Anatoxin-a, Anatoxin-a(s), BMAA, Cylindrospermopsin, Microcystins & Saxitoxin Report

Project: Utah DEP – Division of Water Quality

Lab ID	Station ID	Description	Collection Date
160734-01	SLC 1	NA	7/16/16
160734-02	SLC 2	NA	7/16/16
160734-03	SLC 3	NA	7/16/16
160734-04		JR @ 7200 South	7/16/16
160734-05	4994520	JR @ Bangerter	7/16/16
160734-06	4994740	JR @ Outlet	7/16/16
160734-07	4992970	Big Cottonwood AB JR	7/16/16
160734-08	4993580	Little Cottonwood AB JR	7/16/16
160734-09	4993470	JR @ 4500 S	7/16/16
160734-10		7800S 4000W	7/16/16
160734-11		8700S 3400W	7/16/16
160734-12	4917370	1 mile east Pelican Point	7/14/16
160734-13	4917500	3 miles WNW Lincoln Beach	7/14/16
160734-14	4917620	Goshen Bay	7/14/16
160734-15	4917700	2.5 miles NE Lincoln Point	7/14/16
160734-16	4917700	Outside Proud Bay	7/14/16
160734-17		Lindon Harbor	7/14/16
160734-18	4917520	2 miles E of Saratoga Springs	7/14/16
160734-19		Water Ski Park	7/14/16
160734-20	4917390	1 mile west of Proud Harbor	7/14/16
160734-21	4917310	½ mile W of Geneva	7/14/16
160734-22		Jordon River @ Narrows	7/15/16
160734-23		Jordon River @ Narrows	7/16/16
160734-24	UTL3	Lincoln Harbor Integrated	7/15/16
160734-25	UTL1	1 mile W of Airport	7/15/16
160734-26	UTL2	Mouth of Proud Harbor	7/15/16
160734-27	4994790	Jordon River BL Utah Lake	7/15/16
160734-28	4917770	Outside Proud Bay	7/15/16
160734-29	4917390	1 mile W of Proud Harbor (2)	7/15/16
160734-30	4917390	1 mile W of Proud Harbor (29)	7/15/16
160734-31	4917310	Utah Lake Geneva	7/15/16
160734-32	4917450	Proud Bay	7/15/16
160734-33	4917520	Utah Lake Saratoga (2)	7/15/16
160734-34	4917500	Utah Lake Lindon Beach (2)	7/15/16
160734-35	UTL4	Saratoga Spring Private Marina	7/15/16
160734-36	4917370	Utah Lake @ Pelican Point	7/15/16
160734-37	UTL 5	Lincoln beach	7/15/16
160734-38	UTL 6	Lincoln Harbor Surface Scum	7/15/16
160734-39	UTL 7	Sandy Beach	7/15/16

Analytes: Anatoxin-a (ANTX-A), anatoxin-a(s) (ANTX-A(s)), β-*N*-methylamino-L-alanine (BMAA), Cylindrospermopsin (CYN), Microcystins (MC), Saxitoxins (STX)

Sample Prep

The samples were ultra-sonicated to lyse cells. Aliquots were analyzed for STX with approximately 20% spiked (lab fortified matrices – LFMs) with STX standard at 0.2 µg/L. Six samples were prioritized for BMAA and Anatoxin-a(s) analysis. Nine samples were analyzed for ANTX-A, CYN and MC (highlighted in red). LFMs were also prepared with 0.1 µg/L ANTX-A (160734-03 & 160734-24), CYN (1.0 µg/L) and MCLR (1.0 µg/L). An internal standard (d3BMAA) was spiked in the samples at a concentration of 10 ppb. Cation solid phase extraction (SPE) was used to pre-concentrate samples for BMAA analysis (10x). Samples were diluted 1:2 with MeOH for the OP/Carbamate assay.

Analytical Methodology

ANTX-A

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 product ions (*m/z* 56, 91, 107, 131 & 149) were monitored.

ANTX-A(s)

An organophosphate (OP)/Carbamate plate assay was used for the detection of a wide range of organophosphates, including anatoxin-a(s). The sample was run (in duplicate) in conjunction with both positive (5 ppb diazinon) and negative controls. A plate reader was used to measure the absorbance values in comparison to controls.

BMAA

LC-MS/MS was utilized for the determination of BMAA. The $[M+H]^+$ ion for BMAA (*m/z* 119) was fragmented and the product ions (*m/z* 73, 76, 88, 101 and 102) were monitored. Transitions of BMAA isomers (DAB, AEG & BAMA), were also monitored (*m/z* 119→46, 73, 76, 88, 101 and 102). Differentiation of isomers was made by retention time and relative abundance of product ions.

MC

The Adda (Abraxis) 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 recoveries of laboratory fortified blanks (LFB) spiked with 1 µg/L MCLR were 107%, 117% and 105%.

CYN
A cylindrospermopsin ELISA (Abraxis) was utilized for the quantitative detection of CYN. The current assay is sensitive down to a LOD/LOQ limit of 0.10 µg/L for CYN. The average LFB recovery was 110%.

STX
A saxitoxin enzyme linked immunosorbent assay (ELISA) was utilized for the quantitative detection of STX. The current assay is sensitive down to a LOD/LOQ limit of 0.05 µg/L STX. The average LFB recoveries were 95%, 105% and 100%.

Summary of Results

Sample	Anatoxin-a(s) (µg/L)	BMAA (µg/L)	ANTX-A (µg/L)	CYN (µg/L)	MC (µg/L)
160734-03	NA	ND	ND	ND	**0.18**
160734-08	–	ND	ND	ND	ND
160734-24	NA	ND	ND	ND	**9.5**
160734-25	–	ND	ND	ND	**0.44**
160734-28	–	ND	ND	ND	ND
160734-33	NA	ND	ND	ND	ND
160734-34	–	ND	ND	ND	ND
160734-37	–	ND	ND	ND	**3.6**
160734-38	–	ND	ND	ND	**698**
Detection Limits (µg/L)	*NA*	*10*	*0.05*	*0.1*	*0.15*

ND = Not detected above the detection limit
\+ = positive response
– = positive response
NA = not applicable
TBD = to be determined

Summary of STX Results

Detection Limit = 0.05 µg/L

LAB ID	STX (µg/L)	LAB ID	STX (µg/L)	LAB ID	STX (µg/L)
160734-01	ND	160734-14	ND	160734-28	ND
160734-02	ND	160734-15	ND	160734-29	ND
160734-03	ND	160734-16	ND	160734-30	ND
160734-04	ND	160734-17	ND	160734-31	ND
160734-05	ND	160734-18	ND	160734-32	ND
160734-06	ND	160734-19	ND	160734-33	ND
160734-07	ND	160734-20	ND	160734-34	ND
160734-08	ND	160734-21	ND	160734-35	ND
160734-09	ND	160734-22	ND	160734-36	ND
160734-10	ND	160734-23	ND	160734-37	ND
160734-11	ND	160734-24	ND	160734-38	ND
160734-12	ND	160734-25	ND	160734-39	ND
160734-13	ND	160734-26	ND		

ND = Not detected above the detection limit

Due to the lack of available standards for quantitation, the analysis of anatoxin-a(s) was qualitative in nature, without method detection limits. The assay absorbance values indicated negligible inhibition of the enzyme acetylcholinesterase when compared to the control solutions. Therefore the samples did not have detectable organophosphates, such as anatoxin-a(s).

Submitted by: 

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Date: 7/26/16