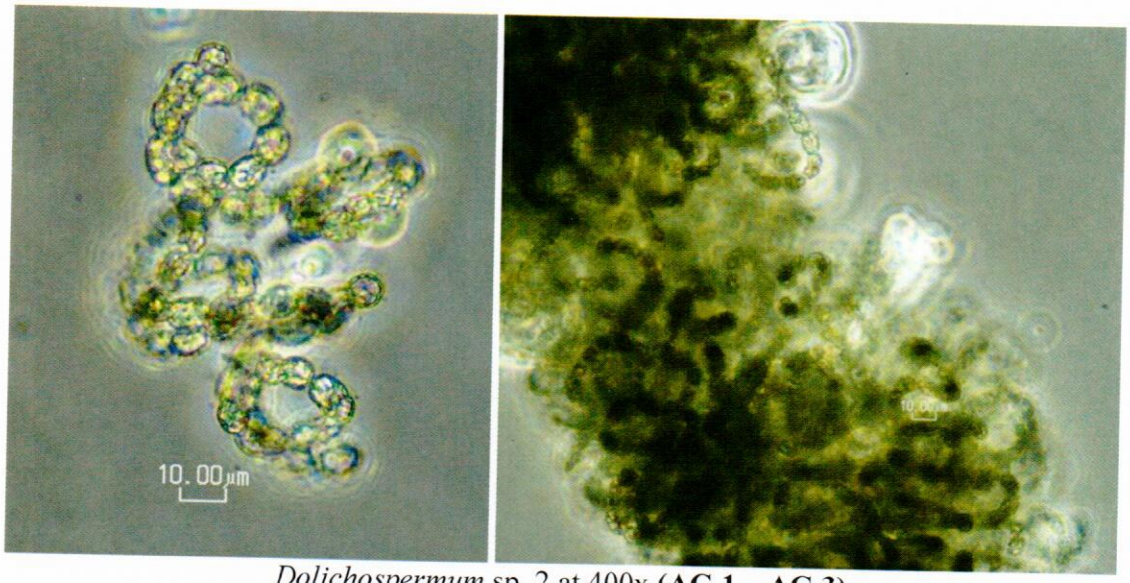
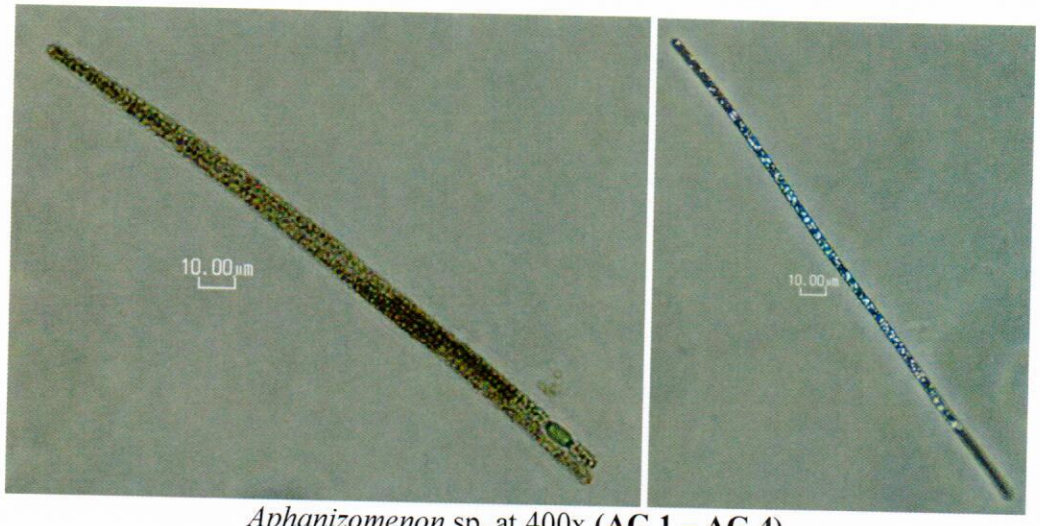



