

**MISSOURI DEPARTMENT OF NATURAL RESOURCES  
DIVISION OF ENVIRONMENTAL QUALITY  
ENVIRONMENTAL SERVICES PROGRAM  
Standard Operating Procedure**

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SOP TITLE: Sample Collection and Handling of Cyanobacteria for Identification, Enumeration, and Cyanotoxin Analysis

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REVISED BY: N/A – This is a new SOP

APPROVED BY: Original signed by Brian Allen, Director, ESP

SUMMARY OF REVISIONS: N/A – This is a new SOP

APPLICABILITY: This SOP applies to all ESP staff involved in the collection and handling of samples for cyanobacteria identification and cyanotoxin analysis.

DISTRIBUTION: MoDNR Intranet  
SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed:				
Initials:				

## 1.0 SCOPE AND APPLICABILITY

Cyanobacteria (i.e. blue-green algae) have the potential to produce toxins known as cyanotoxins. These cyanotoxins can cause illness and death in humans and animals through exposure to contaminated recreational waters and drinking water supplies. Of the many known cyanotoxins, the most commonly identified in the United States are microcystin, cylindrospermopsin, anatoxin-a, and saxitoxin.

This Standard Operating Procedure (SOP) provides guidance on the collection and handling of surface water and drinking water samples to be analyzed for cyanotoxins by the Enzyme Linked Immunosorbent Assay (ELISA) analysis method. The procedures outlined in this SOP are adapted from methods developed by the U.S. Geological Survey (Graham, Loftin, Ziegler, & Meyer, 2008), the U.S. Environmental Protection Agency (Zaffiro, Rosenblum, & Wendelken, 2016), and Abraxis, Inc.

## 2.0 SUMMARY OF METHOD

Sample container type, residual chlorine, light exposure, and pH can all negatively affect cyanotoxins and result in inaccurate data. This SOP will outline the sampling and handling requirements for the most common cyanotoxins: microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin. These procedures are for total (free and cell bound) toxin analysis. If quantitative analysis for either fraction is desired, contact the Environmental Services Program (ESP), Water Quality Monitoring Section (WQMS) for further instruction.

## 3.0 DEFINITIONS AND ABBREVIATIONS

- ELISA- Enzyme Linked Immunosorbent Assay
- ESP – Environmental Services Program
- MoDNR – Missouri Department of Natural Resources
- $\text{Na}_2\text{S}_2\text{O}_3$  – Sodium thiosulfate
- PPE – Personal protective equipment
- SOP – Standard Operating Procedures
- WQMS – Water Quality Monitoring Section

## 4.0 HEALTH AND SAFETY REQUIREMENTS

- 4.1 Algal toxins include neurotoxins, hepatotoxins, and dermatotoxins that have been known to cause illness and death in humans and wildlife. PPE, such as disposable gloves and waders, should always be worn. Glove length should be determined based upon sampling requirements. If sampling is conducted from a boat, a personal floatation device should be worn at all times while in the boat.
- 4.2 Skin and eye contact should be avoided. If eye exposure occurs, immediately flush eyes with clean water for at least 15 minutes. Immediately wash any areas of skin exposure with soap and clean water.

- 4.3 Studies have shown that toxins can become aerosolized through wave action created by wind and/or boating. Inhalation of aerosolized toxins may result in allergy or asthma-like symptoms. Whenever possible, waterbodies experiencing an algal bloom should be approached from the windward (upwind) direction to minimize contact with any fine mists. If using a boat, “no wake” speed should be used in the affected areas. Individuals with respiratory illnesses such as asthma or other respiratory diseases are more susceptible to breathing difficulties and may experience more severe symptoms. Such individuals should consider avoiding exposure if possible. If one uses a rescue inhaler, they should ensure it is readily available. Although wearing an N95 mask or respirator may reduce respiratory exposure, there is no documented evidence that it will completely eliminate the risks.
- 4.4 At any time during a bloom investigation or sampling event, if staff experience adverse side effects such as headaches, skin irritation, respiratory irritation, etc., the staff should vacate the area and notify their supervisor. If sampling is required, the event shall then be conducted by staff equipped with additional PPE.
- 4.5 Any equipment, such as waders or secondary sampling devices, should be washed as soon as possible to prevent spreading of scums and future contact by staff. If decontamination cannot be conducted onsite, contaminated equipment should be bagged and sealed until decontamination is performed.
- 4.6 After collection, sample containers must be wiped with a clean paper towel to prevent possible contamination of other containers and staff who will handle the sample container.
- 4.7 Sampling locations may include streams and lakes where the sample collector should maintain awareness of their surroundings such as weather, stream conditions, and boat traffic.

## 5.0 PERSONNEL QUALIFICATIONS

Sample collectors shall have attended the department’s Basic Sampling Training class, observed more experienced personnel in the field, and have reviewed and be knowledgeable of the following SOPs.

- MDNR-ESP-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations*
- MDNR-ESP-002 *Field Sheet and Chain-of-Custody Record*
- MDNR-ESP-003 *Sample Numbering and Labeling*
- MDNR-ESP-004 *Field Documentation*
- MDNR-ESP-005 *General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows*

- MDNR-ESP-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab*

## 6.0 SUPPLIES AND EQUIPMENT

- Amber glass containers (120 mL wide mouth bottle or 40 mL VOA vial)
- Sodium thiosulfate
- Ascorbic acid
- Anatoxin-a/Saxitoxin sample diluent (10x) concentrate
- Lugol's solution (Lugol's iodine)
- Cooler with ice
- Sample labels
- Chain of custody
- Field notebook
- Disposable gloves
- Respirator or N95 mask – see 4.3

## 7.0 PROCEDURE

### 7.1 Collection

#### 7.1.1 Surface Water Toxin Analysis

In order to collect a sample that represents a potential risk from recreational exposure, the water should be gently mixed from the surface to approximately 6 inches below. Fill the sample container within the mixed 6-inch water column, cap, preserve according to Section 7.2, and place the sample immediately on ice and protect from light.

