# 2024 South Carolina Cyanotoxin Distribution Project

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Cover Photo: Goose Creek Reservoir (Hanahan, South Carolina)

# 2024 South Carolina Cyanotoxin Distribution Project

by

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# Executive Summary

Harmful algal blooms (HABs) are a concern in the United States and are generally caused by excessive growth of cyanobacteria, or blue-green algae. Cyanobacteria blooms can degrade water quality through increased water column turbidity that reduces light availability for ecologically important vegetation. Die-offs of these blooms can reduce oxygen levels that can lead to fish kills. Some cyanobacteria species produce toxins (cyanotoxins) that are harmful to humans, livestock, and wildlife. In high enough concentrations, algal blooms can also cause nuisance taste and odor issues in drinking water and increase the cost of water treatment.

In 2018, the South Carolina Department of Environmental Services (SCDES), formerly the Department of Health and Environmental Control, initiated the HABs Monitoring Program to investigate the effects that cyanotoxins have on human health and the environment within the State. SCDES aimed to:

- Continue collecting baseline data of cyanotoxin distribution in State reservoirs and estuaries,
- Monitor drinking water intakes with a history of HABs and/or taste and odor issues,
- Issue recreational advisories for waterbodies that exceed SCDES's state standards, and
- Identify potential correlative relationships between cyanotoxin concentrations and other physicochemical water quality parameters.

In 2024, samples were collected and analyzed for microcystins from 110 monthly-monitored sites across South Carolina reservoirs, estuaries, and influent streams. Microcystin samples were collected during the May 1 to October 31 growing season. Five of the 110 stations were sampled starting in April due to a special nutrient study on Lake Monticello and Parr Reservoir. The monthly-monitored sites were coordinated in conjunction with routine sampling conducted by SCDES regional field staff, which allowed data comparison to other parameters collected contemporaneously. In addition to monthly monitoring of lake and estuarine sites, samples were collected from an additional three lakes at four drinking water intakes with past algal issues, including taste and odor complaints. Eleven waterbodies were sampled in response to the occurrence of possible HAB conditions (event-driven samples) from February through October.

Monthly-monitoring concentrations were less than 1 microgram per liter (µg/L) for microcystins. Concentrations greater than the analytical detection level (≥ 0.100 µg/L for ADDA ELISA method or ≥ 0.016 µg/L for SAES ELISA method) were observed in 72% of samples analyzed for microcystins. Toxin concentrations in all monthly-monitoring samples were less than SCDES's state recreational standard of 8 µg/L for microcystins.

Microcystins were also detected at all four drinking water intakes. The drinking water intakes at Lake Rabon (Laurens Commissions of Public Works) and Lake Murray (City of Columbia and City of West Columbia) each had at least three samples that exceeded the USEPA 10-day drinking water health advisory value of 0.3 µg/L for microcystins; however, the treatment processes at all drinking water intakes can remove microcystins at these low concentrations.

There was one recreational advisory issued in 2024 at Lake Rabon for toxin concentrations greater than the recreational standard. The advisory was removed once the microcystin concentrations were below 8 µg/L and the blooms had dissipated. Recreational watches were issued in 2024 at Lake Woodcross, Lake Greenwood, Twin Lakes, Lake Wateree, and Goose Creek Reservoir. Recreational watches are issued when a potential toxin producing bloom is identified on a waterbody but microcystin or cylindrospermopsin concentrations are less than state standards, or the identified algal species could potentially be producing algal toxins that are not in SCDES's state standards.

Correlation analyses were conducted for monthly monitoring microcystin concentration data for Lake Hartwell, Lake Murray, Parr Reservoir, Lake Wylie and Lake Wateree. No strong relationships were determined for microcystin concentrations and water quality parameters including dissolved oxygen, pH, temperature, total phosphorous, nitrogen: phosphorus ratio, and chlorophyll-*a* for any of the lakes.

This assessment builds on the past years studies and broadens the baseline understanding of cyanotoxin distributions across the State. Future goals of the HABs Monitoring Program include evaluating additional toxins, such as anatoxins and saxitoxins, expanding sampling to large rivers and streams, and including probability-based, or "random", lake sampling stations. This will further enhance the State's growing understanding of cyanotoxin distributions.

# Introduction and Background

Harmful algal blooms (HABs) are an increasing concern in U.S. waters. These blooms occur when algae grow excessively in response to elevated nutrient concentrations, typically from non-point and point source runoff due to a variety of land-uses. In high enough densities, blue-green algae, or cyanobacteria, can impact aquatic life and human health by degrading water quality and producing cyanotoxins. There is growing recognition of the need for increased monitoring of cyanotoxin concentrations in waterbodies and in the water treatment process (Jetto, Grover, & Krantxberg, 2015). The U.S. Environmental Protection Agency (USEPA) has issued health advisory criteria (U.S. Environmental Protection Agency, 2019) and recreational advisory criteria (U.S. Environmental Protection Agency, 2015b,c) for two cyanotoxins (microcystins and cylindrospermopsin). Exposure to high levels of microcystins can lead to liver, reproductive, developmental, kidney, and gastrointestinal effects (U.S. Environmental Protection Agency, 2019). Exposure to high levels of cylindrospermopsin can affect the liver, kidneys, and potential deformation of red blood cells (U.S. Environmental Protection Agency, 2019).

The South Carolina Department of Environmental Services (SCDES, formerly Department of Health and Environmental Control[1]) has maintained a robust surface water monitoring network since the 1950s. With the advancement of cyanotoxin analytical methods, SCDES established the HABs Monitoring Program in 2018 to monitor cyanotoxins statewide. A primary objective of the HABs Monitoring Program is to establish a statewide baseline and context for interpretation of cyanotoxin concentrations in South Carolina's waters, which was accomplished with the adoption of the USEPA's recreational advisory criteria (Table 1) in SCDES's State standards in 2020.

[1] On July 1, 2024, the South Carolina Department of Health and Environmental Control was dissolved into two separate agencies, creating the South Carolina Department of Environmental Services and South Carolina Department of Public Health.

**Table 1**: SCDES recreational water quality advisory criteria for microcystins and cylindrospermopsin. Recreational water activities include swimming, rowing, fishing, boating, etc.

| SCDES Recreational Water Quality Advisory Criteria | | |
|---|---|---|
| **Microcystin Concentration (µg/L) [a, b]** | **Cylindrospermopsin Concentration (µg/L) [a, b]** | **Duration** |
| 8 | 15 | Recreational advisories will remain in place until two (2) consecutive samples report back as less than the advisory criteria |

a. SCDES Regulation 61-68
b. µg/L = micrograms per liter (parts per billion)

## Purpose of Assessment

The purpose of the 2024 assessment was to examine cyanotoxin distributions in South Carolina reservoirs and estuaries to determine potential risks for recreational and aquatic life uses for waterbodies of the State. Cyanotoxin concentrations were also compared to USEPA drinking water health advisories (Table 2) to identify potential hazards to drinking water facilities. The data were used to identify reservoirs of potential concern and will guide future assessment activities. In 2024, monitoring activities primarily focused on analyzing microcystin toxins based on results from the previous six years. Cylindrospermopsin toxins were detected at one lake in 2024 as a result of sampling an algal bloom complaint.

**Table 2**: USEPA 10-day health advisory values for microcystins and cylindrospermopsin in drinking water.

| **Cyanotoxin** | **USEPA 10-day Drinking Water Health Advisory [a, b]** | |
|---|---|---|
| | **Bottle Fed Infants and pre-school children (µg/L)** | **School age children and adults (µg/L)** |
| Microcystins | 0.3 | 1.6 |
| Cylindrospermopsin | 0.7 | 3.0 |

a. U.S. Environmental Protection Agency, 2015b, c
b. µg/L = micrograms per liter (parts per billion)

## Methods

SCDES Bureau of Water (BOW) Aquatic Science Division (ASD) analyzed cyanotoxin samples from January 2024 to November 2024 for microcystins. Three types of sampling were conducted as part of the 2024 study: monthly-monitoring at waterbodies throughout the

State, sampling at drinking water intakes with a history of algal issues (drinking water lake source monitoring), and sampling in response to complaints (event-driven). A total of 22 freshwater lakes and 41 estuaries and influent streams were sampled during the monthly-monitoring component, four drinking water lake intakes, and event-driven samples were collected at eleven different water bodies. Event-driven sampling in 2024 included visually observed algal blooms and a fish kill in response to citizen and stakeholder complaints. In 2020, the USEPA criteria for recreational water quality and swimming advisories for microcystins and cylindrospermopsin were adopted as State water quality standards.

## Monthly-Monitoring

One hundred and ten sites were sampled monthly from May 2024 to October 2024 (Table 3 and Figure 1). These sites were selected from the 2024 list of Ambient Water Quality Monitoring Program sites (SCDHEC, 2024b). The 2024 Ambient Water Quality Monitoring Program collected monthly samples from a total of 240 Base Sites for water quality parameters including temperature, chlorophyll *a*, nutrients, metals, etc. providing an opportunity to compare cyanotoxin results to other water quality parameters. Five of the 110 sites were sampled from April 2024 to October 2024 due to a special nutrient study being conducted on Lake Monticello and Parr Reservoir, which were sampled according to SCDES BOW Parr Shoals and Monticello Reservoirs 2024 Nutrient Study Quality Assurance Project Plan (SCDES, 2025b).

A total of 671 samples were analyzed for microcystins. Sample collection, field analysis, handling, preservation, and Chain of Custody (COC) followed SCDES Determination of Total Microcystins and Cylindrospermopsin in Ambient Water Standard Operating Procedure (SOP) (Appendix 1). The field manager oversaw the transportation of the samples and the COCs to the SCDES ASD laboratory. Samples were frozen at –20°C for a holding time not to exceed two (2) weeks.

Samples were analyzed for microcystins using the Enzyme Linked Immunosorbent Assay (ELISA) technique described in SCDES Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1). The analysis is based on USEPA method 546 (U.S. Environmental Protection Agency, 2015a) with guidance from the assay provider, Abraxis. Microcystins/Nodularins ADDA ELISA and SAES ELISA plates were used for this analysis, with detection limits of 0.100 ug/L and 0.016 ug/L, respectively.

