Rapid Response Survey of Cyanobacteria Toxin Levels Downstream of North Fork Shenandoah River Algal Bloom After Tropical Storm Ida, 2021

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Interstate Commission on the Potomac River Basin (ICPRB)

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Summary

The Virginia Department of Health (VDH) issued a Harmful Algae Bloom (HAB) Advisory for a 53-mile stretch of the North Fork of the Shenandoah River on August 10, 2021 (Figure 1, left). Samples from multi-species algal mats on the river bottom contained harmful levels of toxins produced by cyanobacteria. Three weeks later, Tropical Storm Ida passed over the North Fork, dumping torrential rain on the watershed. Sharply rising streamflows were expected to scour the benthic algal mats, potentially lysing their cells and releasing toxins as they washed downstream. The ICPRB's Emergency River Spill Model (ERSM) indicated the scoured material's leading edge would reach the Potomac River mainstem by September 2nd - 4th and Great Falls near Washington, D. C. by September 3rd - 6th.

Virginia Department of Environmental Quality (VADEQ) staff confirmed the algal mats were scoured off the river bottom. Water samples collected by ICPRB at the Shenandoah River mouth (Figure 1, right) indicate the storm's high flows diluted the algal cells and their associated toxins to below-detection levels before they reached the Potomac River. If flows had been less intense, we hypothesize the scoured material and toxins could potentially have reached the Potomac River mainstem. More advanced flow modeling and additional sampling during algal blooms could better characterize the potential transport of scoured or senescing algal blooms in the Shenandoah River under different river conditions.

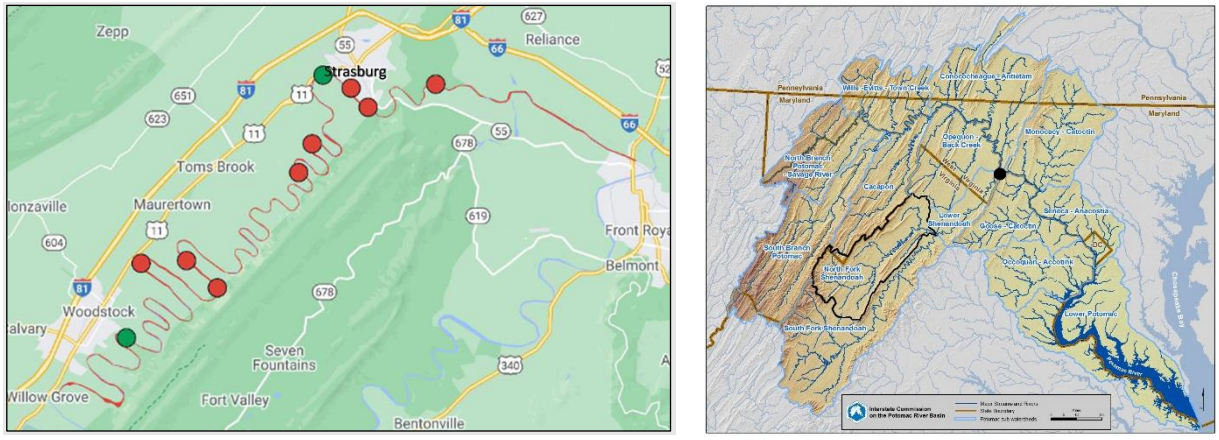

Figure 1. *North Fork Shenandoah River.* ***Left****, Known presence of the cyanobacterial toxins (red dots) in the North Fork Shenandoah River (VDH 2021c).* ***Right****, Watershed of the North Fork (black outline) with respect to the Potomac River basin, the ICPRB survey sampling location at the Shenandoah River mouth near Harper's Ferry, WV (black dot), and Washington D.C. (from ICPRB).*

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Introduction

Cyanobacteria can produce anatoxins, saxitoxins, and microcystins which can impact wildlife, recreation, human health, drinking water supply and ecosystem services. Contact with cyanotoxins causes skin rashes and gastrointestinal illness; ingestion can be fatal. Virginia Department of Environmental Quality (VADEQ) found extensive multi-species benthic algal mats in the North Fork Shenandoah River at Bethel Road and Strasburg, VA, on July 13 and 19, 2021. Analysis showed the mats contained several cyanobacteria taxa and high levels of cyanotoxins.

