

Dermatoxins Report*Project: Florida Department of Environmental Protection*

Submitted to: FDEP Laboratory
Organization: Florida Department of Environmental Protection
Address: 2600 Blaire Stone Rd, MS 6512, Tallahassee, FL, 32399-2400
Email: magan.nbowden@floridadep.gov; Kathleen.lurding@floridadep.gov
Sample Receipt Date: 23 July 2021
Sample Condition: 4.1 °C upon arrival
Report#: 210721_FDEP
Date Prepared: 26 July 2021
Prepared by: Amanda Foss

Table 1: Samples analyzed

<u>Sample ID</u>	<u>Site/Description</u>	<u>Collection Date</u>
2264387	HAB-SD-07212-0900	21 July 2021
2264388	HAB-SD-07212-0920	21 July 2021
2264389	HAB-SD-07212-1400	21 July 2021

Analytes: Lyngbyatoxin-a (LA), Debromoaplysiatoxin (DAT), Aplysiatoxin (AT)

Abbreviations

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

Sample Preparation

Water Sample Freeze-Thaw

The samples were received, inverted to mix, and aliquots transferred to 40 mL vials. Three freeze thaw cycles were employed prior to solid phase extraction (SPE).

Solid Phase Extraction (SPE)

LA/DAT/AT (Dermatoxins)

Aliquots (2 mL) were transferred to glass vials with LFSMs (Table 2). Preconditioned Strata X Polymeric SPE (100 mg) was loaded with sample, rinsed with deionized water, and eluted 100% MeOH. Elutions were blown to dryness (N₂ at 60°C) and reconstituted in 50% MeOH (0.5 mL).

Analytical Techniques

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

Dermatoxins (LA/AT/DAT)

High performance chromatography coupled with tandem mass spectrometry was used for a targeted lyngbyatoxin-a, debromoaplysiatoxin, and aplysiatoxin analysis. The [M+Na]⁺ ion for DAT (*m/z* 615) was fragmented and the product ions (*m/z* 597, 583, 481, and 393) were monitored. The [M-H]⁻ ion for AT (*m/z* 670) was fragmented and product ions (*m/z* 509, 626) were monitored. The [M+H]⁺ ion for LA (*m/z* 438) was fragmented and product ions (*m/z* 410, 395, 302, 271) were monitored. External standard curves were used to determine LFSM returns.

Quality Control

Table 2: LFSM QC samples prepared for analyses pre-extraction.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
DAT	5	2264387	LFSM	53% ^N
LA	5	2264387	LFSM	20% ^N
AT	5	2264387	LFSM	56% ^N
DAT	5	2264389	LFSM	49% ^N
LA	5	2264389	LFSM	28% ^N
AT	5	2264389	LFSM	68% ^N

*Control limits: water LFSM $\pm 30\%$; complicated matrix LFSM and when LFSM within $2 \times \text{MDL} \pm 50\%$; IS $\pm 50\%$

The N Qualifier is used as spike returns were low ($<70\%$) for all dermatotoxins, likely due to losses during SPE. The MRLs reflect the reported spike returns.

Qualifier	Flag
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

Summary of Results

Table 5: Summary of results in ng/mL for debromoaplysiatoxin (DAT), lyngbyatoxin-A (LA), and aplysiatoxin (AT).

Sample ID	DAT (ng/mL)	LA (ng/mL)	AT (ng/mL)
2264387	ND	ND	ND
2264388	ND	ND	ND
2264389	ND	ND	ND
<i>MRL (ng/mL):</i>	<i>0.5</i>	<i>0.1</i>	<i>0.3</i>
<i>Analyst Initials:</i>	<i>AF</i>	<i>AF</i>	<i>AF</i>
<i>Date Analyzed:</i>	<i>7/26/2021</i>	<i>7/26/2021</i>	<i>7/26/2021</i>

Interpretations:

Dermatoxins (lyngbyatoxin-a, debromoaplysiatoxin aplysiatoxin) were not detected in the submitted samples above the method reporting limits.