**Table 3**: Sampling site locations for 2024 monthly-monitoring.

| Site | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| B-327 | Midlands | Lake Monticello | 34.3297 | -81.3026 |
| B-339 | Greenville | Lake Bowen | 35.1128 | -82.0455 |
| B-340 | Greenville | Lake Bowen | 35.1099 | -82.0991 |
| B-345 | Midlands | Parr Reservoir | 34.2621 | -81.3354 |
| B-346 | ASP | Parr Reservoir | 34.3049 | -81.3552 |

| Site | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| B-354 | Lancaster | Lake Whelchel | 35.1069 | -81.6315 |
| B-885 | Lancaster | Lake Whelchel | 35.1130 | -81.6359 |
| B-889 | ASP | Parr Reservoir | 34.3218 | -81.3786 |
| B-890 | ASP | Parr Reservoir | 34.3159 | -81.3178 |
| CL-019 | Greenville | Lake Jocassee | 34.9599 | -82.9236 |
| CL-041 | Greenville | J. Strom Thurmond | 33.6699 | -82.2076 |
| CL-064 | Aiken | Lake Edgar Brown | 33.2482 | -81.3693 |
| CL-069 | Midlands | Langley Pond | 33.5223 | -81.8432 |
| CL-089 | Midlands | Lake Wateree | 34.3368 | -80.7049 |
| CSTL-069 | Beaufort | Ashepoo River | 32.7437 | -80.5561 |
| CSTL-102 | Charleston | Ashley River | 32.9584 | -80.2010 |
| CSTL-107 | Beaufort | Coosawhatchie River | 32.5883 | -80.9238 |
| CW-016F | Lancaster | Fishing Creek Reservoir | 34.6777 | -80.8772 |
| CW-033 | Midlands | Cedar Creek Reservoir | 34.5426 | -80.8777 |
| CW-057 | Lancaster | Fishing Creek Reservoir | 34.6053 | -80.8910 |
| CW-174 | Midlands | Cedar Creek Reservoir | 34.5581 | -80.8917 |
| CW-197 | Midlands | Lake Wylie | 35.1376 | -81.0594 |
| CW-201 | Midlands | Lake Wylie | 35.0281 | -81.0477 |
| CW-207 | Midlands | Lake Wateree | 34.4025 | -80.7884 |
| CW-207B | Midlands | Lake Wateree | 34.4039 | -80.7827 |
| CW-208 | Midlands | Lake Wateree | 34.4219 | -80.8674 |
| CW-230 | Midlands | Lake Wylie | 35.0225 | -81.0087 |
| CW-231 | Midlands | Lake Wateree | 34.5365 | -80.8749 |
| LCR-02 | Midlands | Lake Wateree | 34.4858 | -80.8998 |
| MD-001 | Beaufort | Beaufort River | 32.4456 | -80.6632 |
| MD-004 | Beaufort | Beaufort River | 32.3653 | -80.6779 |
| MD-043 | Charleston | Cooper River | 32.9629 | -79.9212 |
| MD-045 | Charleston | Cooper River | 32.8453 | -79.9335 |
| MD-049 | Charleston | Ashley River | 32.8758 | -80.0815 |
| MD-052 | Charleston | Ashley River | 32.7966 | -79.9719 |
| MD-069 | Charleston | Intracoastal Waterway | 32.7728 | -79.8422 |
| MD-077 | Florence | Sampit River | 33.3574 | -79.2940 |
| MD-115 | Charleston | Wando River | 32.9228 | -79.9273 |
| MD-116 | Beaufort | Broad River | 32.3848 | -80.7838 |
| MD-117 | Beaufort | Chechessee | 32.3741 | -80.8361 |
| MD-118 | Beaufort | New River | 32.2360 | -81.0129 |
| MD-120 | Beaufort | Dawho River | 32.6366 | -80.3418 |
| MD-125 | Florence | Intracoastal Waterway | 33.8533 | -78.6539 |
| MD-129 | Beaufort | Great Swamp | 32.4061 | -81.0187 |
| MD-130 | Charleston | Folly River | 32.6596 | -79.9433 |
| MD-142 | Florence | Waccamaw River | 33.4083 | -79.2171 |
| MD-173 | Beaufort | May River | 32.2104 | -80.8423 |
| MD-174 | Beaufort | Broad Creek | 32.1804 | -80.7740 |
| MD-175 | Beaufort | Calibogue Sound | 32.1371 | -80.8249 |
| MD-176 | Beaufort | Colleton River | 32.3323 | -80.8774 |

| Site | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| MD-202 | Charleston | Stono River | 32.7857 | -80.1075 |
| MD-206 | Charleston | Stono River | 32.6744 | -80.0046 |
| MD-209 | Charleston | Bohicket Creek | 32.6223 | -80.1643 |
| MD-248 | Charleston | Cooper River | 32.8905 | -79.9627 |
| MD-252 | Beaufort | Combahee River | 32.5643 | -80.5570 |
| MD-253 | Beaufort | Ashepoo River | 32.5330 | -80.4484 |
| MD-256 | Beaufort | Unnamed Creek | 32.3399 | -80.5078 |
| MD-257 | Beaufort | Ramshorn Creek | 32.1288 | -80.8890 |
| MD-258 | Beaufort | Ramshorn Creek | 32.1110 | -80.8986 |
| MD-259 | Beaufort | Wright River | 32.0943 | -80.9489 |
| MD-260 | Beaufort | S. Edisto River | 32.5673 | -80.3901 |
| MD-261 | Charleston | Yonges Island Creek | 32.6947 | -80.2229 |
| MD-262 | Charleston | N. Edisto River | 32.6059 | -80.2293 |
| MD-264 | Charleston | Wando River | 32.8584 | -79.8959 |
| MD-266 | Charleston | Casino Creek | 33.0751 | -79.3941 |
| MD-267 | Charleston | Five Fathom Creek | 33.0366 | -79.4769 |
| MD-269 | Charleston | Sewee Bay | 32.9367 | -79.6550 |
| MD-271 | Charleston | Hamlin Sound | 32.8269 | -79.7746 |
| MD-273 | Charleston | Kiawah River | 32.6080 | -80.1274 |
| MD-275 | Florence | Pee Dee River | 33.4222 | -79.2246 |
| MD-277 | Florence | Parsonnage Creek | 33.5529 | -79.0339 |
| MD-278 | Florence | Winyah Bay | 33.2735 | -79.0340 |
| MD-281 | Beaufort | Parrot Creek | 32.4953 | -80.5553 |
| MD-282 | Beaufort | Morgan River | 32.4438 | -80.6069 |
| PD-325 | Florence | Black River | 33.4138 | -79.2504 |
| PD-327 | Lancaster | Lake Robinson | 34.4675 | -80.1698 |
| RL-01008 | ASP | Goose Creek Reservoir | 32.9649 | -80.0358 |
| RL-04370 | ASP | Parr Reservoir | 34.3656 | -81.3229 |
| S-022 | Greenville | Lake Greenwood | 34.3278 | -82.0849 |
| S-024 | Greenville | Lake Greenwood | 34.3079 | -82.1101 |
| S-131 | Greenville | Lake Greenwood | 34.2791 | -82.0587 |
| S-211 | Midlands | Lake Murray | 34.0984 | -81.4765 |
| S-213 | Midlands | Lake Murray | 34.1251 | -81.4337 |
| S-222 | Midlands | Lake Murray | 34.0802 | -81.5625 |
| S-279 | Midlands | Lake Murray | 34.0763 | -81.4724 |
| S-280 | Midlands | Lake Murray | 34.0713 | -81.3942 |
| S-308 | Midlands | Lake Greenwood | 34.3467 | -82.1088 |
| S-309 | Midlands | Lake Murray | 34.1315 | -81.6048 |
| S-310 | Midlands | Lake Murray | 34.1151 | -81.5999 |
| S-311 | Greenville | Boyd Mill Pond | 34.4547 | -82.2019 |
| S-326 | Midlands | Lake Murray | 34.0682 | -81.5869 |
| ST-005 | Charleston | North Santee River | 33.2091 | -79.3839 |
| ST-006 | Charleston | South Santee River | 33.1839 | -79.4058 |
| ST-032 | ASP | Goose Creek Reservoir | 32.9324 | -80.0112 |
| ST-033 | ASP | Goose Creek Reservoir | 32.9348 | -80.0223 |

| Site | Regional Lab | Description | Latitude | Longitude |
|---|---|---|---|---|
| SV-098 | Greenville | Lake Russell | 34.0704 | -82.6429 |
| SV-200 | Greenville | Lake Hartwell | 34.6117 | -83.2262 |
| SV-236 | Greenville | Lake Hartwell | 34.5954 | -82.9078 |
| SV-268 | Greenville | Lake Hartwell | 34.5972 | -82.8218 |
| SV-331 | Greenville | Lake Secession | 34.3319 | -82.5758 |
| SV-335 | Greenville | Lake Jocassee | 35.0320 | -82.9151 |
| SV-336 | Greenville | Lake Jocassee | 34.9959 | -82.9793 |
| SV-338 | Greenville | Lake Keowee | 34.8269 | -82.8977 |
| SV-339 | Greenville | Lake Hartwell | 34.5112 | -82.8098 |
| SV-340 | Greenville | Lake Hartwell | 34.4032 | -82.8391 |
| SV-357 | Greenville | Lake Russell | 34.1920 | -82.6309 |
| SV-361 | Greenville | Lake Keowee | 34.7339 | -82.9183 |
| SV-363 | Greenville | Lake Hartwell | 34.4800 | -82.9454 |
| SV-372 | Greenville | Stephens Creek Reservoir | 33.5928 | -82.1233 |
| SV-374 | Greenville | Lake Hartwell | 34.5721 | -82.8299 |

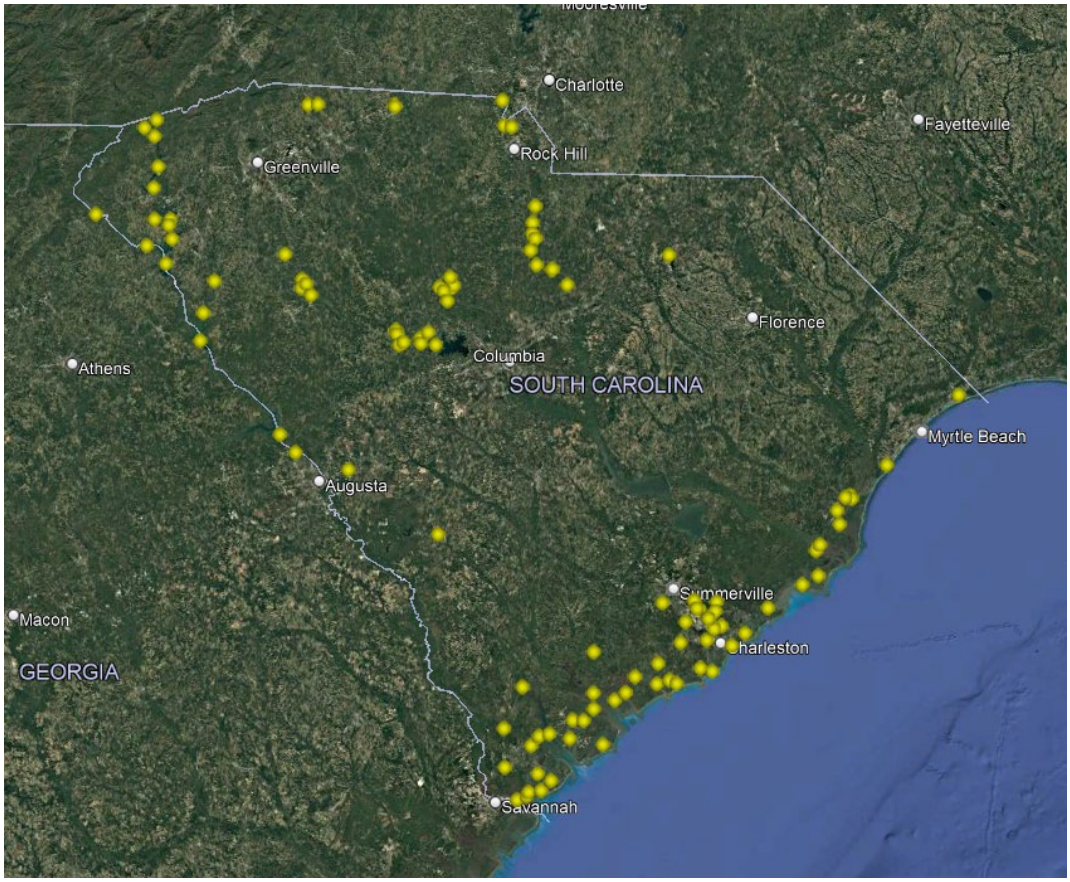


**Figure 1**: 2024 monthly-monitoring sampling site locations.

## Drinking Water Lake Source Monitoring

Three lakes were sampled monthly from May through October 2024 near four drinking water facility intakes (Table 4). The lake and drinking water intake sampling sites were selected based on prior algal issues and taste and odor complaints. A total of 22 samples were collected from the drinking water lakes and analyzed for microcystins. Sampling was focused near the drinking water facility intakes; however, additional samples were collected at other parts of the lakes if algal blooms were observed to examine bloom dynamics.

Drinking water sample collection, field analysis, handling, preservation, and laboratory analysis followed the same procedures as described above for Monthly-Monitoring samples.

