ASSESSMENT OF UV LIGHT FOR THE TREATMENT OF CYANOTOXINS IN SMALL-SCALE DRINKING WATER TREATMENT SYSTEMS

By

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ABSTRACT

ASSESSMENT OF UV LIGHT FOR THE TREATMENT OF CYANOTOXINS IN SMALL-SCALE DRINKING WATER TREATMENT SYSTEMS

Ryan Spencer McLintock

Harmful Algal Blooms (HABs) are commonly caused by the rapid growth of cyanobacteria in fresh waterways, which many people rely on for drinking water. When a HAB occurs, a variety of cyanotoxins can be produced and released into sources of drinking water, which can make people sick or die if not properly treated. Two of the most common toxins are microcystin-LR (MC-LR) and anatoxin-a (A-a), for which the World Health Organization (WHO) recommends a maximum allowable concentration of 1 μg/L in drinking water to avoid health risks. The recommendation for maximum allowable concentration was calculated specifically for microcystins, but is currently used as a limit for all cyanotoxins due to a lack of research on other toxin varieties. Treatment of drinking water to remove cyanotoxins requires special knowledge and equipment that may not be available to people who do not have access to a community-scale water system and use a small-scale treatment system such as a slow-sand filter. The lack of special training and equipment leaves members of underserved communities, such as the Hoopa and Yurok tribes in Humboldt County, at risk of drinking untreated water contaminated with cyanotoxins. The purpose of this project was to assess the effectiveness of using ultra-violate (UV) light for the treatment of two of the most

prevalent cyanotoxins (MC-LR and A-a) in a small-scale drinking water treatment system.

MC-LR and A-a were each dissolved separately in solutions of Nanopure water and water from the Klamath River after being treated by a slow-sand filter at concentrations of 1, 10, 100, 1000, and 5000 μg/L. Each concentration of toxin was then exposed to UV light focused at a wavelength of 254 nm. Doses of UV light applied were: 60, 750, 1500, and 4000 mJ/cm^2. A successful UV dose would lower MC-LR and A-a concentrations to below 1 μg/L, which means a 4-log removal for the highest concentrations. The concentration of MC-LR and A-a in each sample was determined using ELISA test kits, which are specific to microcystin congeners and anatoxin congeners, respectively. The work reported here showed that the highest doses of UV light applied could not achieve even a 2-log removal and showed a pattern of diminishing returns between 1500 and 4000 mJ/cm^2. When the starting concentration of toxins was 10 μg/L or less, then the highest dose of UV light was sufficient to degrade the cyanotoxins below the WHO guideline. The results of this study suggest that UV treatment may require prohibitively high doses to be relied upon for treatment of cyanotoxins on its own, but may be an effective polishing step after some other primary treatment has occurred. Further testing is required to find the optimal UV dose to provide treatment in a small-scale drinking water treatment system.

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INTRODUCTION

Cyanobacteria, also called blue-green algae, are prokaryotic, photosynthetic, single-celled microorganisms commonly found in freshwater habitats such as rivers, lakes, ponds and reservoirs, as well as brackish and marine habitats (Demirel and Sukatar 2012). Under certain conditions, such as stagnant water with warm temperatures and high nutrient load, cyanobacteria populations can increase dramatically over a short time, causing a Harmful Algal Bloom (HAB) (U.S. EPA 2014). When a HAB occurs, cyanobacteria can produce a variety of toxins, collectively called cyanotoxins. These toxins may exit each individual cell, persist in the waterway after the bloom has subsided, and harm lifeforms that come into contact with the water downstream of the bloom (Hudnell and Dortch 2008). While the conditions that allow a HAB to occur are most common during summer months, HABs can occur at any time without spatial or temporal consistency. However, in recent years HABs have occurred more frequently and in more areas around the globe (U.S. EPA 2014). In Humboldt County, California, HABs have occurred in the Klamath and Trinity rivers and released microcystin-LR (MC-LR) and anatoxin-a (A-a), respectively (CSWRCB 2019). For example, the Trinity River was reportedly contaminated with A-a in August of 2014 (Crandall 2014). Consequently, in Humboldt County, cyanotoxins are an issue of concern to people that treat their own drinking water, such as some members of the Hoopa Tribe and Yurok Tribe who are not connected to the community water supply and instead draw their drinking water directly from the Trinity or Klamath rivers.

