Hydrilla in Lake Thurmond is colonized by cyanobacteria linked to avian vacuolar myelinopathy (AVM), a disease affecting several avian species including bald eagles and American coots. The cyanobacteria produce a neurotoxin that causes brain lesions and adverse effects including the inability to fly, swim, and walk. Prior studies have established that the toxin can be ingested through the hydrilla, but this test aims to determine the presence of the cyanobacterial toxin in the water. An experiment was designed using an ENVI™ SPE Disk (C18 bonded phase) that successfully extracted the toxin from a previously spiked water sample. Water samples were then collected from the lake during the Fall 2016 AVM season and analyzed for the presence of the toxin with the previously developed SPE disk method. This study was developed in order to create a method to test the water samples for toxin and identify different routes of exposure to the toxin and evaluate its environmental effects.

**Introduction**

- Avian vacuolar myelinopathy (AVM) is linked to cyanobacteria (Anabaena variabilis) that grows on hydrilla in Lake Thurmond. The cyanobacteria produce a toxin (or toxins) that researchers hypothesize is responsible for AVM. While the toxin primarily affects birds, other studies have found that fish, turtles, and some invertebrates are also susceptible.
- AVM causes brain lesions that lead to neurological dysfunction. These adverse effects include but are not limited to a decline in motor function and a decrease in coordination.
- All affected animals that have been studied to date have been exposed via ingestion of the hydrilla in the lake. If the toxin is present in the water, additional species may be affected.
- Prior studies have determined that the cyanobacteria are most prevalent during the months of November and December.
- The model used for toxin detection in this study was a C6 rat glioma cell line. Previous studies have established that the toxin induces cell cycle arrest, and this endpoint can be used to track toxicity.

**Objectives**

- The first objective was to create a method to identify the presence of the cyanobacterial toxin(s) in water samples.
- Once a method had been developed, samples collected from Lake Thurmond were tested to determine the presence of the AVM toxin.

**Methods**

**Method Development**

- The first part of this study was to create a method to successfully extract the AVM toxin from a water sample. With toxin extracts obtained from UGA, 1 liter samples of deionized water were spiked with 100µL of the extract. Methanol was added to create a final concentration of 5%.
- An SPE ENVl-Disk™ (C18 bonded Phase) was placed in an apparatus that would drain into a Buchner funnel, which was connected to a vacuum. (See Figure 2)
- The SPE disk was conditioned with 10 mL of Methanol that was suspended for 10 seconds on the disk until the spiked sample was ready to be filtered.
- The 1 liter sample was poured into the apparatus and run through the SPE Disk until all the water had passed. The disk was then dried and placed back into the apparatus with a smaller test tube and 10 mL of methanol was run through the SPE disk to elute the toxin. The 10 mL was transferred to a small 100 mL beaker and left to dry overnight in the fume hood.
- The extracts were then re-suspended in 600µL of methanol, transferred to a 1500µL test tube, and placed into the centri-vap for approximately 45 minutes. After drying, the sample was re-suspended into the original volume of 100µL.
- The extracted samples and original extracts were tested on a C6 cell line from concentrations of 1:1 to 1:1024 to compare toxicity. Only a slight loss in toxicity was seen, so this method was then used to test water samples from Lake Thurmond.

**Environmental Sample Analysis**

- Samples were collected in 1 liter bottles from Cherokee Recreation Area, J. Strom Thurmond Lake, GA on three dates: 11/11/16 (F1), 12/21/16 (F2), and 12/17/16 (F3). (See Figure 1.)
- The collected samples were pre-filtered through Whatman™ GF/C glass microfiber filters (1.2 µm pore size) to remove cells and particulates.
- The toxin was extracted from each sample according to the method outlined in previous section.
- The extracted samples were re-suspended in 100 µL of methanol and tested on C6 cell line at the following concentrations: 2µL, 1µL, 0.5µL, and 0.25µL in 200µL media.
- The F1 samples from November 11, 2016, showed the highest toxicity, demonstrating that the toxin is both on the hydrilla and present in the water.
- The F1 samples from November 11, 2016, showed the highest toxicity, inducing cell cycle arrest at lower concentrations than F2 and F3. This could be an indication of when the toxin is at its peak, but may also be due to the random sampling method of water collected at the boat ramp.
- The presence of the toxin in the water poses concerns for many more species who do not necessarily ingest the hydrilla, but are now known to be exposed via consumption of the water.

**Results**

- The method development was successful for this experiment, and with few alterations can be used for future studies.
- Each of the three collected water samples contained the toxin.
- The F3 samples from November 11, 2016, showed the highest toxicity, inducing cell cycle arrest at lower concentrations than F2 and F3. This could be an indication of when the toxin is at its peak, but may also be due to the random sampling method of water collected at the boat ramp.

**Discussion**

- For future research, the extraction method can be tweaked to be more time manageable. SPE filtration of the 1L sample often took longer than expected, possibly due to loss of conditioning. The percentage of methanol could be increased up to approximately 25% to maintain a stronger SPE disk conditioning. This may also improve toxin recovery.
- sediment samples collected from the same location and dates as the water samples will also be analyzed. If the toxin is found in the sediment it could pose a threat to even more organisms that may live or feed from the sediment at the bottom of the lake.

**Future Research**

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**References**