Figure 2. Rt. 340 bridge over Shenandoah River near Harpers Ferry, WV (R. Bourassa, ICPRB).

Based on these results, Virginia Department of Health (VDH) declared a health advisory on July 23, 2021, for the river between Bethel Road and Strasburg (VDH 2021a). With additional sampling, the advisory was expanded on August 10, 2021, to encompass a 53-mile stretch of the river (Figure 1, left) (VDH 2021b, c). At the time, the mats were intact and did not appear to be releasing measurable toxins to the water column. In addition to cautioning recreational users against contact with the river water, VDH worked with several townships along the North Fork to ensure their drinking water supplies were not affected by cyanotoxins (e.g., VADEQ 2021a, Paullin 2021).

Tropical Storm (TS) Ida came through the North Fork Shenandoah River watershed on September 2, 2021, dumping up to 3.9 inches of rain on parts of the watershed and sharply raising flow rates. Anticipating the storm's potential to scour the algal mats and wash them downstream, the Interstate Commission on the Potomac River (ICPRB) implemented a survey to determine if measurable algal toxin levels reached the Potomac River mainstem after the storm. Samples were collected from the Rt 340 bridge (Figure 2) near the mouth of the Shenandoah River when time-of-travel estimates indicated the scoured North Fork algal material would be passing.

Methods and Materials

The ERSM Model created by ICPRB is based on time of travel dye studies conducted by the U. S. Geological Survey (ICPRB 2015). It was used to estimate hypothetical travel times of the scoured material's leading edge down the Shenandoah to the river's confluence with the Potomac River mainstem and eventually to Great Falls near Washington D.C. (Figure 1, right). Four ERSM model runs reflecting different river conditions were made as TS Ida approached the region to understand the range of possible future conditions and as more accurate forecasts became available. Model runs were based on the following assumptions:

- "Spill" referred to scouring and mobilization of benthic algae in the river.
- The spill started in the Shenandoah River just upstream of Front Royal at noon on Wednesday, September 1, 2021.
- The spill occurred as a 1-hour instantaneous event to understand time of travel of the leading edge.

• Spill material was dissolved and instantly and uniformly mixed across a stream channel and through the water column at the initial spill point and also when a tributary joins a larger stream.
• Streamflows were constant throughout the modeled time period.
• Very high or very low river flows can affect model accuracy. High flows simulated as part of several model runs described here were outside of the calibrated range of the model.
• Accuracy also is dependent on the quality of information provided, including spill location, contaminant type and amount, and duration of event.
• Toxin decay was not included in the simulation.

Water samples were collected twice daily between September 4 and 7, 2021, from the Rt 340 bridge. The bridge crosses the Lower Shenandoah River approximately 105 km (65.2 mi) downstream of Strasburg, VA, and 1.25 km (0.78 mi) upstream of the river's confluence with the Potomac mainstem. Samples were collected via rope and bucket from the Shenandoah River Bridge at ¼, ½, and ¾ river width (Figure 2). The three bridge samples were combined and mixed well in a large sampling vessel. From the integrated sample, one 250ml (dark plastic Nalgene specimen bottle) and one 100ml (dark glass bottle) aliquot were collected and immediately put on ice. Samples were kept near freezing from time of collection to date of shipment to GreenWater Laboratories in Palatka, Florida on September 8, 2021.

GreenWater Laboratories personnel first scanned 1 ml aliquots of each sample for known toxin-producing taxa (PTOX), then tested the samples for total microcystins / nodularins (MCs/NODs) as measured through the MMPB method, anatoxin-a (ATX), cylindrospermopsin (CYN), saxitoxins (STX) and the dermatoxins (debromoaplysiatoxin (DAT), lyngbyatoxin-A (LA), aplysiatoxin (AT)). The laboratory methods are described in the Appendix.