Cyanotoxins can cause myriad health problems for people of all ages including nausea, skin rashes, liver and kidney failure, and potentially death depending on the type and concentration of cyanotoxin that was contacted (WHO 2003). The most common routes of exposure are through drinking contaminated water or contact with contaminated water through recreational means (Hudnell and Dortch 2008). Additionally, there is a lower chance of exposure through eating contaminated fish and shellfish (Hudnell and Dortch 2008). Symptoms from cyanotoxin exposure can occur at very low concentrations, so the World Health Organization recommends a maximum allowable concentration for combined microcystins in drinking water to be 1 μg/L or less, while the U.S. EPA set a guideline value of 1.6 μg/L (WHO 1998; Stanton 2018). Due to a lack of information there are no recommendations for anatoxins from WHO or the U.S. EPA, so the recommendation for microcystins is used during this experiment (WHO 1998; Hitzfeld et al. 2000). In the U.S., the EPA has published guidelines for treating cyanotoxins in drinking water, however these guidelines are non-binding and there are currently no water treatment regulations at either the State or Federal level regarding cyanotoxins (U. S. EPA 2017; Stanton 2018). Consequently, few have explored establishing protocols for the treatment of such toxins, especially on a small-scale drinking water treatment system serving 1-4 homes and operated by non-professionals, leaving many vulnerable to health problems caused by cyanotoxins.

Treatment methods, such as oxidation techniques with ozone or free chlorine, and the use of activated carbon, have shown varying degrees of success in treating both MC-LR and A-a (U.S. EPA 2016). The use of these methods has occurred mostly in

community-scale water treatment systems where obtaining the necessary chemicals and calculating proper doses is a reasonable expectation. For a small-scale treatment system, these are large hurdles to overcome, especially when considering the unpredictable timing and nature of HABs that cause cyanotoxins. Each of these methods has other side effects that also may deter their use. Oxidation with free chlorine results in by-products such as halogenated trihalomathanes and haloacetic acids, and oxidation with ozone may result in the formation of aldehydes and aldo, keto, and carboxylic acids, all of which may be harmful to human health (Weinberg 1999; Gopal et al. 2006). Since activated carbon adsorbs the toxins to its surface, the used carbon becomes a toxic hazard that must be disposed of properly, increasing the cost and difficulty of use (He et al. 2016). Ultra-Violet (UV) light treatment may offer protection from cyanotoxins equal to other commonly used treatment methods without succumbing to the same drawbacks.

When using UV light to degrade cyanotoxins, treatment starts with the flip of a switch, and no toxic residual compounds or hazardous materials are formed during the process, making it a potentially favorable treatment option for small-scale water treatment systems (Kaya and Sano 1997). UV treatment alone is not a favorable treatment method for community-scale treatment systems because the dose needed to degrade cyanotoxins is at least an order of magnitude greater than the dose needed for disinfection (< 40 mJ/cm^2) (AWWA 2016). There are two ways to meet the higher required dosage of UV Light; increase the output of the UV bulb, or increase the time the water is exposed to the light. Many community-scale drinking water treatment plants may not be able to increase the treatment time and still meet their water demand, so using more powerful

UV bulbs remains their only option. Higher intensity bulbs are more expensive, require more maintenance, and use considerably more electricity for the same operating time, which may make the use of UV light cost prohibitive. A small-scale treatment plant, however, may be able to increase the time water is exposed to UV light and still meet the demand for drinking water, allowing small-scale systems to achieve high doses of UV light without running a more expensive UV treatment system, keeping the cost of operations relatively low.