Submitted by: 
Mark T. Aubel, Ph.D.
Lab Director
Date: July 28, 2021

*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.*

Chandrasekhar, Thekkekalathil

From: amandafoss@greenwaterlab.com
Sent: Thursday, August 5, 2021 4:49 PM
To: Chandrasekhar, Thekkekalathil
Cc: 'Mark Aubel'
Subject: RE: Dermatoxin Data 7/21/21

The extractions began at 1200, finished 1600. Analyzed shortly thereafter.

From: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Sent: Thursday, August 5, 2021 4:41 PM
To: amandafoss@greenwaterlab.com
Subject: RE: Dermatoxin Data 7/21/21

Do you not record the exact time a sample prep has begun?

Chandra

From: amandafoss@greenwaterlab.com <amandafoss@greenwaterlab.com>
Sent: Thursday, August 5, 2021 4:29 PM
To: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Cc: 'Mark Aubel' <markaubel@greenwaterlab.com>
Subject: RE: Dermatoxin Data 7/21/21

Hi Chandra,
The samples were all extracted on 7/26/2021 between 1200 – 1600.
Amanda

From: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Sent: Thursday, August 5, 2021 10:38 AM
To: amandafoss@greenwaterlab.com
Cc: Mark Aubel <markaubel@greenwaterlab.com>
Subject: FW: Dermatoxin Data 7/21/21
Importance: High

Amanda,

Could you please send us the sample preparation date and time for the samples in the attached report. Thank you.

Chandra

T. M. Chandrasekhar, Ph.D.
Quality Assurance Officer
DEP Laboratory, Scientific Support Services Program

Florida Department of Environmental Protection
2600 Blair Stone Road, Tallahassee, FL 32399-2400
Thekkekalathil.Chandrasekhar@floridadep.gov
Office: 850.245.8075

From: Chandrasekhar, Thekkekalathil
Sent: Wednesday, August 4, 2021 9:28 AM
To: Mark Aubel <markaubel@greenwaterlab.com>; amandafoss@greenwaterlab.com; Bowden, Magan <Magan.Bowden@dep.state.fl.us>; Lurding, Kathleen <Kathleen.Lurding@FloridaDEP.gov>
Subject: RE: Dermatoxin Data 7/21/21

Mark,

Does this analysis had a preparation? If so, could you please provide a prep date and time for the three samples. Thank you.

Chandra

From: Mark Aubel <markaubel@greenwaterlab.com>
Sent: Wednesday, August 4, 2021 2:19 AM
To: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>; amandafoss@greenwaterlab.com; Bowden, Magan <Magan.Bowden@dep.state.fl.us>; Lurding, Kathleen <Kathleen.Lurding@FloridaDEP.gov>
Subject: RE: Dermatoxin Data 7/21/21

Hi Chandra,

I apologize for the delayed response. Method blanks (fortified or not) are typically not employed in routine analyses unless positive data needs to be validated or recoveries are too low to establish an acceptable MRL (i.e. troubleshooting). In this case, multiple LFSMs were utilized to determine MRLs and thus confirm the absence of toxins above the MRLs. Instrument blanks and CCCs are always employed for analysis but typically not reported. We would be glad to discuss further.

Regards,
Mark

Contact Info:
Mark T. Aubel, Ph.D.
GreenWater Lab/**Cyano**Lab
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Palatka, FL 32177
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algal toxin analysis...research...consulting

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From: Chandrasekhar, Thekkekalathil [<mailto:Thekkekalathil.Chandrasekhar@dep.state.fl.us>]

Sent: Thursday, July 29, 2021 3:09 PM

To: amandafoss@greenwaterlab.com; Bowden, Magan; 'Mark Aubel'; Lurding, Kathleen

Subject: RE: Dermatotoxin Data 7/21/21

Amanda,

Your report does not include any QC data on the method blank. Did you also run a lab-fortified blank in addition to the matrix spikes?