**Table 4**: Sampling site locations for three lakes that were monitored at their respective drinking water source intakes.

| Lake | Drinking Water Facility | Latitude | Longitude |
|---|---|---|---|
| Lake Murray | City of Columbia | 34.0215 | -81.2326 |
| | City of West Columbia | 34.0978 | -81.2313 |
| Lake Rabon | Laurens Commissions of Public Works | 34.4785 | -82.1398 |
| Lake Wylie | City of Rock Hill | 35.0169 | -81.0099 |

## Event-Driven Samples

Eleven waterbodies were sampled in response to complaints reporting algal blooms, fish kills, and/or taste and odor issues during the 2024 sampling season. Toxin samples and/or phytoplankton tow nets were collected after a complaint was received. Samples were observed under the microscope for algal identification at the SCDES ASD laboratory and analyzed for microcystin and/or cylindrospermopsin if the species identified was a potential toxin producing species.

## Advisories and Watches

In 2024, there was one recreational advisory issued on Lake Rabon due to an exceedance of SCDES's microcystin recreational water quality advisory criteria.

Recreational watches were issued when a potential toxin producing bloom was identified on a waterbody but toxins for microcystin or cylindrospermopsin were less than SCDES's state standards. Watches are also issued when the identified algal species could potentially be producing algal toxins, such as anatoxins and saxitoxins, that are not in SCDES's state standards. In 2024, there were five recreational watches issued (Appendix 3). Recreational watches were monitored monthly and removed once the bloom dissipated.

Recreational advisories and watches were posted on the Harmful Algal Bloom Monitoring GIS Application: https://gis.dhec.sc.gov/hab_viewer

## Quality Assurance/ Quality Control

In total, 615 of the 671 samples analyzed for microcystins in 2024 passed quality control requirements. Quality Control Requirements can be found in section 10.5 of SCDES's Determination of Total Microcystins and Cylindrospermopsin in Ambient Water SOP (Appendix 1). SCDES also participated in the Abraxis Cyanotoxins Proficiency Testing Program for recreational water as a check on the accuracy of ASD's routine sample analysis. Performance was evaluated by calculating a z-score metric based on the analysis results of three surface water standards fortified with purified Microcystin-LR, Microcystin-RR, Microcystin-YR, and/or nodularins (toxins produced by *Nodularia sp.*, a cyanobacterium). The z-score metric is as follows:

$$z = \frac{(x - X)}{\sigma}$$

Where:

$z$= the z score (Standard score)
$x$= the reported value of analyte
$X$= the assigned value, the best estimate of the *true* concentration
$\sigma$= the estimate of variation (proficiency standard deviation)

The following interpretations for z-scores in proficiency testing schemes are recommended:

| Results Obtained | Rating |
|---|---|
| $z \leq 2$ | Satisfactory |
| $2 < z < 3$ | Questionable |
| $z \geq 3$ | Unsatisfactory |

The results for SCDES's proficiency testing for each of the three (3) samples are listed in the table below.

| Sample Number | Result (µg/L) [a] | Z-Score | Evaluation |
|---|---|---|---|
| 1 | 7.45 | -0.12 | Satisfactory |
| 2 | 0.798 | -0.24 | Satisfactory |
| 3 | 8.34 | -0.29 | Satisfactory |

a. µg/L = micrograms per liter (parts per billion)

## Statistical Analyses

Pearson correlation coefficients were calculated to determine if there were linear relationships between concentrations of microcystins and pH, dissolved oxygen (mg/L), temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L) in water bodies that met the sample size requirement of three detectable samples per month. Only

detectable data (toxin concentration values greater than or equal to the method detection limit) were used for analyses. Microcystin concentration data were considered detectable when result(s) were ≥ 0.016 ug/L for SAES ELISA plates and ≥0.100 ug/L for ADDA ELISA plates.

Sixty-five waterbodies across the State were sampled as part of the 2024 monthly-monitoring program. Due to different hydrologic characteristics among the water bodies, lakes were analyzed individually. Water bodies meeting the minimum sample size requirement (three detectable samples per month) over the course of six months included Lake Hartwell, Lake Murray, Parr Reservoir, Lake Wylie and Lake Wateree.

Pearson correlation matrix output values range from -1 to 1, where values closer to -1 indicate a strong inverse relationship and values closer to 1 indicate a strong positive relationship. Matrix values that are closer to zero indicates no linear relationship. All data analyses were made using Microsoft Excel.

# Results

## Monthly-Monitoring

From April 2024 through October 2024, a total of 671 samples were collected for microcystins. Of the 615 samples meeting QA/QC guidelines for microcystins, 72% had concentrations greater than or equal to the method detection limit. The maximum microcystin concentration was 0.877 µg/L at station B-354 on Lake Whelchel in July 2024. All monthly-monitoring microcystin concentrations were less than 1 µg/L and all microcystin concentrations were less than the SCDES recreational action level of 8 µg/L.

A total of 41 estuarine sites were sampled during the 2024 monitoring season. Thirty-seven of the 41 estuarine sites had more than one sample with detectable amounts of microcystins (Figure 2). North Santee River had the highest average detectable microcystin concentration (mean ($\bar{x}$)=0.140 µg/L, standard error (SE)=0.034). The Intracoastal Waterway had the lowest average detectable microcystin concentration ($\bar{x}$=0.027 µg/L, SE= 0.004). Refer to Appendix 2 to see the microcystin concentrations of individual sites analyzed each month, organized based on estuarine location.

All 22 freshwater lakes had more than one sample with detectable amounts of microcystins (Figure 3). Lake Whelchel had the highest average microcystin concentration ($\bar{x}$=0.470 µg/L, SE=0.059); Lake Robinson had the lowest average microcystin concentration ($\bar{x}$=0.022 µg/L, SE=0.0005). Refer to Appendix 2 to see the microcystin concentrations of individual sites analyzed each month, organized based on lake location.

Microcystins did not strongly correlate with dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, or chlorophyll *a* in Lake Hartwell, Lake Murray, Parr Reservoir, Lake Wylie and Lake Wateree with coefficients ranging from -0.54 to 0.56 (Table 5).

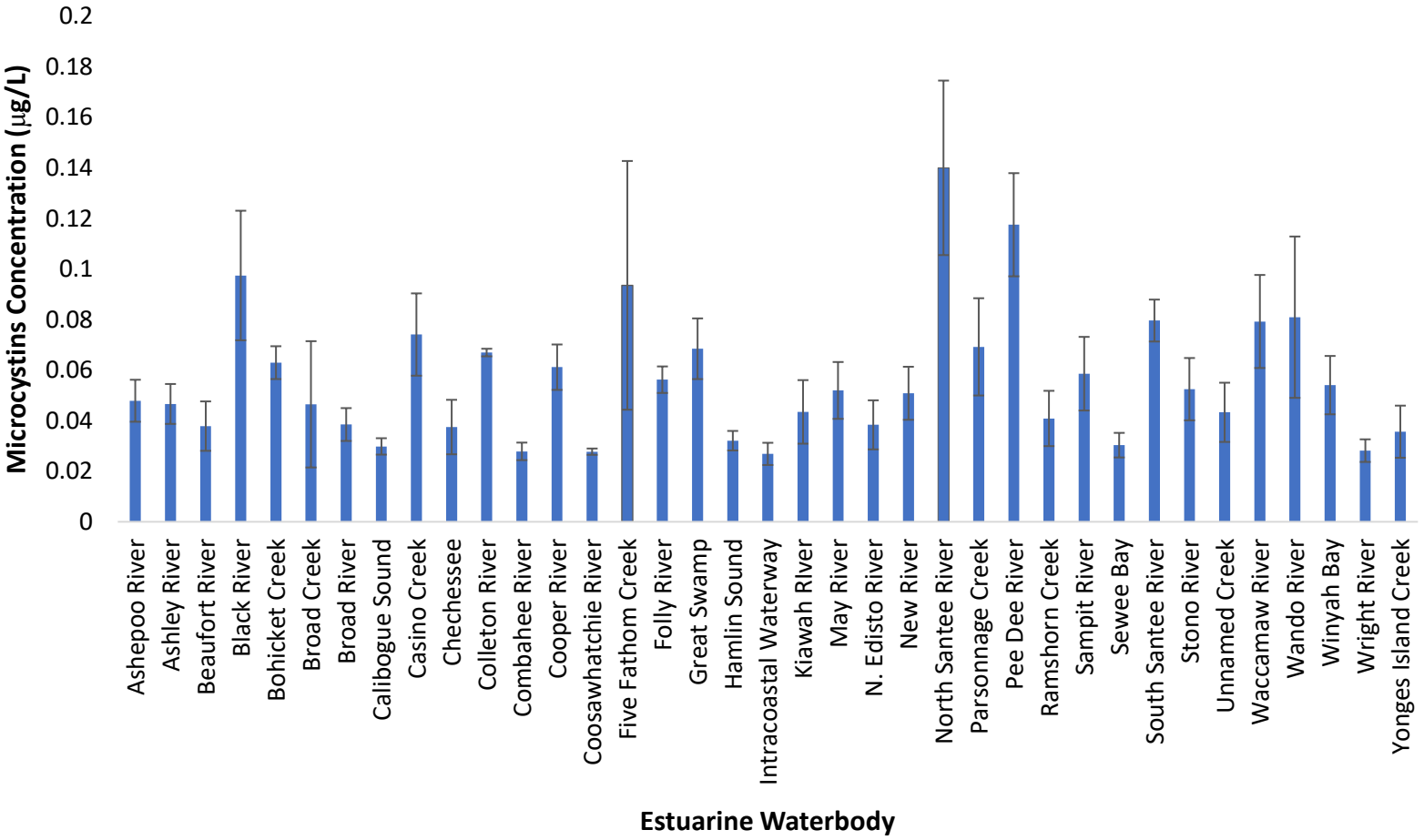


**Figure 2**: Average detectable microcystin concentrations (µg/L) per estuarine site sampled in 2024. There were 37 estuary sites that had more than one sample with quantifiable concentrations. The error bars represent +/- one standard error

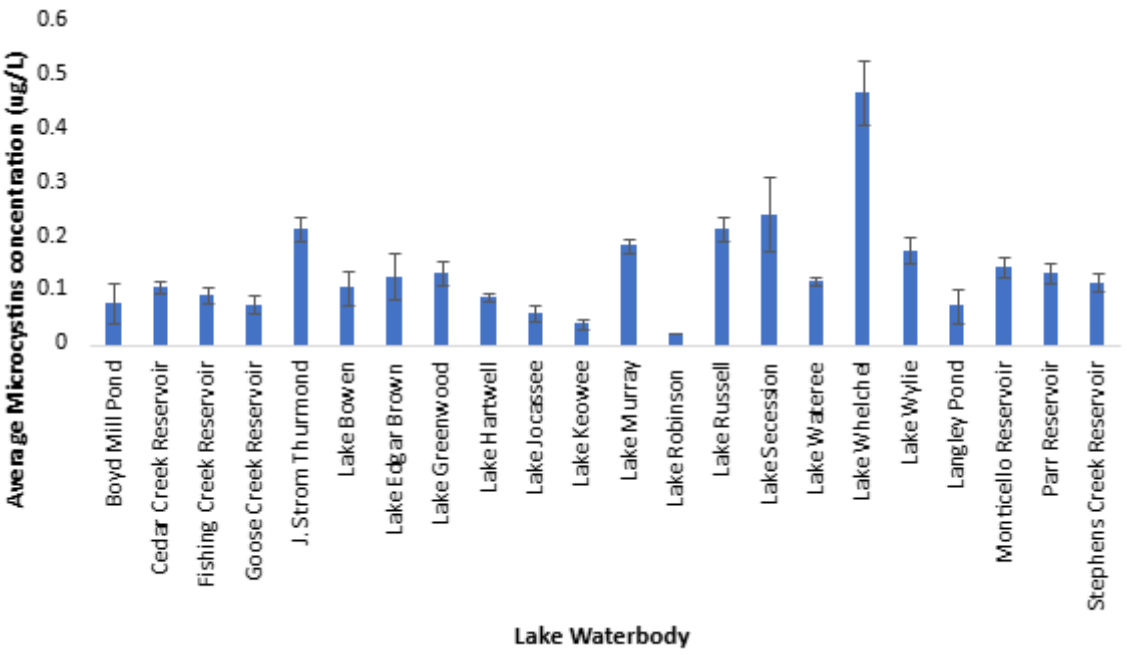


**Figure 3**: Average detectable microcystin concentrations (µg/L) per freshwater lake in 2024. There were 22 lakes that had more than one sample with concentrations above the detection limit. The error bars represent +/- one standard error.