Results

ERSM model results indicated the leading edge of scoured North Fork algal material could arrive between the evening of September 2nd and late night on September 4th at the raw water intake for Brunswick, MD, located on the Potomac River a few miles downstream of the Rt. 340 bridge and the Shenandoah-Potomac confluence. Algal material was projected to rapidly pass several other Potomac raw water intakes and start arriving at the Washington Aqueduct water supply intakes between the afternoon of September 3rd and late night on September 6th (Table 1). The wide range of arrival times is due to uncertainty in future flow conditions at the time of the model runs. The magnitude, extent, and timing of storm conditions were expected to affect actual arrival times. Due to the approximately 53-mile length of the North Fork bloom, scoured algal material was expected to continue passing the Rt 340 bridge sampling location for several days after the leading edge arrived.

Table 1. Potential arrival (mil time) of leading edge of scoured North Fork Shenandoah algal material at water supply intakes along the Potomac River mainstem below Harpers Ferry, WV.

	Early Arrival Time Scenario*	Late Arrival Time Scenario**
Brunswick, MD	Thurs, Sep 02, 1900	Sat, Sep 04, 2330
Frederick Co., MD	Fri, Sep 03, 0200	Sun, Sep 05, 0930
Leesburg, VA	Fri, Sep 03, 0630	Sun, Sep 05, 1900
Fairfax Water, VA	Fri, Sep 03, 1000	Mon, Sep 06, 0330
Washington Suburban San. Commission, MD	Fri, Sep 03, 1200	Mon, Sep 06, 0930
Rockville, MD	Fri, Sep 03, 1230	Mon, Sep 06, 1100
Washington Aqueduct, Great Falls	Fri, Sep 03, 1300	Mon, Sep 06, 1300
Washington Aqueduct, Little Falls	Fri, Sep 03, 1630	Mon, Sep 06, 2230

* Flows based on the value exceeded 5 percent of the time in the observed gage record.
** Flows based on the value exceeded 35 percent of the time in the observed gage record.

GreenWater Laboratory found no detectable toxigenic algae in the eight water column samples collected September 4 – 7, 2021 at the Rt. 340 bridge. The laboratory also found no detectable concentrations of cyanotoxins in 4 representative daily samples. A single filament of the toxigenic *Geitlerinema splendidum* was found in a one milliliter aliquot of the afternoon sample collected on September 5, 2021. The GreenWater Laboratory reports are in the Appendix.

Discussion

Analysis of VADEQ July and August samples of the benthic algal mats in the North Fork Shenandoah River identified multiple cyanobacteria capable of producing toxins, including *Geitlerinima*, *Lygnbya*, *Microcoleus*, *Pseudanabaena*, *Oscillatoria*, *Phormidium*, *Planktothrix*, and *Limnothrix*. The sum of the mat concentrations of microcystin, cylindrospermopsin, anatoxin-a and saxitoxin was estimated to be as high as 2,806 ppb, though laboratory methods are evolving and this number is not a precise quantification (VDH/ODU August 23, 2021). *Microcoleus*, previously *Phormidium* and a known anatoxin producer, was the most prevalent cyanobacteria taxa (T. Egerton VDH, pers. comm.). Concentrations of anatoxin-a within the mats were highest of the four cyanotoxins and the reason for the advisories.

Oscillatoria, *Phormidium*, *Planktothrix*, and *Limnothrix* filaments were found in July and August in the water column samples from the 53-mile bloom reach. However, their cell densities were not high (500 – 7,800 cells per liter). Cyanotoxin concentrations in the water column samples were typically below the analysis method detection limits (bdl). None exceeded the Virginia Department of Health's draft cyanobacteria bloom recreational advisory thresholds, i.e., 8 ppb for microcystin, 15 ppb for cylindrospermopsin, 8 ppb for anatoxin-a, and 4 ppb for saxitoxin (VDH 2021d).