Chandra

T. M. Chandrasekhar, Ph.D.

Quality Assurance Officer

DEP Laboratory, Scientific Support Services Program

Florida Department of Environmental Protection

2600 Blair Stone Road, Tallahassee, FL 32399-2400

Thekkekalathil.Chandrasekhar@floridadep.gov

Office: 850.245.8075

From: amandafoss@greenwaterlab.com <amandafoss@greenwaterlab.com>

Sent: Thursday, July 29, 2021 2:23 PM

To: Bowden, Magan <Magan.Bowden@dep.state.fl.us>; 'Mark Aubel' <markaubel@greenwaterlab.com>; Lurding, Kathleen <Kathleen.Lurding@FloridaDEP.gov>

Cc: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>

Subject: RE: Dermatotoxin Data 7/21/21

Hi Magan,

Here are the times of analysis:

Sample ID	Date	Time
2264387	7/26/2021	1621
2264387 LFSM	7/26/2021	1634
2264388	7/26/2021	1647
2264389	7/26/2021	1700
2264389 LFSM	7/26/2021	1713

Let us know if you need anything else,
Amanda

From: Bowden, Magan <Magan.Bowden@dep.state.fl.us>
Sent: Thursday, July 29, 2021 9:24 AM
To: Mark Aubel <markaubel@greenwaterlab.com>; Lurding, Kathleen <Kathleen.Lurding@FloridaDEP.gov>
Cc: amandafoss@greenwaterlab.com; Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Subject: RE: Dermatoxin Data 7/21/21

Mark,

Would you happen to know the analysis time for the samples?

Thanks,

Magan S. Bowden

Environmental Specialist III
Purchasing & Overflow Coordinator
DEP Laboratories
2600 N. Blair Stone Road
Tallahassee, FL 32399
Office: 850-245-8095

From: Mark Aubel <markaubel@greenwaterlab.com>
Sent: Wednesday, July 28, 2021 11:04 PM
To: Bowden, Magan <Magan.Bowden@dep.state.fl.us>; Lurding, Kathleen <Kathleen.Lurding@FloridaDEP.gov>
Cc: amandafoss@greenwaterlab.com
Subject: Dermatoxin Data 7/21/21

Hi Magan & Kathleen,

The dermatotoxin report is attached for the samples (2264287, 2264388, 2264389) collected on 7/21/21. Please let us know if you have any questions.

Regards,
Mark

Contact Info:
Mark T. Aubel, Ph.D.
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205 Zeagler Drive, Suite 302
Palatka, FL 32177
www.greenwaterlab.com



algal toxin analysis...research...consulting

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Chandrasekhar, Thekkekalathil

From: amandafoss@greenwaterlab.com
Sent: Friday, August 13, 2021 6:18 PM
To: Chandrasekhar, Thekkekalathil
Cc: 'Mark Aubel'; 'Shirley Rodman'
Subject: RE: Dermatoxin Data 7/29/21

Follow Up Flag: Follow up
Flag Status: Flagged

Chandra,
DAT = 0.01 ng/mL
LA = 0.02 ng/mL
AT = 0.02 ng/mL
Using S:N = 3
Amanda

From: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Sent: Friday, August 13, 2021 4:56 PM
To: amandafoss@greenwaterlab.com
Cc: 'Mark Aubel' <markaubel@greenwaterlab.com>; 'Shirley Rodman' <shirleyrodman@greenwaterlab.com>
Subject: RE: Dermatoxin Data 7/29/21

Amanda,

What are the MDLs of the analytes in the attached report? Only the reporting limits are mentioned in the report. Thank you.

Chandra

From: amandafoss@greenwaterlab.com <amandafoss@greenwaterlab.com>
Sent: Thursday, August 12, 2021 3:21 PM
To: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Cc: 'Mark Aubel' <markaubel@greenwaterlab.com>; 'Shirley Rodman' <shirleyrodman@greenwaterlab.com>
Subject: RE: Dermatoxin Data 7/29/21

Chandra,
Per my below email, I will address the MDLs tomorrow when I have eyes on the raw data.
Amanda

From: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Sent: Thursday, August 12, 2021 3:13 PM
To: amandafoss@greenwaterlab.com
Subject: RE: Dermatoxin Data 7/29/21

Amanda,

Thank you for your explanation on holding times. Could you provide us information on MDLs from an earlier report (see attached). The most recent report had information on MDLs in the Analysis Techniques section on page 2. This was not the case for the earlier report. Thank you.