**Table 5:** Pearson correlation coefficient results comparing microcystin concentrations (µg/L) in Lake Hartwell, Lake Murray, Parr Reservoir, Lake Wateree, and Lake Wylie to dissolved oxygen (mg/L), pH, temperature (°C), total phosphorous (mg/L), N:P ratio, and chlorophyll *a* (µg/L).

| **Water Body** | **Microcystin Concentrations Correlation for Respective Water Quality Parameters** | | | | | |
|---|---|---|---|---|---|---|
| | Dissolved Oxygen | pH | Temperature | Total Phosphorous | N:P | Chlorophyll *a* |
| Lake Hartwell | -0.19 | -0.05 | -0.19 | -0.43 | 0.08 | 0.24 |
| Lake Murray | -0.25 | -0.05 | 0.36 | -0.37 | 0.07 | -0.34 |
| Parr Reservoir | 0.13 | 0.56 | 0.44 | -0.29 | 0.01 | 0.22 |
| Lake Wateree | -0.31 | -0.06 | 0.24 | -0.22 | 0.41 | 0.02 |
| Lake Wylie | -0.54 | 0.05 | 0.22 | -0.34 | 0.43 | -0.09 |

Summary of Monthly-Monitoring Findings

- 72% of the 615 samples analyzed for microcystins were detectable (≥ 0.100 µg/L for ADDA ELISA or ≥ 0.016 µg/L for SAES ELISA method).
- All microcystin samples were less than the SCDES recommended recreational action level of 8 µg/L.
- There were no strong correlations between microcystin concentrations and dissolved oxygen, pH, temperature, total phosphorous, N:P ratio, and chlorophyll *a* in, Lake Hartwell, Lake Murray, Parr Reservoir, Lake Wylie or Lake Wateree.

## Drinking Water Lake Source Monitoring

From May through October 2024, 22 samples were collected for microcystins at three different lakes for four different drinking water intakes. Samples collected near the City of West Columbia drinking water intake at Lake Murray had the highest average microcystin concentration ($\bar{x}$=0.321 µg/L, SE=0.0423); the City of Rock Hill drinking water intake samples at Lake Wylie had the lowest average microcystin concentration ($\bar{x}$=0.178 µg/L, SE=0.057). All Lake Wylie (City of Rock Hill) samples were below the USEPA 10-day drinking water health advisory values of 0.3 µg/L for bottle fed infants and pre-school aged children and 1.6 µg/L for school age children and adults (U.S. Environmental Protection Agency, 2019) (Figure 4).

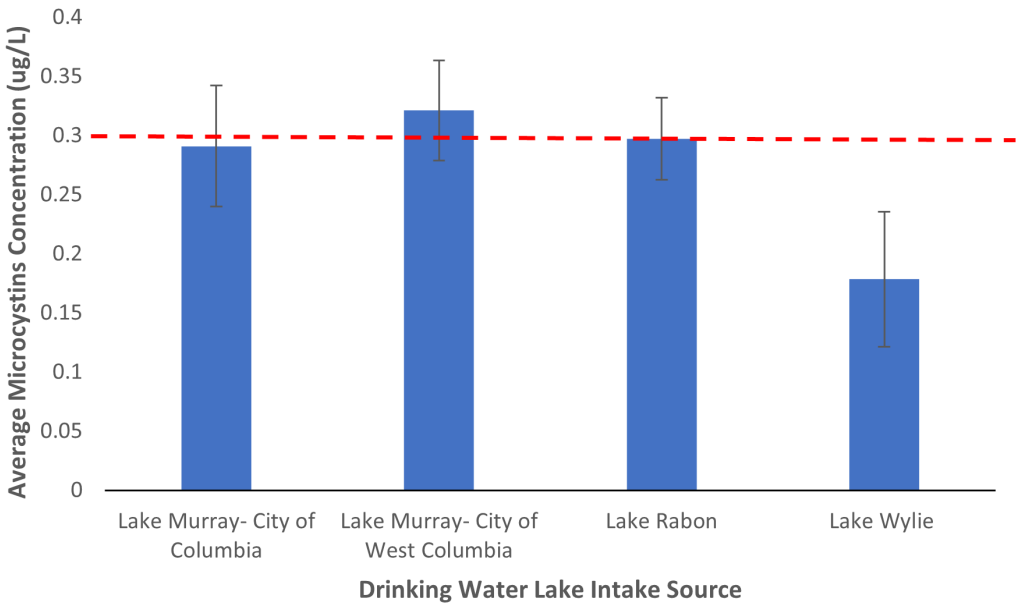


**Figure 4**: Average detectable microcystin concentrations (µg/L) per drinking water source intake in 2024. There were three lakes sampled for four different drinking water facilities. The red line indicates the USEPA drinking water 10-day health advisory values of 0.3 for bottle fed infants and pre-school aged children. The error bars represent +/- one standard error.

Summary of Drinking Water Lake Source Sample Findings

- Microcystins were detected in samples collected near all four drinking water intakes in 2023 (≥ 0.100 µg/L for ADDA ELISA or ≥ 0.016 µg/L for SAES ELISA method).
- Lake Wylie (City of Rock Hill) samples were below the USEPA 10-day drinking water health advisory values of 0.3 µg/L for bottle fed infants and pre-school aged children and 1.6 µg/L for school age children and adults (Figure 4).

## Event-Driven Samples

Throughout the 2024 season, the SCDES BOW ASD section received complaints on eleven waterbodies (Table 6). Samples that did not have blooms identified in the sample were still analyzed for microcystins to rule out the presence of this toxin. The highest concentration of microcystins (1.82 µg/L) was at Lake Greenwood.

Four of the eleven complaint blooms were tested for cylindrospermopsin toxins based on the presence of cyanobacteria. One of these four samples had detectable levels of cylindrospermopsin (Table 6).

**Table 6:** Description and microcystin and cylindrospermopsin concentration (µg/L) results from 2024 algal bloom complaints with the associated date of the HAB.

| Sample Location | Sample Description | Collection Date | Microcystins (µg/L) [a] | Cylindrospermopsin (µg/L) [a] |
|---|---|---|---|---|
| North Augusta | *Aphanizomenon sp.* | 02/13/2024 | 0.460 | BDL[c] |
| House Creek, North Myrtle Beach | Filamentous, non-harmful algae | 02/13/2024 | 0.127 | N/A[b] |
| Lake Keowee | Green algae, *Zygnema sp.* | 04/01/2024 | N/A[b] | N/A[b] |
| Lake Woodcross, Columbia | *Dolichospermumsp.* and *Worchinia.* | 04/16/2024 | 0.406 | 0.049 |
| Fishing Creek Reservoir | Green Algae, *Carteria sp.* | 06/14/2024 | 0.168 | N/A[b] |
| Anne Springs Greenway, Fort Mill | *Planktothrix sp.* | 06/18/2024 | 0.048 | N/A[b] |
| Twin Lakes, Spartanburg | *Planktothrix sp.* | 06/20/2024 | 1.43 | N/A[b] |
| Lake Murray | *Lyngbya sp.* and aquatic plant, Fanwort | 08/14/2024 | 0.265 | N/A[b] |
| Lake Greenwood | *Lyngbya sp.* and *Oscillatoria sp*. | 08/20/2024 | 1.82 | N/A[b] |
| H. Cooper Black Park | *Hapalosiphon, Planktothrix, Dolichospermum, Kamptonema, Lyngbya* | 08/21/2024 | N/A[b] | BDL[c] |
| Lake Wylie | Bloom not identified in sample | 10/08/2024 | 0.200 | BDL[c] |

a. µg/L = micrograms per liter (parts per billion)
b. N/A= Not Applicable
c. BDL= below detection limit

Summary of Event-Driven Sample Findings

- Nine of the eleven HAB complaint samples were analyzed for microcystins, and all nine samples had detectable levels of microcystins (≥ 0.100 µg/L for ADDA ELISA or ≥ 0.016 µg/L for SAES ELISA method).
- Four of the eleven HAB complaint samples were analyzed for cylindrospermopsin toxins. One of the four samples had detectable limits of cylindrospermopsin (≥ 0.040 µg/L).

### Advisories and Watches

The recommended USEPA recreational water quality and swimming advisory criteria for microcystins and cylindrospermopsin (Table 1) were adopted as enforceable State water quality standards in 2020. One recreational advisory was issued in 2024 for microcystin concentrations higher than SCDES's state standard of 8 µg/L

The advisory was issued at Lake Rabon on September 18, 2024 following a microcystin concentration of 10.2 µg/L. The advisory was lifted on October 10, 2024 when the second consecutive sample had a microcystin concentration below 8 µg/L (microcystin concentration of 0.240 µg/L).

Recreational watches were issued in 2024 at Lake Woodcross, Lake Greenwood, Twin Lakes, Lake Wateree, and Goose Creek Reservoir (Appendix 3). The watches on these reservoirs did not result in any recreational advisories.

Summary of Advisories and Watches

- A recreational advisory was issued in September 2024 at Lake Rabon for a microcystin concentration exceeding SCDES's state standard of 8 µg/L. The advisory was lifted on October 10, 2024.
- Recreational watches were issued in 2024 on Lake Woodcross, Lake Greenwood, Twin Lakes, Lake Wateree, and Goose Creek Reservoir

## Discussion

A primary goal of the HAB Monitoring Program is to establish cyanotoxin spatial distribution data in South Carolina waterbodies. These 2024 results have (a) contributed to a cyanotoxin concentration baseline for South Carolina waterbodies and (b) provided insight towards cyanotoxin presence/absence expectations. The total number of samples analyzed for microcystins increased by 5% from 2023 to 2024 and microcystins were detected in 72% of the samples that passed QA/QC.

Overall, the results from the 2024 monthly-monitoring for microcystins in lakes showed toxin concentrations less than 1 µg/L, well below SCDES's recreational standards of 8 µg/L. Estuaries were monitored for cyanotoxins for the fifth consecutive year in 2024. While all microcystin concentrations for estuaries were below 1 µg/L, these data are important milestones in establishing baseline toxin levels along the coast. The low cyanotoxin concentrations observed as part of the monthly-monitoring data suggest that generally recreational activities in South Carolina are not an immediate concern. Maintaining and expanding monthly-monitoring in the future will help in identifying localized elevated cyanotoxin concentrations in various environments. A limitation of the monthly-monitoring sampling sites is that they are fixed open-water locations. Cyanobacteria blooms often occur in shallow coves or along shorelines.

The event-driven sampling is a more targeted component of the HAB Program, which provides insight into potential cyanotoxin producing HABs in nearshore environments. Microcystin concentrations in event-driven samples ranged from below detection limit to 1.82 µg/L. One of the four complaint samples tested for cylindrospermopsin had detectable limits of toxins. One recreational advisory was issued in 2024 on Lake Rabon. Recreational watches were issued in 2024 on Lake Woodcross, Lake Greenwood, Twin Lakes, Lake Wateree, and Goose Creek Reservoir (Appendix 3).