The North Fork Shenandoah River is the raw water source for several towns including Woodstock, Strasburg, and Winchester, VA. Further downstream, the Lower Shenandoah River is the raw water source for Charleston and Harpers Ferry, WV. Anatoxin-a was detected in raw and/or finished water samples at Strasburg on August 3rd, 9th, and 12th but not detected in subsequent samples (Table 2).

*Table 2. Cyanotoxin concentrations (µg/L) measured in the raw water and [finished water] at drinking water utilities on the North Fork Shenandoah River; nd, non-detect; *, filtered raw water. One microgram per liter (µg/L) equals one part per billion (ppb). Data from VDH/ODW (2021).*

Date	Location	Test	Anatoxin-a	Cylindro-spermopsin	Microcystin	Nodularin	Saxitoxin
8/3/2021	Strasburg	ELISA	1.057 [*0.277*]	nd [*0.062*]	nd [*nd*]		nd [*nd*]
8/9/2021	Strasburg	ELISA	[*0.316*]				
8/10/2021	Winchester	L-231	nd*	nd*	nd*	nd*	
8/11/2021	Winchester	L-231	[*nd*]	[*nd*]			
8/12/2021	Strasburg	ELISA	0.248 [*nd*]				
8/16/2021	Strasburg	ELISA	nd [*nd*]	nd [*nd*]	nd [*nd*]		nd [*nd*]
8/16/2021	Winchester	L-231	nd [*nd*]	nd [*nd*]	nd [*nd*]	nd [*nd*]	
8/18/2021	Woodstock	ELISA	[*nd*]	[*nd*]	[*nd*]		[*nd*]
8/19/2021	Strasburg	ELISA	nd [*nd*]	nd [*nd*]	nd [*nd*]		nd [*nd*]
8/19/2021	Winchester	ELISA	[*nd*]	[*nd*]	[*nd*]		[*nd*]
8/19/2021	Winchester	L-231	nd [*nd*]	nd [*nd*]	nd [*nd*]	nd [*nd*]	
8/23/2021	Strasburg	ELISA	nd [*nd*]	nd [*nd*]	nd [*nd*]		nd [*nd*]
8/23/2021	Winchester	L-231	nd [*nd*]	nd [*nd*]	nd [*nd*]	nd [*nd*]	
8/24/2021	Woodstock	ELISA	nd [*nd*]	nd [*nd*]	nd [*nd*]		nd [*nd*]
8/24/2021	Winchester	ELISA	[*nd*]	[*nd*]	[*nd*]		[*nd*]
8/25/2021	Strasburg	ELISA	nd [*nd*]	nd [*nd*]	nd [*nd*]		nd [*nd*]
8/31/2021	Winchester	ELISA	[*nd*]	[*nd*]	[*nd*]		[*nd*]
9/1/2021	Strasburg	ELISA	nd [*nd*]	nd [*nd*]	nd		nd [*nd*]
9/1/2021	Woodstock	ELISA	nd [*nd*]	nd [*nd*]	nd [*nd*]		nd [*nd*]

Cylindrospermopsin was detected in one finished water sample at Strasburg on August 3rd (0.062 µg/L). Neither toxin exceeded VDH's advisory thresholds. Microcystin, nodularin and saxitoxin were not detected in any samples.

The relatively low cyanotoxin levels in the water column and water supply intake samples suggest the benthic algal mats were still intact for the most part in August and not releasing measurable toxins to the water column. Field observation notes on August 16th and 23rd, however, suggest cyanobacteria mats at one site, 1BNFS017.93 (38.9573, -78.3832), may have been starting to senesce (die) (VADEQ September 14, 2021). As algal mats senesce in late summer or autumn, disintegrating algal cells and their associated toxins are mixed into the water column where they can be transported downstream.

TS Ida was a particularly strong, wet storm. It made landfall near New Orleans on August 29th as a Category 4 hurricane and weakened to a tropical depression as it moved through the eastern United States to New England and out to sea. In response to the storm's very high rainfall totals, stream flows rose to extreme levels along its entire path (Figure 3). The North Fork Shenandoah was squarely in the storm's path.