The objective of this research is to assess the effectiveness of a small-scale UV water treatment system to degrade two of the most prevalent cyanotoxins, MC-LR and A-a, found in surface waters used as drinking water sources. To assess the effectiveness of UV light for degrading MC-LR and A-a, an array of samples with different concentrations of each toxin was dissolved in both Nanopure water and in water collected from the Klamath River watershed and treated by a slow-sand filter. Each sample was then treated with a variety of doses of UV light. The final toxin concentration after treatment was determined using enzyme-linked immunosorbent assay (ELISA) testing procedures.

REVIEW OF LITERATURE

Harmful Algal Blooms

Cyanobacteria, also known as blue-green algae, are a phylum of photosynthetic bacteria that are commonly found in fresh, marine, and brackish water bodies across the globe (Newcombe 2009; Bouma-Gregson et al. 2018). These bacteria play an important role in the health of any ecosystem by fixing nitrogen, producing oxygen, and supporting the base of the food chain in whichever environment they are found (Chorus and Bartram 1999). When freshwater is warm, stagnant, nutrient rich, and exposed to sunlight, cyanobacteria can grow and proliferate very quickly, creating a scum or mat on the surface of the water known as a Harmful Algal Bloom (HAB) (Chorus and Bartram 1999; Sliwinska-Wilczewska et al. 2019). Not all cyanobacterial blooms are harmful, yet it is not possible to tell the species or composition of the bloom by appearance or if it will be harmful (Bouma-Gregson 2017). Some blooms do produce a wide variety of toxins that leech into the surrounding water body, causing serious health effects for animals and humans that drink or make contact with contaminated water (Meriluoto and Codd 2005). A single bloom can contain several species of cyanobacteria, and a single species may produce multiple different toxins in a single bloom, so identifying the toxins produced by a bloom, if any are produced at all, has proven very challenging (Butler et al. 2012; Bernard 2017; Bouma-Gregson 2017). Concentrations of cyanotoxins during a HAB event have been observed to vary from undetectable to over 25,000 µg/L (Fastner et al. 1999). The factors that lead to a cyanobacterial bloom producing toxins are not well

known (Newcombe 2009). However, once a bloom does produce toxins and becomes a HAB, it poses a serious health hazard to humans and animals including pets and cattle that may come into contact with the contaminated water (Francis 1878; Hudnell and Dortch 2008; Bernard 2017). Cyanotoxins have caused fish kills in many waterways, and even if the fish don't die, the toxins can bioaccumulate in fish and shellfish tissues, creating a health hazard for anyone that consumes contaminated organisms (Poste et al. 2011; Bernard 2017). Cases of exposure through the ingestion of algal dietary supplements have also been reported (WHO 1998). In Humboldt County, HABs have occurred in the Eel, Klamath, Trinity, and Van Duzen Rivers and have caused the deaths of over 11 dogs and several head of cattle since 2001 (Butler et al. 2012; Crandall 2017).

Cyanobacteria live in almost all water bodies, but blooms occur in warm, stagnant, shallow waters with a high nutrient load, especially phosphorous (He et al. 2016; Bouma-Gregson 2017). These conditions are most commonly seen in the northern hemisphere during the months of July through September. HABs have been documented since at least 1878, but have occurred much more frequently in the last 30 years due to effects of climate change, river management, and increased water diversions (Francis 1878; Sliwinska-Wilczewska et al. 2019). Worldwide, increases in HABs create challenges for water management and water treatment facilities since cyanotoxins can persist for several weeks after a bloom has occurred. Many water agencies have developed programs to identify and monitor HABs as they occur to help keep the public and water treatment facilities informed of cyanobacteria blooms (AWWA 2016; U.S. EPA 2061). Given that not every cyanobacteria bloom produces toxins, this is a difficult

task (Hudnell and Dortch 2008; U.S. EPA 2017). In the instance that a HAB has been identified, water treatment facilities are faced with further challenges since such a wide variety of toxins can be produced, and each toxin poses different treatment needs.