SCDES's HAB Monitoring Program collaborated with four drinking water facilities in 2024 to monitor drinking water intakes at three lakes: Lake Murray, Lake Rabon, and Lake Wylie. Microcystins were detected at all drinking water intakes, sample greater than the USEPA 10-day drinking water health advisory value of 0.3 µg/L for bottle fed infants and pre-school aged children. As HABs continue to expand and increase in frequency and duration, monitoring drinking water intakes and collaborating with drinking water facilities will continue to be a vital component of the HAB Monitoring Program.

No strong relationships were observed in the monthly-monitoring correlation results comparing microcystin concentrations to dissolved oxygen, pH, temperature, total phosphorus, N:P ratio, and chlorophyll-*a* for Goose Creek Reservoir, Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. The lack of a clear relationship among these monitoring variables is consistent with the analyses from past seasons (SCDHEC, 2020a; SCDHEC, 2021; SCDHEC, 2022; SCDHEC, 2023; SCDHEC, 2024a, (SCDES, 2025a)). The lack of a relationship suggests that the periodic occurrence of toxin producing cyanobacteria species is more complex than a single variable correlation in the same time and space (Davis, Berry, Boyer, & Gobler, 2009; Paerl & Otten, 2012; Wiltsie, Schnetzer, Green, Vander Borgh, & Fensin, 2018) or is related to environmental variables not routinely measured as part of the ambient monitoring program. Further, these lake-by-lake datasets are small and likely not robust enough for meaningful correlation. A more comprehensive dataset analysis of the past five years may provide a clearer understanding of patterns in cyanotoxin production.

## Conclusion

The monthly-monitoring cyanotoxin results were generally lower than the SCDES state recreational standards, suggesting recreational activities in South Carolina were not an immediate concern. The 2024 season was the fifth full season for cyanotoxin monthly-monitoring in estuaries and sixth full season for cyanotoxin monitoring in lakes. While initial microcystin concentrations were low, continuing to monitor the estuarine environment in future years will improve and expand SCDES's understanding of harmful cyanobacteria presence along the coast.

SCDES issued recreational watches when a potential toxin producing bloom was identified on a waterbody, but the toxin concentrations were less than SCDES's state standards or the algal species could potentially be producing algal toxins that are not in SCDES's state standards, such as anatoxin and saxitoxin. SCDES continued to work with drinking water facilities to monitor four different drinking water intakes at three lakes for microcystins. Microcystins were present at each drinking water intake, but the drinking water treatment processes at all drinking water intakes are able to remove microcystins at these low concentrations. Even though no strong correlations between microcystin concentrations and other environmental parameters were discerned in this assessment, a larger dataset over several years may provide better insight into relationships among these variables.

The HAB Monitoring Program continues to work on educating South Carolina residents on HABs by creating the HAB Monitoring GIS Application. Future goals of the HABs Monitoring Program include expanding the statewide cyanotoxin study to include other toxins, such as anatoxin and saxitoxin as well as incorporating large rivers and streams into the cyanotoxin monitoring program.

Overall Summary:

- The 2024 season completed the sixth full year of the HAB Monitoring Program. The data gathered from 2018 to 2024 will be used to inform future sampling plans and provide insights into lakes that the agency may consider monitoring more frequently.
- The monthly-monitoring sampling suggests no immediate concern for recreation activities due to the low concentrations of microcystins in open water settings.
- 2024 was the fifth full year for monthly-monitoring of microcystins of estuarine water bodies and sixth full year for monthly-monitoring in lakes.
- One recreational advisory was issued in 2024 for Lake Rabon. Recreational watches were issued for five waterbodies. Watches were issued when a potential toxin producing bloom was identified but was producing toxins for microcystin or cylindrospermopsin less than SCDES's state standards, or the identified algal species could potentially be producing algal toxins that are not in SCDES's state standards (Appendix 3).
- There were no strong correlations between microcystin concentrations and other parameters measured in Goose Creek Reservoir, Lake Greenwood, Lake Hartwell, Lake Murray, and Lake Wateree. Future analyses would benefit from a larger data set that also includes samples from algal blooms.

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**Appendix 1:** Standard Operating Procedure for Determination of Total Microcystins and Cylindrospermopsin in Ambient Water


# Determination of Total Microcystins and Cylindrospermopsin in Ambient Water

## Bureau of Water- Aquatic Science Programs

**Revision 2.0**

**February 28, 2022**

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02/28/22
Date

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2022-03-01
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# 1. SCOPE AND APPLICATION

## 1.1 Method Description

These methods are used for the determination of algal toxins in ambient water, including (extracellular and intracellular) microcystins and cylindrospermopsin via enzyme-linked immunosorbent assay (ELISA). The detection limit for the Microcystin ADDA assay is 0.10 ppb (µg/L) and the detection limit for the Microcystins ADDA SAES assay is 0.016 ppb (µg/L). The detection limit for the Cylindrospermopsin assay is 0.040 ppb (µg/L). The detection limit for using the seawater sample treatment solution for Cylindrospermopsin is 0.015ppb (ug/L).

# 2. METHOD SUMMARY

The method is an immunoassay for the quantitative and sensitive cogener-independent detection of Microcystins and Nodularins and Cylindrospermopsin in ambient water samples. The testing is completed in a 96-well microtiter plate.

## 2.1 Microcystins

The test is an indirect competitive ELISA for the congener-independent detection of Microcystins and Nodularins. It is based on the recognition of Microcystins, Nodularins, and their congeners by specific antibodies. Microcystins, nodularins, and their cogeners when present in a sample and a Microcystins-protein analogue immobilized on the plate compete for binding sites of antibodies in solution. The plate is then washed and a second antibody-HRP label is added. After a second washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Microcystins present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

## 2.2 Cylindrospermopsin

The test is a direct competitive ELISA for the detection of Cylindospermopsin. It is based on the recognition of Cylindrospermopsin by specific antibodies. Cylindrospermopsin, when present in a sample, and a Cylindrospermopsin-HRP analogue compete for the binding sites of rabbit anti-Cylindrospermopsin antibodies in solution. The anti-Cylindospermopsin antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the wells of the microtiter plate. After a washing step and addition of the substrate solution, a color signal is generated. The intensity of the blue color is inversely proportional to the concentration of Cylindrospermopsin present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

## 3. DEFINITIONS

### 3.1 Analysis Batch

Standards, samples, and quality control elements are assayed on a single 96-well plate using identical lots of reagents and wells. Each plate by definition is an Analysis Batch, regardless of the number of wells included. Quality control samples must be analyzed in each Analysis Batch at the frequencies prescribed. Each Analysis Batch includes the following elements:

- Calibration Standards
- Quality Controls
- Field samples (ambient water)

### 3.2 Well Replicates

Within the Analysis Batch, this method requires each calibration standard, field sample, and QC sample to be assayed in two wells. These two wells are called well replicates. Two values are associated with each well replicate: an absorbance measured by the plate reader, and a concentration calculated from this absorbance.

### 3.3 Use of Well Replicate Absorbance Values

For each set of well replicates, the percent coefficient of variation (%CV) is calculated from the two absorbance values. The %CV of the absorbance values for calibration standards must meet QC criteria. The %CV of the absorbance values for all field and QC samples must meet the limits. Refer to Table 2 for QC criteria.

### 3.4 Use of Well Replicate Concentrations

For each set of well replicates, the mean is calculated from the two concentration values. The mean concentration must be used for reporting field sample results. The mean must be used in all method calculation and for evaluating results against QC limits.

### 3.5 Calibration Standards

Solutions of Microcystin and Cylindrospermopsin toxins provided in the ELISA kit or prepared in the laboratory that are appropriate for the measurement range of the ELISA kit.

### 3.6 Calibration Curve

The calibration points are modelled using a four-parameter logistic function, relating concentration (x-axis) to the measured absorbance in the wells (y-axis). Note the inverse relationship between concentration and response. The zero calibration standard gives the highest absorbance and the highest calibration standard gives the lowest absorbance. Note also that the slope, or sensitivity, of the ELISA response is greatest in the middle of the curve and tends toward zero slope at extreme low and high concentrations.

### 3.7 Four-parameter Logistic Equation

$$y = \frac{(a-d)}{1+(\frac{x}{c})^b} + d$$

y= absorbance
x= concentration
a= absorbance at the bottom plateau
b= slope related term at the inflection point
c= concentration at the inflection point= $EC_{50}$
d= absorbance at the top plateau

The coefficients, a, b, c, and d, are calculated by the data reduction software using regression analysis.

### 3.8 Quality Control Sample (QCS)

A solution containing microcystin toxins or cylindrospermopsin toxins at a known concentration that is obtained from a source different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibrations standards.

## 4. HEALTH AND SAFETY WARNINGS

### 4.1 Microcystins

The standard solution in the test kit contains small amounts of Microcystins. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 4.2 Cylindrospermopsin

The standard solutions in the test kit contain small amounts of Cylindrospermopsin. The substrate solution contains tetramethylbenzidine (TMB) and the stop solution contains diluted sulfuric acid. Avoid contact of the TMB and stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water.

### 4.3 Cylindrospermopsin Seawater Sample Reagent

Irritant to skin and mucous membranes. May cause eye irritation in susceptible persons. The chemical, physical, and toxicological properties of this reagent have not been thoroughly investigated.

**4.4** Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of any chemicals used in this method. A reference file of Safety Data Sheets should be made available to all personnel involved in the analysis. Handle samples and standards using appropriate personal protective equipment.

## 5. INTERFERENCES

**5.1** Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test or QuikLyse. However, due to high variability of compounds that may be found in water samples, test interferences caused by matrix effects cannot be completely excluded.

**5.2** Samples containing methanol must be diluted to a concentration <1% methanol to avoid matrix effects.

**5.3** Mistakes in handling the test can cause errors. Possible sources for such errors include: inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, and extreme temperatures during the test performance (lower than 10ºC or higher than 30ºC). The assay procedure should be performed away from direct sunlight.

**5.4** To avoid cross contamination between samples, do not reuse plastic syringes for filtering. Thoroughly clean glass containers if they are reused. Do not reuse septa from bottle containing ambient water samples.

**5.5** As with any analytical technique, positive results requiring regulatory action should be confirmed by an alternative method.

## 6. SAMPLE HANDLING, PRESERVATION, AND STORAGE

**6.1** Collect samples in 500 mL polyethylene terephthalate glycol (PETG) containers with Polytetrafluoroethylene (PTFE) lined septa lids. Use of other types of plastic collection and/or storage containers may result in adsorptive loss of Microcystins, producing inaccurate (falsely low) results. Ambient water samples do not need to be treated after collection. Freeze samples upon arrival at the laboratory. Samples can be stored in the freezer for up to 2 weeks. When freezing, allow adequate volume for expansion and place the sample container on its side to prevent breakage.

**6.2** Place samples on ice immediately. The temperature blank in the cooler must not exceed 10ºC during the first 48 hours after collection. A temperature of greater than 10ºC is acceptable if transit time is short and the samples do not have sufficient time to chill. In this case, examine the ice packs in the cooler. If they remain frozen, the samples are valid. Based on holding time (see section 6.1), refrigerate or freeze samples upon arrival to the laboratory.

**6.3** Samples may be filter and assayed any time after lysing if within 14 days of collection. If not assayed immediately, store lysed samples by freezing in glass vials with PTFE-faced septa, for example, 1 mL of lysed and filtered sample held in a 4mL vial.