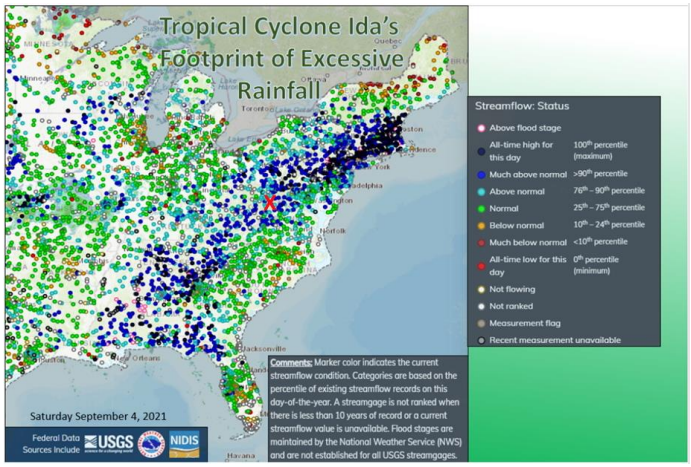

Figure 3. Streamflow status in the eastern United States on September 4, 2021 (USGS). Red X indicates the North Fork Shenandoah River watershed.

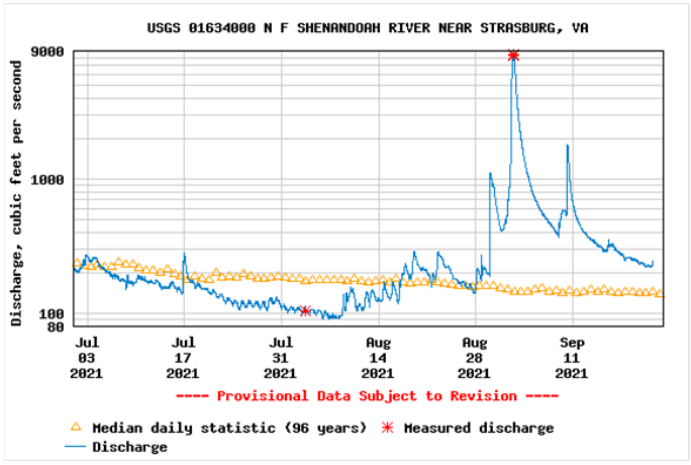

Figure 4. Streamflow at the US Geological Survey Strasburg gage in the North Fork Shenandoah River watershed, July 1 – Sept 22, 2021. Tropical Storm Ida passed over the watershed September 1-2, 2021 (USGS).

The Mid-Atlantic River Forecast Center (MARFC) forecast on August 30th estimated the center of TS Ida would move through the Mid-Atlantic region on September 1 – 2, 2021, with 2 – 4 inches of rain expected for much of the region. Between September 1st and 3rd, the US Weather Service recorded 3.9 inches of rain falling at Harrisonburg, VA, near the headwaters of the North Fork. Discharge at the Strasburg USGS gage 01634000 (established 1998), located near the downstream end of the 53-mile HAB advisory reach, showed a steep rise in flow beginning mid-day on Wednesday, September 1st and peaking at 8,420 cfs at 9:00 am on Thursday, September 2nd (Figure 4). The storm's peak flow was above the 99.5th percentile of all earlier instantaneous flow observations at Strasburg and well above the maximum instantaneous flow recorded for that date (2,470 cfs).

VADEQ field observations at various sites on the North Fork Shenandoah River before and after TS Ida confirm the storm's high flows scoured the cyanobacteria mats (VADEQ September 14, 2021). Before the storm, on August 23rd and 31st, cyanobacteria mats were present throughout the HAB advisory reach, between river miles 0.57 and 17.93 (Figure 1, left). Mats were also

present in places as far upstream as Timberville, VA, at river mile 87.02. Dense growths of filamentous green algae (FGA) and submerged aquatic vegetation (SAV) co-occurred with the mats at several locations. After the storm, no algal mats were observed in the HAB advisory reach or upstream. The SAV and FGA beds were also gone. VADEQ reported that North Fork "samples collected September 14, 2021, contained cyanobacteria cells and toxins below safe contact levels" (VDH 2021e) and the health advisory for the entire North Fork was lifted on September 16, 2021 (VDH 2021f).