Cyanotoxin Descriptions

Toxins produced by cyanobacteria, known collectively as cyanotoxins, include cytotoxins, dermatotoxin, endotoxins, hepatotoxins, and neurotoxins (Carmichael 1991; Pantelic et al. 2013). Two of the most common toxin families found in Humboldt County are the microcystins, a family of hepatotoxins (liver toxins), and anatoxins, a family of neurotoxins (Federal 2002).

Microcystins are a group of monocyclic heptapeptides (a molecule with a single ring and 7 amino acids linked by peptide bonds), which affect the liver (Pantelic et al. 2013). To date more than 80 variants have been identified (Carmichael 1991; Demirel and Sukatar 2012). The microcystin variants are differentiated by which two amino acids are present in the carbon ring. The best studied and most common variant is known as microcystin-LR (MC-LR) with chemical formula of $C_{49}H_{74}N_{10}O_{12}$ and has leucine (L) and arginine (R) as the variable amino acid groups (Figure 1) (Harada 1996; Demirel and Sukatar 2012). MC-LR has a molecular weight of 995.2 g/mol, is highly soluble in water, and has a maximum absorption of UV light at 238 nm (Harada 1996). Since the molecule is so large and stable, it may persist in shaded regions of water bodies for months after a bloom dissipates due to the lack of photodegradation (Pantelic et al. 2013). When exposed to sunlight, MC-LR has a half-life of about one week, since the molecule can be degraded by the UV rays in sunlight and through biodegradation (WHO 2018). The most

common pathway for exposure to MC-LR is through ingestion of drinking water, followed by exposure through recreational use of freshwater bodies (WHO 1998; WHO 2018). Due to the size of the molecule, absorption though skin contact is unlikely, but there is some evidence of bioaccumulation of MC-LR in fish and shellfish, so exposure through ingestion of contaminated food is a possibility (WHO 1998; Poste et al. 2011). While there have been many instances of people becoming sick through exposure to MC-LR, there are no known instances of death to humans (WHO 2018). It has been shown that when MC-LR breaks down under UV light, one of three isomers can be created, each of which is not toxic to humans, so the resulting water is safe to drink (Kaya and Sano 1997).