## 7. INSTRUMENTATION AND EQUIPMENT

**7.1** Adda ELISA Test Kits- 96-well Microtiter Plates

- **7.1.1** Microcystins/Nodularins- Abraxis PN 520011
- **7.1.2** Microcystins-ADDA SAES- Abraxs PN 520011SAES
- **7.1.3** Cylindrospermopsin- Abraxis PN 522011
- **7.1.4** Standards

1. Microcystins ADDA: (6): 0, 0.15, 0.40, 1.0, 2.0. 5.0 ppb, 1mL each
2. Microcystins ADDA SAES: (6): 0, 0.05, 0.15, 0.4, 1.5, 5.0 ppb, 1mL each
3. Cylindrospermopsin: (7): 0, 0.05, 0.10, 0.25, 0.50, 1.0, 2.0 ppb, 1mL each

**7.1.5** Control:
1. Microcystins: 0.75 ± 0.185 ppb, 1 mL
2. Cylindrospermopsin: 0.75 ± 0.15ppb, 1 mL

**7.1.6** Sample Diluent, 25 mL, for use as a Laboratory Reagent Blank and for dilution of samples above the range of the standard curve

**7.1.7** Antibody Solution
1. Microcystins ADDA: 6mL
2. Microcystins ADDA SAES, 6mL
3. Cylindrospermopsin: rabbit anti-Cylindrospermopsin, 6 mL

**7.1.8** Conjugate Solution
1. Microcystins ADDA: Anti-Sheep-HRP conjugate solution, 12 mL
2. Microcystins-ADDA SAES Conjugate Solution, 12mL
3. Cylindrospermopsin: Cylindrospermopsin-HRP conjugate solution (vortex before use), 6 mL

**7.1.9** Wash Buffer (5X) Concentrate, 100 mL, must be diluted prior to use

**7.1.10** Substrate (Color) Solution (TMB), 12 mL

**7.1.11** Stop Solution
1. 6 mL for Microcystins
2. 12mL for Cylindrospermopsin

**7.1.12** Cylindrospermopsin Seawater Sample Treatment Solution, 45 test

**7.2** QuikLyse Cell Lysis for Microycstins/Nodularins ELISA microtiter plate
- **7.2.1** Lysis Reagent A, 2.5 mL
- **7.2.2** Lysis Reagent B, 0.5 mL
- **7.2.3** Disposable Pipettes, 45
- **7.2.4** Filtering Tips, 45

**7.3** Cyanotoxin Manual Assay System- Abraxis PN 475010S. Includes:
- **7.3.1** Microplate Reader, Model 4303
- **7.3.2** Pipette, transfer, 10-100 µL, adjustable
- **7.3.3** Pipette, repeating, manual
- **7.3.4** Pipette, multichannel, 8-tip, adjustable
- **7.3.5** Basin, reagent, for multichannel, 50/bag
- **7.3.6** Rack for 4mL vials, 48-postion (4x12)

**7.4** Disposable plastic tips for pipettes
- **7.4.1** Cartridges, Repeater, 1mL, bx/100- PN 70468
- **7.4.2** Tips, Pipette, 10-200µL, 96/bx- PN 300002
- **7.4.3** Tips, Pipette, 30-300µL, 96/bx- PN 300004

**7.5** Vials for freezing samples

**7.5.1** Vials, Glass, Clear, 4 mL with caps
**7.5.2** Vials, Glass, Clear, 40mL with caps
**7.6** Syringes and Filters for Lysing
**7.6.1** All plastic Luer-Lok syringes, 3mL, from Thermofisher Scientific
**7.6.2** Glass Fiber Syringe Filters, 25mm, 1.2µm,
**7.7** 500 mL PETG containers with PTFE septa lined lids
**7.8** Parafilm for plate covering

## 8. REAGENTS, STANDARDS, AND CONSUMABLE MATERIALS

### 8.1 Analysis Kit

Store kits according to manufacturer's instructions. Standards and reagents may be used until the manufacturer's expiration date.

**8.1.1** Both the Microcystin and Cylindrospermopsin kits should be stored in the refrigerator (4-8ºC). The solutions must be allowed to reach room temperature (20-25 ºC) before use. Consult state, local, and federal regulations for proper disposal of all reagents.

**8.1.2** QuikLyse reagents should be stored in the refrigerator (2-8ºC). The remaining components in the QuikLyse kit require no special storage conditions and may be stored separately from the reagents to conserve refrigerator space. Discard samples according to loca, state, and federal regulations. Allow the QuikLyse reagents to warm to room temperature before use.

## 9. INSTRUMENT CALIBRATION PROCEDURES

### 9.1 Micropipetters

Micropipetters must be verified each year for accuracy. Verification of accuracy is done by pipetting DI water and then weighing to determine if it is accurate. This check must be done for 50µL, 100 µL, and 250 µL.

### 9.2 Calibration Procedure

A calibration is required with each Analysis Batch. Use the concentrations stated in the kit instructions. Do not add additional calibration levels or eliminate any levels. Use the calibration standards provided in the original kit. Each calibration standard must be added to at least two wells.

### 9.3 Calibration Acceptance Criteria

The calibration curve is validated by evaluating the %CV of the absorbance values for the well replicates representing each calibration level, and the correlation coefficient of the four-parameter logistic curve. Calculate the %CV for each of the paired absorbance values, including the "zero" standard. The %CV for each pair must be less than, or equal to, 10%. However, one pair is allowed to exceed 10% providing the %CV is less than, or equal to, 15%. The square of the correlation coefficient ($r^2$) of the four-parameter curve must be greater than, or equal to, 0.98.

If the calibration fails, the %CV limits or $r^2$ is less than 0.98, then the entire Analysis Batch is invalid. Assay the samples in a subsequent Analysis Batch.

Freeze the filtered samples if this Analysis Batch cannot be completed on the same day as the original attempt. Each sample must be within the 14-day holding time for the repeat assay.

## 10. Procedures

### 10.1 Sample Lysing Procedure by Freeze-Thaw

**10.1.1** Mix samples thoroughly and immediately transfer 5 to 10 mL of each field sample into a 40 mL vial to begin three freeze-thaw cycles. If the sample was previously frozen, only two freeze-thaw cycles are needed (once it has thawed, it has undergone the first freeze/thaw cycle). Smaller vials may be use but reduce the sample volume to less than 25% of vial capacity.

**10.1.2** Once sample is completely frozen, remove from freezer and thaw. To speed up the process, vials may be immersed in a 35ºC in a water bath until completely thawed. Ensure samples are completely frozen and completely thawed during each cycle.

**10.1.3** Filter 1 to 2 mL of each lysed sample into a 4mL vial using a glass-fiber syringe filter. Samples are ready for immediate analysis.

### 10.2 Sample Lysing Procedure by Abraxis QuikLyse

**10.2.1** Transfer 1 mL of sample to a glass vial

**10.2.2** Add 100 uL of QuikLyse Reagent A to the sample in the vial. Cap and shake for 2 minutes. Incubate for 8 minutes at room temperature.

**10.2.3** Add 10 uL of QuikLyse Reagent B to the sample in the vial. Cap and shake for 2 minutes. Incubate for 8 minutes at room temperature.

**10.2.4** Draw less than half of the treated sample into a disposable pipette. Place a filtering tip firmly onto the disposable pipette. Sample will leak if pipette and tip are not pressed tightly together.

**10.2.5** Squeeze the pipette bulb gently, filtering the sample dropwise into a clean glass vial. The filtering tip can be removed and reattached to filter the entire lysed sample, if desired

**10.2.6** The lysed, filtered sample is now ready for analysis with one of the Abraxis Microcystins ELISA Microtiter Plate Kits.

1. Results obtained with samples prepared using the QuikLyse system must be multiplied by 1.11 to correct for sample dilution from the QuikLyse reagents.

### 10.3 Seawater Sample Preparation

**10.3.1** Microcystins

1. No matrix effects have been observed with seawater salinities (salinity up to 38 parts per thousand) using the ADDA SAES ELISA plate

**10.3.2** Cylindrospermopsin

1. Weigh 0.1 g of Cylindrospermopsin Seawater Sample Treatment reagent into a clean, appropriately labeled 4mL glass vial
2. Add 1mL of brackish water or seawater sample to the vial
3. Vortex for 1 minute. Allow the sample to settle for 10 minutes
4. Pipette the supernatant into an appropriately labeled microcentrifuge tube. Centrifuge for 5 minutes at 13,000 rpm. The sample will separate into 3 laters: a solid, white precipitate (bottom layer), a clear liquid (center layer), and a very thin white film (on top of the liquid layer).
5. Pipette the clear liquid (center layer) into a clean, appropriately labeled 4mL glass vial. Avoid pipetting the very thin white film
6. Dilute the supernatant 1: 3 with DI H2O (I.e. 333 uL supernatant and 667 ul DI H2O). The sample can then be analyzed using the Abraxis Cylindrospermopsin ELISA Kit.

**10.4 Test Preparation**

**10.4.1** Verify kit standards and reagents are used prior to the expiration date. Allow the reagents and samples to reach ambient temperature before analysis. The assay procedure must be performed away from direct sunlight.

**10.4.2** Remove the number of microtiter plate strips required from the resealable pouch. The remaining strips are stored in the pouch with the desiccant (tightly sealed)

**10.4.3** The standards, control, sample diluent, antibody enzyme conjugate, substrate, and stop solutions are ready to use and do not require any further dilutions

**10.4.4** Dilute the wash buffer (5X) concentrate at a ratio of 1:5 with deionized or distilled water. If using the entire bottle (100mL), add to 400mL of deionized or distilled water and mix thoroughly.

**10.4.5** The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously. See Table 1.

**10.5 Assay Procedures**

**10.5.1 Microcystins**

1. Add 50µL of the standard solutions, control, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add 50µL of the antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the

contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 90 minutes at room temperature.

3. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips three times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
4. Add 100 µL of the enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strip for 30 minutes at room temperature.
5. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strip three times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
6. Add 100 µL of substrate (color) solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from sunlight.
7. Add 50 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel pipette or a stepping pipette.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

**10.5.2 Cylindrospermopsin**

1. Add 50 µL of the standards, control (QCS), LRB, or samples into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.

2. Add 50 µL of the enzyme conjugate solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette.
3. Add 50 µL of the antibody solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill the contents. Incubate the strips for 45 minutes at room temperature.
4. Remove the covering, decant the contents of the wells into a sink, and blot the inverted plate on a stack of paper towels. Wash the strips four times using the diluted wash buffer. Please use at least a volume of 250 µL of 1X wash buffer for each well and each washing step. Blot the inverted plate after each wash step on a stack of paper towels. After the last wash/blot, check the wells for any remaining buffer in the wells, and if necessary, remove by additional blotting.
5. Add 100 µL of substrate (color) solution to the individual wells successively using a multi-channel, stepping, or electronic repeating pipette. Cover the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping or electronic repeating pipette.
6. Add 100 µL of stop solution to the wells in the same sequence as for the substrate (color) solution using a multi-channel, stepping, or electronic repeating pipette.
7. Read the absorbance at 450nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

### 10.6 Running an Assay

**10.6.1** Place the plate instrument with well A-1 at the rear right corner so that row 1 is going into the reader first. As you press the first row back and down you will feel slight tension on the plate stretching the carrier so that the front fits in. The plate requires a snug fit.

**10.6.2** When using a strip tray, make sure wells are pushed down into tray so that they will not cause the plate to jam or entry. Use care that well tabs do not extend over other wells. Do not place the tabbed ends of strips in row 1; they should be in row 12. Be sure to place the strips in the order in which Blanks, Calibrators and Samples are to be read.

**10.6.3** For best results, do not fill wells completely; 200-250 µL depending on well total volume is the maximum fill recommended when the mixing feature is used.

**10.6.4** Plate Layout is the default window for Abraxis Reader and displays when the program is started. There are several options: Load Plate, Save Plate, Reset, Re-Assign, Read Plate or Remove. Once samples

have been assigned, press the Read Plate button to run. Results are displayed as delta Abs for fixed time read, and delta Abs/min for non-fixed time kinetic. Refer to the "AReader Abraxis Model 4303 Operators Manual" for more information on running an assay.

**10.6.5** Sample analyses resulting in a higher concentration than the highest standard in the calibration curve must be diluted within the calibration range and reanalyzed to obtain accurate results. Samples may not be diluted in the well plate. If a sample is diluted, the final values must be calculated by multiplying the result by the proper dilution factor. Report calculated values.

**10.6.6** Save and print a copy of the calibration curve and sample results as part of the laboratory's record maintenance protocol.