The fact that the North Fork HAB bloom was washed out by TS Ida and cyanotoxins were not detected as the suspended algal material passed the Shenandoah River mouth suggests "dilution was the solution" for this HAB event. Specifically, **TS Ida's very high flows diluted the cyanotoxins in the scoured algal mats to non-detectable levels before they reached the Potomac mainstem.** We posit this might not be the case when streamflows are lower, as is more typical of late summer and early autumn, and cyanobacteria blooms are senescing. Further investigation of the downstream transport of cyanotoxins in the Potomac River and its tributaries could be done with river flow models that are more advanced than the ERSM model. While adequate for the purposes of this rapid response survey, the ERSM is a relatively simple "spill model" with built-in assumptions that are not necessarily met and features that cannot be changed to reflect actual river conditions. Additional field observations and algal bloom sampling would also provide a better understanding of how rapidly cyanotoxins decompose once they are released into the water column and exposed to different river conditions.

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Appendix

GreenWater Laboratory cyanobacteria taxa and cyanotoxin reports to ICPRB

Potentially Toxigenic (PTOX) Cyanobacteria Screen

Project: ICPRB

Submitted to: Gordon Selckmann
Organization: ICRPB
Address: 30 West Gude Dr., Rockville, MD, 20850
Email: GMSelckmann@icprb.org

Sample Receipt Date: 09 September 2021
Sample Condition: 16.2 ºC upon arrival
Report# 210904-210905-210906-210907_PTOX_ICPRB
Date Prepared: 09 September 2021
Prepared by: Alyssa Garvey

Sample ID	Site	Collected
Harp-BR1P	Harpers Ferry, WV	04 September 2021
Harp-BR2P	Harpers Ferry, WV	04 September 2021
Harp-BR3P	Harpers Ferry, WV	05 September 2021
Harp-BR4P	Harpers Ferry, WV	05 September 2021
Harp-BR5P	Harpers Ferry, WV	06 September 2021
Harp-BR6P	Harpers Ferry, WV	06 September 2021
Harp-BR7P	Harpers Ferry, WV	07 September 2021
Harp-BR8P	Harpers Ferry, WV	07 September 2021

Method

A one mL aliquot of each non-preserved sample was prepared using a Sedgewick Rafter cell. The samples were scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon TE200 Inverted Microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs.

Results

Harp-BR1P

Potentially toxigenic (PTOX) cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

Harp-BR2P

PTOX cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

Harp-BR3P
PTOX cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

Harp-BR4P
The PTOX cyanobacteria *Geitlerinema splendidum* (1 filament per mL) was observed. The sample also contained abundant particulate matter, diatoms (Bacillariophyta), green algae (Chlorophyta), and flagellated algae.

Harp-BR5P
PTOX cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

Harp-BR6P
PTOX cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

Harp-BR7P
PTOX cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

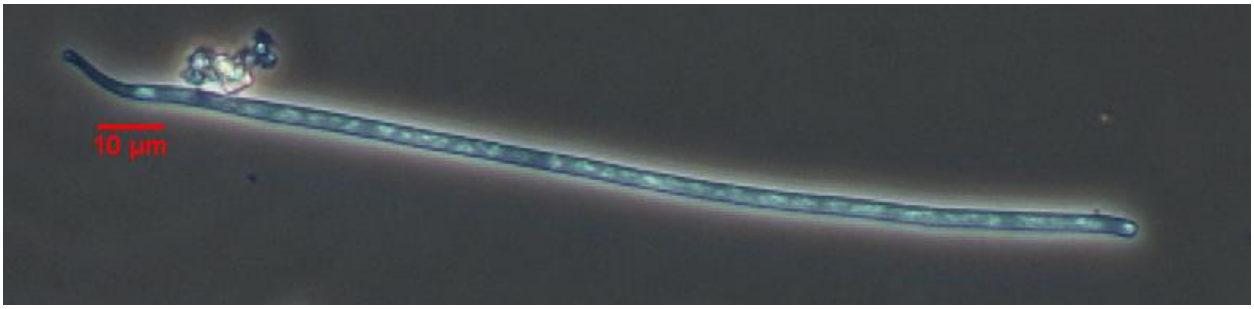
Harp-BR8P
PTOX cyanobacteria were not observed. The sample contained abundant particulate matter, diatoms (Bacillariophyta), and flagellated algae.