#### 7.1.2 Drinking Water Toxin Analysis

- Source Drinking Water

Fill containers prior to any treatment processes and preserve according to Section 7.2. Place the sample(s) immediately on ice and protect from light.

- Finished Drinking Water

Fill containers after all treatment processes. Preserve according to Section 7.2, ensuring proper neutralization of chlorine. Place the sample immediately on ice and protect from light.

### 7.1.3 Algal Identification or Enumeration

If algal identification or enumeration is desired, an additional 100 mL aliquot of sample must be collected and preserved according to 7.2.4. Samples should be placed on ice, but NOT frozen.

## 7.2 Preservation

### 7.2.1 Residual Chlorine

Samples collected from drinking water systems or any other sample with a residual chlorine value greater than 0.1 mg/L, must be treated to quench the chlorine. For samples with little or no residual chlorine, collecting in a container pre-preserved with sodium thiosulfate or ascorbic acid will not negatively impact the sample.

- Microcystins/Cylindrospermopsin Samples – Use 10 mg of sodium thiosulfate per 100 mL of sample. On the sample label, circle  $Na_2S_2O_3$  as the preservative.
- Anatoxin-a/Saxitoxin Samples – Use 10 mg of ascorbic acid per 100 mL of sample. On the sample label, circle *Other* and write *Ascorbic Acid* as the preservative.

### 7.2.2 Microcystins/Cylindrospermopsin Samples

Other than requirements for residual chlorine and temperature, no additional preservation is needed for microcystins or cylindrospermopsin samples.

### 7.2.3 Anatoxin-a/Saxitoxin Samples

Samples collected for anatoxin-a and/or saxitoxin must be preserved with the Abraxis Anatoxin-a/Saxitoxin Sample Diluent (10X) Concentrate (1 mL per 9 mL of water sample). Pre-preserved containers with the Anatoxin-a/Saxitoxin Sample Diluent (10X) Concentrate are available from ESP/WQMS. On the sample label, circle *Other* and write *A/S* as the preservation method. If also using ascorbic acid to quench any residual chlorine, write *A/S* in addition to *Ascorbic Acid*.

### 7.2.4 Algal Identification or Enumeration

Samples for identification and enumeration shall be preserved immediately using Lugol's solution. Pre-preserved bottles are available from the ESP/WQMS. If pre-preserved bottles are not obtained, samples must be preserved with 1 mL of Lugol's solution to 100 mL of sample.

On the sample label, circle “other” and write *Lugol’s* as the preservation method. If collected from a chlorinated source, quenching of residual chlorine is not necessary.

#### 7.2.5 Light

All samples must be protected from natural and artificial light by using amber glass containers and keeping samples in coolers.

#### 7.2.6 Temperature

All samples must be immediately placed on ice or refrigerated after collection. Samples shall be submitted within five days to the laboratory and kept below 10 °C during transport. Samples for toxin analysis (NOT identification or enumeration) may be frozen prior to transport to extend the holding time, but there must be sufficient head space in the container to allow for expansion due to freezing.

### 8.0 SPECIAL CONSIDERATIONS

- 8.1 The ESP has sample containers that are pre-preserved with sodium thiosulfate, ascorbic acid or Lugol’s that may be requested prior to sampling.
- 8.2 Samples collected for anatoxin-a and saxitoxin may be combined into one container. Samples collected for microcystins and cylindrospermopsin may be combined.
- 8.3 All samples shall be collected in amber glass containers.

### 9.0 QUALITY ASSURANCE/QUALITY CONTROL

Quality assurance/quality control processes and methods for sample collection should follow MDNR-ESP-001 and MDNR-ESP-210 *Quality Assurance/Quality Control for Environmental Data Collection*.

### 10.0 REFERENCES

Enzyme-Linked Immunosorbent Assay for the Congener-Independent Determination of Microcystins and Nodularins in Water Samples User Guide. 2014. Abraxis, LLC.

Enzyme-Linked Immunosorbent Assay for the Determination of Anatoxin-a in Water Samples User Guide. 2016. Abraxis, LLC.

Enzyme-Linked Immunosorbent Assay for the Determination of Cylindrospermopsin in Water Samples User Guide. 2015. Abraxis, LLC.

Enzyme-Linked Immunosorbent Assay for the Determination of Saxitoxin (PSP) in Water and Contaminated Samples User Guide. 2015. Abraxis, LLC.

Graham, J. L., Loftin, K. A., Ziegler, A. C., & Meyer, M. T. United States Geological Survey. 2008. Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste-and-Odor Studies in Lakes and Reservoirs. Scientific Investigations Report 2008-5038.

MDNR-ESP-001. 2016. Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-002. 2016. Field Sheet and Chain-of-Custody Record. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-003. 2010. Sample Numbering and Labeling. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-004. 2010. Field Documentation. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-005. 2017. General Sampling Considerations Including the Collection of Grab, Composite, and Modified Composite Samples from Streams and Wastewater Flows. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-018. 2010. Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

MDNR-ESP-210. 2013. Quality Assurance/Quality Control for Environmental Data Collection. Missouri Department of Natural Resources, Division of Environmental Quality, Environmental Services Program.

Zaffiro, A., Rosenblum, L., & Wendelken, S. C. U.S. Environmental Protection Agency. 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. Office of Water.