**10.6.7** Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbances of the standards.

**10.4.7.1** Samples with lower absorbances than a standard will have concentrations of Microcystins or Cylindrospermopsin greater than the standard. Samples which have higher absorbances than a standard will have concentrations of Microcystins or Cylindrospermopsin less than that standard.

## 10. 5 QUALITY CONTROL

QC requirements include the IDC, and QC elements associated with each Analysis Batch. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to satisfy EPA data quality objectives. These QC requirements are considered the minimum acceptable QC protocol. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

### 10.5.1 Initial Demonstration of Capability (IDC)

The IDC must be successfully performed prior to analyzing field samples. A plate with all calibration standards, controls, and LRB, plus 10 field samples, must be ran in duplicate wells for the IDC. The IDC must be performed by each analyst, when a new analyst begins work or whenever a change in analytical performance.

When conducting the IDC, the analyst must meet the calibration requirements specified in section 9 for the standards. The %CV for each pair must be less than, or equal to, 10%. However, one pair is allowed to exceed 10% providing the %CV is less than, or equal to, 15%. All samples must have a %CV of less than 15%. If the analyst fails to meet the %CV limits or $r^2 = 0.98$ for the given standards, then their batch is invalid and they must perform the analysis in a subsequent Analysis Batch. The mean recovery of the QCS must also have a percent recovery ≥70% and ≤130% of the true value. If the analyst fails to meet the percent recovery during the IDC, then the analysis batch is invalid and must be performed again in a subsequent Analysis Batch.

### 10.5.2. Criterion for Replicate Wells

All field and QC samples are added to at least two wells. The %CV of the absorbance values measured for the well replicates must be less than, or equal to, 15%. Calculate the %CV as follows:

$$\%CV = \frac{Standard\ Deviation\ of\ Absorbances}{Mean\ Absorbance} x100\%$$

If the %CV exceeds 15% for a field sample or QC sample, then that sample is invalid. Note that the well replicates of calibration standards must meet a different set of criteria for %CV.

### 10.5.3 Quality Control Standard (QCS)

A secondary source QCS must be analyzed with each batch of samples to verify the concentration of the calibration curve. If a QCS is already included in the kit, it may be used if it has a different lot number than the calibration standards and was prepared from a separate primary stock. Acceptance limits must be within ±25% of true value. QCS values exceeding the acceptance limits require action and reanalysis of sample(s) with results greater than the concentration of an acceptable Low-CV in the same analytical batch. If reanalysis is not possible, all sample concentration results greater than an acceptable Low-CV analyzed in the same batch must be appropriately qualified and noted in the final report.

## 11 DATA REDUCTION, VALIDATION, AND REPORTING

### 11.1 Quantitation

A four-parameter logistic curve fit must be used. Other curve-fitting models are not permitted. Calculate the sample concentration for each well using the multipoint calibration. For each field and QC sample, average the two concentration values from each well. Use this mean to report sample results and to evaluate QC results against acceptance limits. Final results should be rounded to two significant figures.

### 11.2 Exceeding the Calibration Range

If a result exceeds the range of the calibration curve, dilute the sample with reagent water. Analyze the diluted sample in a subsequent Analysis Batch. Incorporate the dilution factor into the final concentration calculations. Report the dilution factor with the sample result.

## 12 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

## 13 REFEREENCES

EPA Method 546, "*Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay*; EPA 815-B-16-011; Office of Water: Cincinnati, OH, August 2016.

## 14 REVISION HISTORY

| Revision | Date | Summary | Section |
|---|---|---|---|
| 1 | 03/05/20 | Added limit detection for Microcystins ADDA-SAES and for use of Cylindrospermopsin seawater sample treatment | 1.1 |
| 1 | 03/05/2020 | Added safety information about the Cylindrospermopsin seawater sample treatment | 4.3 |
| 1 | 03/05/20 | Added limitations with methanol | 5.2 |
| 1 | 03/05/20 | Changed 1 L PETG container to 500mL | 6.1 |
| 1 | 03/05/20 | Added Microcystins ADDA-SAES test kit supplies | 7.1 |
| 1 | 03/05/20 | Added Cylindrospermopsin seawater sample treatment to supplies | 7.1.12 |
| 1 | 03/05/20 | Changed 1 L PETG container to 500mL | 7.6 |
| 2 | 02/28/22 | Add QuikLyse to list of equipment | 7.2 |
| 2 | 02/28/22 | Add QuikLyse reagents | 8.1.2 |
| 2 | 02/28/22 | Add QuikLyse procedure | 10.2 |

## 15 Tables, Figures, and Method Performance Data

**Table 1. Working Scheme of microtiter plate**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | **Std 0** | **Std 4** | **Sample 2** | | | | | | | | | |
| **B** | **Std 0** | **Std 4** | **Sample 2** | | | | | | | | | |
| **C** | **Std 1** | **Std 5** | **Sample 3** | | | | | | | | | |
| **D** | **Std 1** | **Std 5** | **Sample 3** | | | | | | | | | |
| **E** | **Std 2** | **Control** | **Etc.** | | | | | | | | | |
| **F** | **Std 2** | **Control** | **Etc.** | | | | | | | | | |
| **G** | **Std 3** | **Sample 1** | | | | | | | | | | |
| **H** | **Std 3** | **Sample 1** | | | | | | | | | | |

** Note: The working scheme of the Cylindrospermopsin plate contains an additional standard. Thus well G2 and H2 will be used for Standard 6 and the samples will start in the wells in column 3.

**Table 2. Analysis Batch QC Requirements**

| Method Reference | Requirement | Specification and Frequency | Acceptance Criteria |
|---|---|---|---|
| 9 | ELISA Calibration- with provided standards | Use kit-recommended levels and concentrations. Two well replicates per standard. | %CV of absorbance ≤10%; ≤15% allowed for 1 pair.<br><br>$r^2 \geq 0.98$ |
| 3.2 | Well Replicates | Assay field and QC samples in two wells | Sample invalid if %CV of absorbance values > 15% |
| 3.11 | Quality Control Sample (QCS) | Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the $EC_{50}$ with MC-LR from a source independent of the calibration standards. | Percent recovery ≥70% and ≤130% of the true value. |

**Appendix 2**: Results of 2024 microcystin analyses, which are organized by water body, sites within those water bodies, and the analytical results for each of the sites based on the sampling month.

| Water Body | Site | Microcystin Concentration (µg/L)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Apr | May | Jun | Jul | Aug | Sep | Oct |
| Ashepoo River | MD-253 | -[b] | 0.045 | 0.032 | 0.024 | BDL[c] | QCF[d] | 0.054 |
| | CSTL-069 | -[b] | BDL[c] | 0.05 | 0.0825 | BDL[c] | BDL[c] | -[b] |
| Ashley River | MD-049 | -[b] | 0.06 | BDL[c] | BDL[c] | BDL[c] | BDL[c] | BDL[c] |
| | CSTL-102 | -[b] | 0.0185 | 0.066 | 0.099 | 0.02 | 0.0505 | 0.043 |
| | MD-052 | -[b] | 0.023 | BDL[c] | 0.033 | BDL[c] | QCF[d] | 0.0535 |
| Beaufort River | MD-001 | -[b] | BDL[c] | 0.024 | BDL[c] | BDL[c] | QCF[d] | 0.061 |
| | MD-004 | -[b] | BDL[c] | BDL[c] | 0.047 | 0.0195 | QCF[d] | BDL[c] |
| Black River | PD-325 | -[b] | 0.099 | 0.091 | 0.0485 | 0.0565 | -[b] | 0.1925 |
| Bohicket Creek | MD-209 | -[b] | 0.0565 | BDL[c] | 0.0695 | BDL[c] | QCF[d] | BDL[c] |
| Boyd Mill Pond | S-311 | -[b] | BDL[c] | 0.056 | 0.046 | BDL[c] | 0.1885 | 0.024 |
| Broad Creek | MD-174 | -[b] | BDL[c] | BDL[c] | 0.0715 | BDL[c] | QCF[d] | 0.0215 |
| Broad River | MD-116 | -[b] | BDL[c] | 0.032 | BDL[c] | BDL[c] | QCF[d] | 0.045 |
| Calibogue Sound | MD-175 | -[b] | 0.025 | BDL[c] | 0.036 | BDL[c] | QCF[d] | 0.0285 |
| Casino Creek | MD-266 | -[b] | 0.0655 | 0.034 | 0.111 | BDL[c] | QCF[d] | 0.086 |
| Cedar Creek Reservoir | CW-033 | -[b] | 0.099 | 0.0725 | 0.0645 | BDL[c] | 0.128 | 0.0875 |
| | CW-174 | -[b] | 0.149 | 0.083 | 0.1415 | BDL[c] | 0.16 | 0.071 |
| Chechessee | MD-117 | -[b] | 0.028 | 0.0225 | 0.03 | BDL[c] | QCF[d] | 0.0695 |
| Colleton River | MD-176 | -[b] | BDL[c] | BDL[c] | 0.0655 | -[b] | QCF[d] | 0.0685 |
| Combahee River | MD-252 | -[b] | 0.0355 | 0.02 | 0.0245 | 0.0315 | QCF[d] | BDL[c] |
| Cooper River | MD-043 | -[b] | BDL[c] | 0.0595 | 0.1205 | 0.0205 | QCF[d] | 0.0545 |
| | MD-045 | -[b] | BDL[c] | BDL[c] | 0.0845 | 0.0545 | QCF[d] | 0.06 |
| | MD-248 | -[b] | BDL[c] | 0.028 | 0.078 | BDL[c] | QCF[d] | 0.052 |
| Coosawhatchie River | CSTL-107 | -[b] | 0.0265 | BDL[c] | BDL[c] | BDL[c] | QCF[d] | 0.029 |
| Dawho River | MD-120 | -[b] | BDL[c] | BDL[c] | BDL[c] | 0.052 | QCF[d] | BDL[c] |
| Fishing Creek Reservoir | CW-016F | -[b] | 0.043 | 0.0655 | 0.13 | BDL[c] | 0.164 | 0.093 |
| | CW-057 | -[b] | 0.049 | 0.0645 | 0.144 | BDL[c] | BDL[c] | 0.0725 |
| Five Fathom Creek | MD-267 | -[b] | 0.092 | 0.0455 | 0.284 | 0.026 | -[b] | 0.0205 |
| Folly River | MD-130 | -[b] | BDL[c] | BDL[c] | 0.0615 | 0.051 | -[b] | 0.015 |
| | RL-01008 | 0.0245 | BDL[c] | 0.1875 | 0.172 | -[b] | 0.044 | 0.035 |