Chandra

T. M. Chandrasekhar, Ph.D.

Quality Assurance Officer

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Thekkekalathil.Chandrasekhar@floridadep.gov

Office: 850.245.8075

From: amandafoss@greenwaterlab.com <amandafoss@greenwaterlab.com>

Sent: Thursday, August 12, 2021 3:04 PM

To: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>

Cc: 'Mark Aubel' <markaubel@greenwaterlab.com>; 'Shirley Rodman' <shirleyrodman@greenwaterlab.com>

Subject: RE: Dermatoxin Data 7/29/21

Chandra,

I am not sure what hold times you are referencing. Our hold times are arbitrarily 10-days for most the non-traditional algal toxins, but experimental hold times for the dermatoxins have not been established for fresh or saline water samples. It is possible that hold times for these samples might match those assessed for anatoxin-a/cylindrospermopsin by the US EPA, which is 28 days from sample collection, or longer. The aplysiatoxins and lyngbyatoxin are not regulated in any way, official or standard methods have not been published, and very little work has been conducted outside of the research arena.

Upon review of the most recent report, for our arbitrary hold times, there was a 1-day exceedance that I had not included in the report. Please find the attached amended report to reflect this exceedance for your records. A mistake was also made on date of sample receipt which has been corrected and notated.

In the future, please submit samples closer to their collection time to allow us the time for processing, or provide us with a heads up that you will be sending samples for arrival 5 days post collection. This is our busiest time of year and your consideration would be appreciated.

With regard to the detection limits, I will address that question when I return to the lab tomorrow. I do not have the raw data or notes in front of me. The method for marine water (saline) is very different than for fresh water, as you may have read in the reports. The method detection limits will be different.

It would also be greatly appreciated that you provide minimum reporting requirements ahead of sample submission so that we can properly address all of your concerns ahead of time and assure we can accommodate your needs. A little context to the inquiries would also be appreciated.

Regards,
Amanda

From: Chandrasekhar, Thekkekalathil [<mailto:Thekkekalathil.Chandrasekhar@dep.state.fl.us>]
Sent: Thursday, August 12, 2021 1:40 PM
To: Mark Aubel
Cc: Bowden, Magan
Subject: RE: Dermatotoxin Data 7/29/21
Importance: High

Mark,

The sample in this was prepared on Aug 9, four days after the sample prep expired (Aug 5). The method has a 7 day holding time from collection until extraction followed by a 40 day holding time for analysis. The sample was received at your lab on Aug 2. Was there a reason for the delayed extraction? Thank you.

Chandra

T. M. Chandrasekhar, Ph.D.

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From: Bowden, Magan <Magan.Bowden@dep.state.fl.us>
Sent: Thursday, August 12, 2021 10:10 AM
To: Chandrasekhar, Thekkekalathil <Thekkekalathil.Chandrasekhar@dep.state.fl.us>
Subject: FW: Dermatotoxin Data 7/29/21

Results uploaded.

Magan S. Bowden
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Purchasing & Overflow Coordinator
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2600 N. Blair Stone Road
Tallahassee, FL 32399
Office: 850-245-8095

From: Mark Aubel <markaubel@greenwaterlab.com>
Sent: Monday, August 9, 2021 11:50 PM
To: Bowden, Magan <Magan.Bowden@dep.state.fl.us>; Lurding, Kathleen <Kathleen.Lurding@FloridaDEP.gov>

Cc: amandafoss@greenwaterlab.com

Subject: Dermatoxin Data 7/29/21

Hi Magan & Kathleen,

The dermatotoxin report is attached for the water sample (2266193) collected on 7/29/21. Please let us know if you have any questions.

Regards,
Mark

Contact Info:
Mark T. Aubel, Ph.D.
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