Potential toxin producing genera observed include:

Microcystins	Saxitoxins	Anatoxin-a	Cylindrospermopsin
Geitlerinema	*Geitlerinema*	*Geitlerinema*	

Recommendations:
Based on limited PTOX cyanobacterial abundance, toxin analyses are not currently recommended.

Micrographs



Geitlerinema splendidum at 400X (**Harp-BR4P**)

Submitted by: Amanda Foss Date: September 9, 2021

Amanda Foss, M.S.

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.

Anatoxin-a, Cylindrospermopsin, Dermatoxin, Microcystins/Nodularins, and Saxitoxins Report

Project: ICPRB

Submitted to:	Gordon Selckmann
Organization:	ICPRB
Address:	30 West Gude Dr., Rockville, MD, 20850
Email:	GMSelckmann@icprb.org
Sample Receipt Date:	09 September 2021
Sample Condition:	16.2 ºC upon arrival
Report#	210904-0907_ ICPRB
Date Prepared:	16 September 2021
Prepared by:	Amanda Foss

Table 1: Samples analyzed and collection dates

Sample ID	Site	Collected
Harp-BR2P	Harpers Ferry, WV	04 September 2021
Harp-BR4P	Harpers Ferry, WV	05 September 2021
Harp-BR6P	Harpers Ferry, WV	06 September 2021
Harp-BR8P	Harpers Ferry, WV	07 September 2021

Toxins – Anatoxin-a (ATX), Cylindrospermopsin (CYN), Adda Microcystins/Nodularins (MCs/NODs), Saxitoxins (STX/PSTs)**,** 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 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.

ATX/CYN/STX
Samples were prepared neat with LFSMs (Table 3) for analysis with a STX ELISA. Samples for ATX and CYN were prepared as 1 mL aliquots containing the IS with LFSMs (Table 3). Aliquots were filtered (0.2 µm PVDF) prior to analyses.

LA/DAT/AT (Dermatoxins)
Aliquots (1 mL) were diluted 1:1 with MeOH with duplicate subsets prepared as LFSMs (Table 3). Samples were lysed by water bath sonication for 10 min followed by filtration (0.2 µm PVDF) and analysis.

MCs/NODs by MMPB
Aliquots (2 mL) spiked with IS (d_3-MMPB) were oxidized by the addition of 1 mL of a solution containing 0.1 M K_2CO_3, 0.05 M $KMnO_4$ and 0.05 M $NaIO_4$ for 1 hour, stopped with the addition of 40% sodium bisulfite and cleaned using 100 mg Strata X solid phase extraction (SPE). The extracts were reconstituted in water, filtered through 0.2 µm PVDF and analyzed for MMPB.

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

Analytical Techniques

Enzyme-Linked Immunosorbent Assay (ELISA)

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

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

ATX & CYN

High performance chromatography coupled with tandem mass spectrometry was used for a targeted anatoxin-a and cylindrospermopsin 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 CYN (*m/z* 416) was fragmented and the product ions (*m/z* 194, 274, 336) 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 $[M+H]^+$ ion for the internal standard $[^{15}N_5]$CYN (421 *m/z*) was fragmented and the product ion (*m/z* 341) was monitored. The internal standard method was utilized for quantification.