*Dolichospermum* sp. 2 at 400x **(AG 1 – AG 3)**




*Aphanizomenon* sp. at 400x **(AG 1 – AG 4)**

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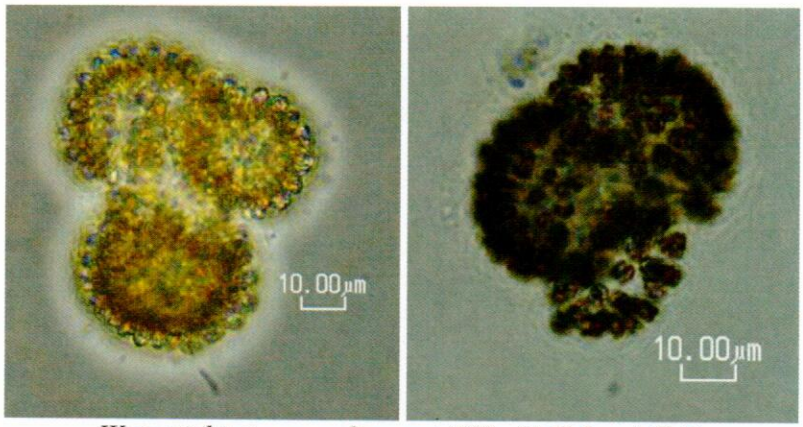
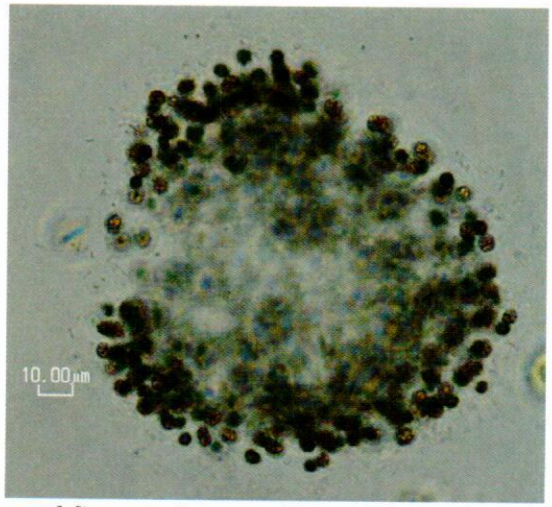



*Woronichinia naegeliana* at 400x **(AG 2 – AG 5)**




*Microcystis* sp. at 400x **(AG 5 only)**

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Sarah Fuller

Date: 9/8/16

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# Anatoxin-a, Cylindrospermopsin, Microcystin & Saxitoxin Report

**Project: Spicer Group**

| Sample ID | Site | Collected |
|---|---|---|
| AG1 | P. P. Lake | 8/30/16 |
| AG2 | P. P. Lake | 8/30/16 |
| AG3 | P. P. Lake | 8/30/16 |
| AG4 | P. P. Lake | 8/30/16 |
| AG5 | P. P. Lake | 8/30/16 |

**Toxins** – Anatoxin-a (ANTX-A), cylindrospermopsin (CYN), microcystin (MC), saxitoxin (STX)

## Sample Prep

The samples were ultra-sonicated to lyse cells and release toxins. Duplicate sub-samples (Lab Fortified Matrix, LFM) were spiked at 0.1 µg/L of ANTX-A, 1.0 µg/L of CYN, 1.0 µg/L MCLR and 0.2 µg/L STX.

## Analytical Methodology

Liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS) was utilized for the determination of ANTX-A. The $[M+H]^+$ ion for ANTX-A (166 *m/z*) was fragmented and the product ions (56.0, 91.1, 107.0, 131.1 & 149.6 *m/z*) were monitored. The current method detection limit is 0.05 µg/L for ANTX-A.

A microcystins enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative and sensitive congener-independent detection of MCs. The current assay is sensitive to down to a LOD/LOQ of 0.15 µg/L for total MCs. The average recovery of a laboratory fortified blank (LFB) spiked with 1 µg/L MCLR was 97%.

A cylindrospermopsin enzyme linked immunosorbent assay (ELISA) was also utilized for the quantitative detection of CYN. The current assay is sensitive down to a LOD/LOQ of 0.1 µg/L for CYN. A lab fortified blank (LFB) spiked with 1.0 µg/L CYN was recovered at 116%.

A saxitoxin enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative detection of saxitoxin. The current assay is sensitive down to a LOD/LOQ of 0.05 µg/L saxitoxin. The LFB (0.2 µg/L STX spike) recovery was 105%.

**Summary of Results**

| Sample | ANTX-A (µg/L) | CYN (µg/L) | MC (µg/L) | STX (µg/L) |
|---|---|---|---|---|
| AG1 | ND | ND | ND | ND |
| AG2 | ND | ND | ND | ND |
| AG3 | ND | ND | ND | ND |
| AG4 | ND | ND | ND | ND |
| AG5 | ND | ND | ND | ND |
| *Limit of Detection (µg/L)* | *0.05* | *0.10* | *0.15* | *0.05* |

ND=Not detected above detection limits

Submitted by: Mark T. Aubel, Ph.D.

Date: 9/16/16