

LakePro Inc., Common Lake Qualitative Algal Analysis Report

Prepared: August 30, 2013

Prepared By: GreenWater Laboratories

Sample ID

40

Collected

8/27/13

Method

Two mL of the sample 40 collected August 27, 2013 was preserved with Lugol's iodine solution and allowed to settle. Preserved sample was observed at 100X, 200X and 400X using a Nikon Eclipse TE100 Inverted Microscope equipped with phase contrast optics.

Results

40

Microscopic observation of the sample 40 revealed was dominated by the potentially toxic (PTOX) cyanobacteria *Anabaena lemmermannii* (Fig1). Other PTOX cyanobacteria observed included *Anabaena circinalis*, *Microcystis* spp. (Fig 2), *Cuspidothrix issatschenkoi* (Fig 3), and *Aphanizomenon* cf. *flos-aquae*. (Fig 4). Other algal groups observed included cryptophytes (Cryptophyta).



Fig. 1 *Anabaena lemmermannii* at 400X (scale 20µm)

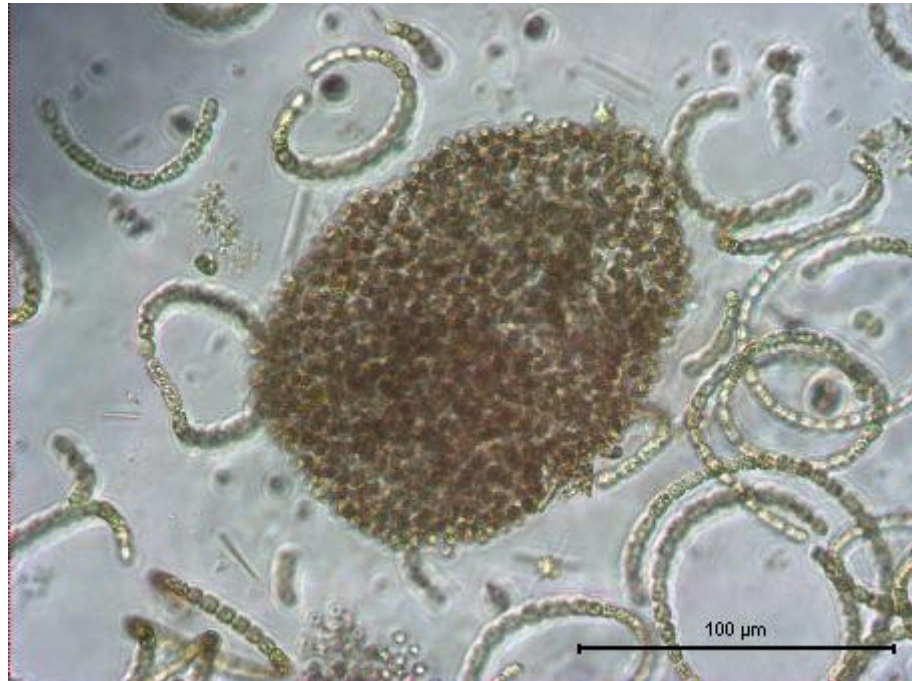


Fig. 2 *Microcystis* sp. at 400X (scale 100μm)



Fig. 3 *Cuspidothrix issatschenkoi* at 400X (scale 20μm)



Fig. 4 *Aphanizomenon* cf. *flos-aquae* at 400X (scale 20 μ m)

Recommendation

Due to the densities of *Anabaena lemmermannii* and *Microcystis* spp., toxin analyses for microcystin, anatoxin, and saxitoxin are recommended at this time.

Submitted by: Nara Rocha de Souza

Nara Souza, M.S.

Date: 8/30/2013

Anatoxin-a/Microcystin/Saxitoxin Report**Project: LakePro Inc.****(Common Lake)**Sample Identification

Common Lake (40)

Sample Collection Date

8/27/13

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

Sample Prep – The sample was ultra-sonicated to lyse all cells and release toxins. Solid phase extraction (SPE) was also utilized for anatoxin-a prep and preconcentration (100x) followed by filtration. Duplicate samples (Lab Fortified Matrix, LFM) were spiked at 0.1 µg/L of ANTX-A, 1.0 µg/L and at 0.2 µg/L STX, which provided quantitative capability and additional qualitative confirmation.

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 (*m/z* 166) was fragmented and the major product ions (*m/z* 149, 131, 107, and 91) provided both specificity and sensitivity. The current methodology established a limit of detection (LOD) of 0.05 µg/L and a limit of quantification (LOQ) of 0.1 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 92%.

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 0.02 µg/L saxitoxin. The LFB (0.2 µg/L STX spike) recovery was 98%.

Summary of ANTX-A/MC/STX Results

<u>Sample</u>	<u>ANTX-A levels</u> (µg/L)	<u>MC levels</u> (µg/L)	<u>STX levels</u> (µg/L)
Common Lake (40)	ND	34.4	ND

ND = Not detected above the LOD

LOD = 0.05 µg/L (ANTX-A & STX), 0.15 µg/L MC

LOQ = 0.1 µg/L ANTX-A, 0.15 µg/L MC, 0.05 µg/L STX

Submitted by:



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Date:

8/30/13

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LakePro, Inc.								
PARALYTIC SHELLFISH TOXINS / SAXITOXIN RESULTS								
Tested on:		8/30/2013						
Method:		Enzyme-Linked ImmunoSorbent Assay (ELISA)						
Analyte:		PSTs (saxitoxins)						
Analyzed by:		Alicia Carter						
Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery(%)	Avg. LFM Recovery (%)	Final Concentration (ug/L)	Average (ug/L)
40-Common Lake	1x	none	ND	1	98	97	ND	ND
8/27/13	1x	none	ND	1	98	97	ND	ND
ND = Not detected above LOD/LOQ LOD/LOQ = 0.05 µg/L LFB = 0.2 µg/L STX LFM = 0.2 µg/L STX								

Submitted by: *Amanda Foss*
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 Date: 8/30/2013

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LakePro, Inc.								
MICROCYSTIN RESULTS								
Tested on:	8/30/2013							
Method:	Enzyme-Linked ImmunoSorbent Assay (ELISA)							
Analyte:	Microcystins							
Analyzed by:	Alicia Carter							
Sample ID/ Date Collected	Initial Conc. Factor	Dilution Ratio	Assay Value, ug/L	Final Dilution Factor	Avg. LFB Recovery(%)	Avg. LFM Recovery(%)	Final Concentration (ug/L)	Average (ug/L)
40-Common Lake	1x	1:100	0.32	100	92	--	31.5	34.4
8/27/13	1x	1:100	0.37	100	92	--	37.2	
ND = Not detected above LOD/LOQ LOD/LOQ = 0.15 µg/L LFB = 1.0 µg/L MCLR LFM = 1.0 µg/L MCLR								

Submitted by: *Amanda Foss*
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