MMPB

The $[M-H]^-$ ion of MMPB (*m/z* 207) was fragmented and the product ion (*m/z* 131) was monitored. The IS (d_3-MMPB) was also fragmented and monitored (*m/z* 210→131). The internal standard method was implemented using a standard curve (0.25 – 10 ng/mL of oxidized MC-LR) to calculate LFSM returns.

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: 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
MC-LR (as MMPB)	1.0	Harp-BR2P	LFSM	70%
d_3-MMPB	1.0	all aliquots	IS	94 ± 7%
CYN	0.1	Harp-BR4P	LFSM	126%
[$^{15}N_5$]CYN	1.0	all aliquots	IS	76 ± 11%
ATX	0.1	Harp-BR4P	LFSM	88%
[$^{13}C_4$]ATX	1.0	all aliquots	IS	77 ± 6%
STX	0.1	Harp-BR2P	LFSM	98%
STX	0.1	Harp-BR8P	LFSM	103%
DAT	10	Harp-BR2P	LFSM	117%
LA	10	Harp-BR2P	LFSM	78%
AT	10	Harp-BR2P	LFSM	141%^{N}

**Control limits: water LFSM ± 30%; complicated matrix LFSM ±50%; IS ± 50%*

Table 4: Raw ELISA Data

Sample ID	Analyte	Dilution Factor	Assay Values (ng/mL)	%CV	Concentration (ng/mL)	Average (ng/mL)
Harp-BR2P	STX	1	0.00	0.0	<0.05	**ND**
		1	0.00		<0.05	
Harp-BR2P	STX	1	0.20	2.5	0.20	**0.20**
LFSM		1	0.19		0.19	
Harp-BR4P	STX	1	0.00	0.0	<0.05	**ND**
		1	0.00		<0.05	
Harp-BR6P	STX	1	0.00	0.0	<0.05	**ND**
		1	0.00		<0.05	
Harp-BR8P	STX	1	0.00	0.0	<0.05	**ND**
		1	0.00		<0.05	
Harp-BR8P	STX	1	0.21	3.5	0.21	**0.21**
LFSM		1	0.20		0.20	

Table 4: STX-ELISA Quality Control Value Table

Date Analyzed:	**14 September 2021**	**Requirement**	**Pass/Fail**
R^2 value:	1.000	≥0.98	PASS
%CV range STDs:	0.1-2.5%	≤15%	PASS
LFB (0.2 ppb) recovery:	80%	±40% True Value	PASS
%CV range LFB:	0.1%	<20%	PASS
Low CCC (0.05 ppb) recovery:	100%	±50% True Value	PASS
LRB	<0.03	<0.03	PASS

Summary of Results

Table 3: Summary of results for total microcystins/nodularins (MCs/NODs) as measured through the MMPB method, anatoxin-a (ATX), cylindrospermopsin (CYN), saxitoxins (STX) and the Dermatoxins (debromoaplysiatoxin (DAT), lyngbyatoxin-A (LA), aplysiatoxin (AT)). All data are reported as ng/mL (ppb).

					Dermatoxins		
Sample	**MCs/NODs**	**ATX**	**CYN**	**STX**	**DAT**	**LA**	**AT**
Harp-BR2P	ND	ND	ND	ND	ND	ND	ND
Harp-BR4P	ND	ND	ND	ND	ND	ND	ND
Harp-BR6P	ND	ND	ND	ND	ND	ND	ND
Harp-BR8P	ND	ND	ND	ND	ND	ND	ND
MRL (ng/mL):	*0.06*	*0.05*	*0.05*	*0.05*	*1.0*	*0.5*	*2.5*
Analyst Initials:	*AF*	*MA*	*MA*	*KC*	*AF*	*AF*	*AF*
Date Analyzed:	*9/17/2021*	*9/13/2021*	*9/13/2021*	*9/14/2021*		*9/15/2021*	

Interpretations

Analytes were not detected above the method detection limits in the total (extra-cellular + intra-cellular) fraction; therefore extra-cellular fractions were not analyzed.

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Date: September 17, 2021

The results in this report relate only to the samples listed above.
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