| Water Body | Site | Microcystin Concentration (µg/L)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Apr | May | Jun | Jul | Aug | Sep | Oct |
| Goose Creek Reservoir | ST-032 | BDL[c] | BDL[c] | 0.134 | 0.0435 | -[b] | 0.0725 | 0.0125 |
| | ST-033 | BDL[c] | BDL[c] | 0.129 | 0.047 | -[b] | BDL[c] | 0.0315 |
| Great Swamp | MD-129 | -[b] | 0.0815 | 0.0795 | 0.0445 | BDL[c] | -[b] | -[b] |
| Hamlin Sound | MD-271 | -[b] | 0.0365 | 0.0435 | 0.027 | 0.021 | -[b] | 0.0325 |
| Intracoastal Waterway | MD-069 | -[b] | BDL[c] | 0.023 | 0.027 | BDL[c] | -[b] | 0.039 |
| | MD-125 | -[b] | 0.0185 | BDL[c] | -[b] | -[b] | -[b] | BDL[c] |
| J. Strom Thurmond | CL-041 | -[b] | 0.145 | 0.227 | 0.31 | 0.19 | 0.229 | 0.183 |
| Kiawah River | MD-273 | -[b] | 0.0185 | BDL[c] | 0.0585 | BDL[c] | 0.0535 | BDL[c] |
| Lake Bowen | B-339 | -[b] | 0.07 | 0.1915 | 0.2645 | 0.117 | 0.1715 | 0.0465 |
| | B-340 | -[b] | BDL[c] | 0.0825 | 0.138 | BDL[c] | 0.172 | 0.0295 |
| Lake Edgar Brown | CL-064 | -[b] | 0.225 | 0.095 | 0.0405 | BDL[c] | 0.2305 | 0.0425 |
| Lake Greenwood | S-022 | -[b] | 0.0785 | 0.0805 | 0.1015 | BDL[c] | 0.186 | 0.165 |
| | S-024 | -[b] | 0.0235 | 0.0245 | 0.1465 | 0.15 | 0.182 | BDL[c] |
| | S-131 | -[b] | 0.027 | 0.0535 | 0.1345 | BDL[c] | 0.271 | BDL[c] |
| | S-308 | -[b] | 0.0535 | 0.0925 | 0.135 | 0.2225 | 0.4075 | BDL[c] |
| Lake Hartwell | SV-200 | -[b] | BDL[c] | 0.0415 | 0.051 | 0.0385 | 0.1415 | BDL[c] |
| | SV-236 | -[b] | BDL[c] | 0.102 | 0.105 | BDL[c] | 0.212 | 0.0885 |
| | SV-268 | -[b] | 0.0205 | 0.055 | 0.032 | -[b] | BDL[c] | BDL[c] |
| | SV-339 | -[b] | 0.0585 | 0.087 | -[b] | 0.118 | 0.122 | 0.106 |
| | SV-340 | -[b] | 0.059 | 0.046 | 0.0625 | BDL[c] | 0.1035 | 0.0815 |
| | SV-363 | -[b] | 0.0445 | 0.1135 | -[b] | 0.084 | 0.117 | 0.1095 |
| | SV-372 | -[b] | BDL[c] | 0.103 | 0.078 | 0.093 | 0.1255 | 0.175 |
| | SV-374 | -[b] | BDL[c] | 0.089 | 0.073 | BDL[c] | 0.1935 | 0.0705 |
| Lake Jocassee | CL-019 | -[b] | 0.0855 | 0.079 | 0.0185 | 0.0325 | BDL[c] | BDL[c] |
| | SV-335 | -[b] | 0.027 | BDL[c] | 0.03 | BDL[c] | BDL[c] | 0.1035 |
| | SV-336 | -[b] | 0.0445 | 0.019 | 0.033 | BDL[c] | BDL[c] | 0.175 |
| Lake Keowee | SV-338 | -[b] | 0.048 | 0.0195 | BDL[c] | BDL[c] | BDL[c] | BDL[c] |
| | SV-361 | -[b] | 0.06 | -[b] | -[b] | 0.0255 | BDL[c] | BDL[c] |
| Lake Murray | S-211 | -[b] | 0.2205 | 0.1665 | 0.2855 | 0.2165 | 0.2005 | 0.1825 |
| | S-213 | -[b] | 0.2615 | -[b] | 0.394 | 0.187 | 0.221 | 0.1765 |
| | S-222 | -[b] | 0.0495 | -[b] | 0.146 | 0.074 | 0.11 | BDL[c] |
| | S-279 | -[b] | 0.2425 | 0.22 | 0.2895 | 0.1855 | 0.139 | 0.1365 |
| | S-280 | -[b] | 0.3025 | 0.141 | 0.41 | 0.128 | 0.214 | -[b] |
| | S-309 | -[b] | 0.1255 | -[b] | 0.123 | 0.1435 | BDL[c] | 0.194 |
| | S-310 | -[b] | 0.0765 | -[b] | 0.0875 | 0.155 | BDL[c] | BDL[c] |
| | S-326 | -[b] | 0.166 | BDL[c] | 0.186 | BDL[c] | 0.191 | 0.0635 |
| Lake Robinson | PD-327 | -[b] | -[b] | 0.024 | 0.022 | 0.0225 | BDL[c] | 0.0215 |
| Lake Russell | SV-098 | -[b] | 0.1305 | 0.25 | 0.1575 | 0.16 | 0.293 | 0.1605 |

| Water Body | Site | Microcystin Concentration (µg/L)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Apr | May | Jun | Jul | Aug | Sep | Oct |
| | SV-357 | -[b] | 0.313 | 0.3665 | 0.217 | 0.1245 | 0.171 | 0.236 |
| Lake Secession | SV-331 | -[b] | -[b] | 0.273 | 0.4795 | 0.1355 | 0.2445 | 0.077 |
| Lake Wateree | CL-089 | -[b] | -[b] | 0.088 | 0.1615 | 0.102 | 0.1445 | 0.0885 |
| | CW-207 | -[b] | 0.0965 | 0.12 | 0.198 | 0.1125 | 0.151 | 0.101 |
| | CW-207B | -[b] | 0.054 | 0.0965 | 0.2555 | 0.085 | 0.1785 | 0.169 |
| | CW-208 | -[b] | 0.065 | -[b] | 0.1385 | BDL[c] | 0.137 | 0.121 |
| | CW-231 | -[b] | 0.154 | 0.0775 | 0.0995 | 0.1005 | BDL[c] | 0.075 |
| | LCR-02 | -[b] | -[b] | -[b] | 0.0995 | 0.1615 | -[b] | -[b] |
| Lake Whelchel | B-354 | -[b] | 0.5705 | 0.5055 | 0.8765 | 0.409 | 0.3385 | 0.1825 |
| | B-885 | -[b] | 0.6415 | 0.556 | 0.6705 | 0.395 | 0.2925 | 0.2005 |
| Lake Wylie | CW-197 | -[b] | 0.04 | 0.24 | 0.1975 | 0.12 | 0.1375 | 0.107 |
| | CW-201 | -[b] | 0.148 | 0.154 | 0.3175 | 0.1705 | 0.2065 | 0.1645 |
| | CW-230 | -[b] | 0.0785 | 0.2315 | 0.474 | 0.0425 | 0.1375 | 0.179 |
| Langley Pond | CL-069 | -[b] | 0.033 | 0.032 | 0.0605 | BDL[c] | 0.1635 | -[b] |
| May River | MD-173 | -[b] | 0.047 | BDL[c] | 0.0735 | BDL[c] | -[b] | 0.0355 |
| Lake Monticello | B-327 | 0.0575 | 0.2935 | 0.208 | 0.158 | 0.134 | 0.2385 | 0.1385 |
| | B-890 | 0.0255 | 0.2515 | 0.3295 | -[b] | BDL[c] | BDL[c] | 0.132 |
| | RL-04370 | 0.038 | 0.3285 | 0.437 | 0.266 | 0.124 | 0.2055 | 0.119 |
| Morgan River | MD-282 | -[b] | BDL[c] | BDL[c] | 0.0335 | BDL[c] | -[b] | BDL[c] |
| N. Edisto River | MD-262 | -[b] | 0.0645 | 0.0385 | 0.0325 | BDL[c] | -[b] | 0.018 |
| N. Santee River | ST-005 | -[b] | 0.0805 | 0.226 | 0.166 | 0.088 | -[b] | 0.194 |
| New River | MD-118 | -[b] | 0.081 | 0.0495 | 0.037 | BDL[c] | -[b] | 0.036 |
| Parr Reservoir | B-345 | -[b] | -[b] | -[b] | 0.1635 | 0.043 | 0.1185 | 0.175 |
| | B-346 | BDL | 0.044 | 0.115 | 0.106 | 0.023 | 0.2045 | 0.1525 |
| | B-889 | 0.0355 | 0.1175 | 0.0905 | 0.065 | BDL[c] | -[b] | 0.025 |
| Parrot Creek | MD-281 | -[b] | BDL[c] | BDL[c] | 0.027 | BDL[c] | -[b] | BDL[c] |
| Parsonnage Creek | MD-277 | -[b] | BDL[c] | 0.05 | 0.0885 | -[b] | -[b] | BDL[c] |
| Pee Dee River | MD-275 | -[b] | 0.183 | 0.134 | 0.096 | 0.06 | -[b] | 0.115 |
| Ramshorn Creek | MD-257 | -[b] | 0.036 | BDL[c] | 0.049 | BDL[c] | -[b] | BDL[c] |
| | MD-258 | -[b] | 0.0215 | BDL[c] | 0.019 | BDL[c] | -[b] | 0.079 |
| S. Edisto River | MD-260 | -[b] | BDL[c] | BDL[c] | BDL[c] | 0.033 | -[b] | BDL[c] |
| S. Santee River | ST-006 | -[b] | 0.0775 | -[b] | -[b] | 0.0665 | -[b] | 0.095 |
| Sampit River | MD-077 | -[b] | 0.096 | 0.0475 | 0.064 | 0.027 | -[b] | -[b] |
| Sewee Bay | MD-269 | -[b] | 0.0265 | 0.0245 | -[b] | BDL[c] | -[b] | 0.04 |
| Stono River | MD-202 | -[b] | 0.0615 | 0.0175 | 0.075 | BDL[c] | BDL[c] | BDL[c] |
| | MD-206 | -[b] | BDL[c] | BDL[c] | 0.056 | BDL[c] | -[b] | BDL[c] |
| Unnamed Creek | MD-256 | -[b] | BDL[c] | BDL[c] | 0.057 | 0.02 | -[b] | 0.053 |

| Water Body | Site | Microcystin Concentration (µg/L)[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Apr | May | Jun | Jul | Aug | Sep | Oct |
| Waccamaw River | MD-142 | -[b] | 0.1385 | 0.0975 | 0.0685 | 0.0285 | -[b] | 0.0635 |
| Wando River | MD-115 | -[b] | BDL[c] | BDL[c] | 0.0795 | BDL[c] | -[b] | 0.0295 |
| | MD-264 | -[b] | BDL[c] | 0.048 | 0.2045 | BDL[c] | -[b] | 0.0435 |
| Winyah Bay | MD-278 | -[b] | 0.0765 | 0.0475 | 0.0245 | 0.037 | -[b] | 0.085 |
| Wright River | MD-259 | -[b] | 0.037 | BDL[c] | 0.0225 | BDL[c] | -[b] | 0.025 |
| Yonges Island Creek | MD-261 | -[b] | 0.056 | BDL[c] | 0.0285 | BDL[c] | -[b] | 0.0225 |

a. µg/L = micrograms per liter (parts per billion)
b. Dashes indicate no data available
c. BDL= below detection limit
d. QCF= quality control failed

**Appendix 3:** Recreational Watches issued on Lake Woodcross, Goose Creek Reservoir, Twin Lakes, Lake Greenwood, and Lake Wateree. Watches remained in place until the bloom was no longer present.

| Lake Name | Location | HAB description | Associated algal toxins | Watch Issued | Watch Lifted |
|---|---|---|---|---|---|
| Lake Woodcross | Entire Lake | *Dolichospermum sp.* and *Worchinia sp.* | Microcystins, Cylindrospermopsin, Anatoxin-a, Saxitoxins | 04/16/2024 | 06/01/2024 |
| Goose Creek Reservoir | Entire Lake | *Dolichospermum sp.* and *Aphanizomenon sp.* | Microcystins, Cylindrospermopsin, Anatoxin-a, Saxitoxins | 06/05/2024 | 07/11/2024 |
| Twin Lakes | Entire Lake | *Planktothrix sp.* | Microcystins, Anatoxin-a | 06/20/2024 | 07/29/2024 |
| Lake Greenwood | Waterloo, SC | *Lyngbya wollei* and *Oscillatoria sp.* | Microcystins, Cylindrospermopsin, Anatoxin-a, Saxitoxins | 08/15/2024 | 10/01/2024 |
| Lake Wateree | From Stillhouse Branch to Wateree Dam | *Phormidium sp.* | Microcystins, Anatoxin-a, Saxitoxins | 08/27/2024 | 09/26/2024 |