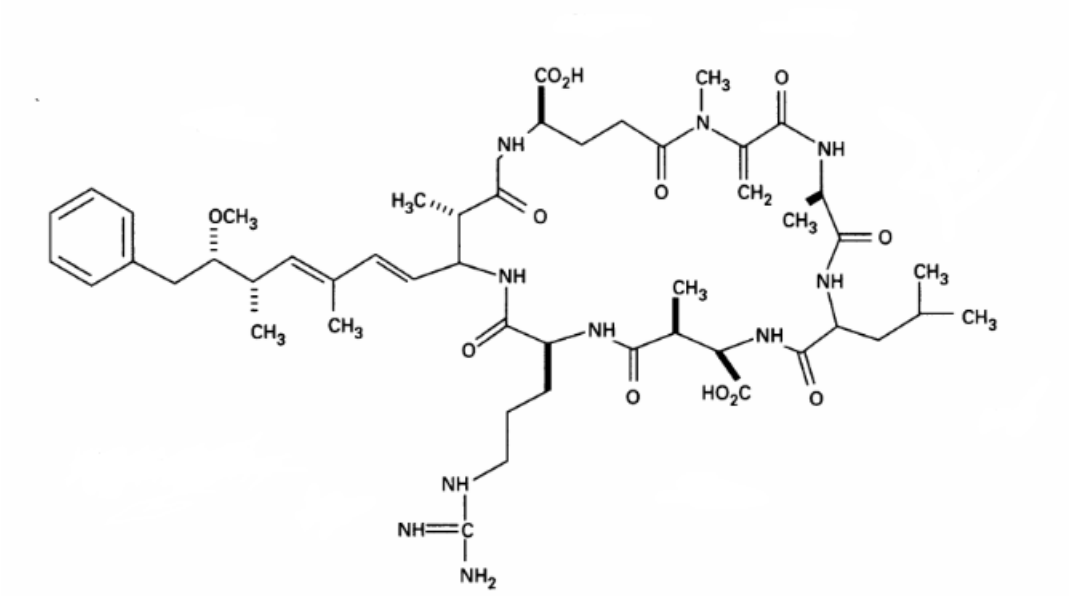

Figure 1: Structure of microcystin-LR molecule (as presented by Hitzfeld et al. 2000)

The most common molecule in the anatoxin family is Anatoxin-a (A-a) with chemical formula of $C_{10}H_{15}NO$, commonly known as the "Very Fast Death Factor" and is

produced by a variety of species of cyanobacteria (Carmichael 1992) (Figure 2). A-a is highly soluble in water, has a molecular weight of 165.23 g/mol and a maximum absorbance at 227 nm (James et al. 1998). Although it has been found in surface waters around the globe, there is limited information on the molecule in treated drinking water (U.S. EPA 2015a). A-a is a neurotoxin that affects the respiratory system. This neurotoxin has been associated with the deaths of many cattle and at least one human, normally through suffocation due to paralysis of the lungs and diaphragm (Gagnon and Pick 2012; Pantelic et al. 2013). In the absence of light, A-a has a half-life varying between several days to several months depending on pH, where alkaline conditions are associated with a shorter half-life (Stevens and Krieger 1991). Unlike other cyanotoxins A-a breaks down readily in the presence of natural sunlight with a half-life between 1 hour and 5 days, again depending on the pH of solution with more alkaline conditions leading to a shorter half-life (Stevens and Krieger 1991; Smith and Sutton 1993). In most instances, exposure to A-a occurs through drinking contaminated water, but can also occur through dermal contact or inhalation during bathing or recreation. To date there have been no known cases of exposure due to ingestion of fish or shellfish, however there have been recorded cases of exposure due to ingestion of dietary supplements containing algae (U.S. EPA 2015a). In the U.S., concentrations of A-a in surface waters have been observed from below the detection limit of 0.05 μg/L to 1,929 μg/L (U.S. EPA 2015a).

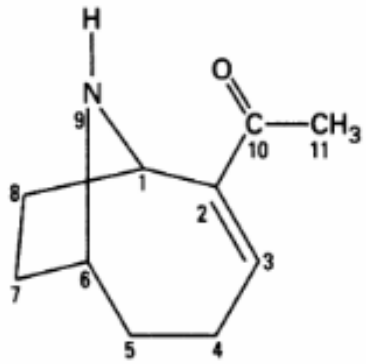

Figure 2: Structure of Anatoxin-a molecule (as presented by Hitzfeld et al. 2000)

Cyanotoxin Detection

Due to the uncertainty of whether or not a cyanobacterial bloom is producing toxins, and the wide variety of toxins that can be produced, it is necessary to be able to test water samples quickly and accurately so that blooms can be properly monitored for public health risks (Gaget et al. 2017). Since there is such a wide variety of toxins, a single analytical method will not suffice for the identification and accurate quantification of every potential toxin in a bloom. Instead, a combination of screening and more sophisticated quantification methods is recommended (WHO 1998; U.S. EPA 2015b). There are many methods available, but each one has a tradeoff between selectivity and sensitivity. Selectivity is a test's ability to identify the presence of a specific molecule while sensitivity refers to how small a concentration of toxin can be identified (Figure 3) (Harada et al. 1999; Sklenar et al. 2016). The most common detection methods used for cyanotoxins are Liquid Chromatography-Mass Spectrometry (LC/MS), and Enzyme-

Linked ImmunoSorbent Assay (ELISA) test kits (Carpenter and Khiari 2015; U.S. EPA 2015b).

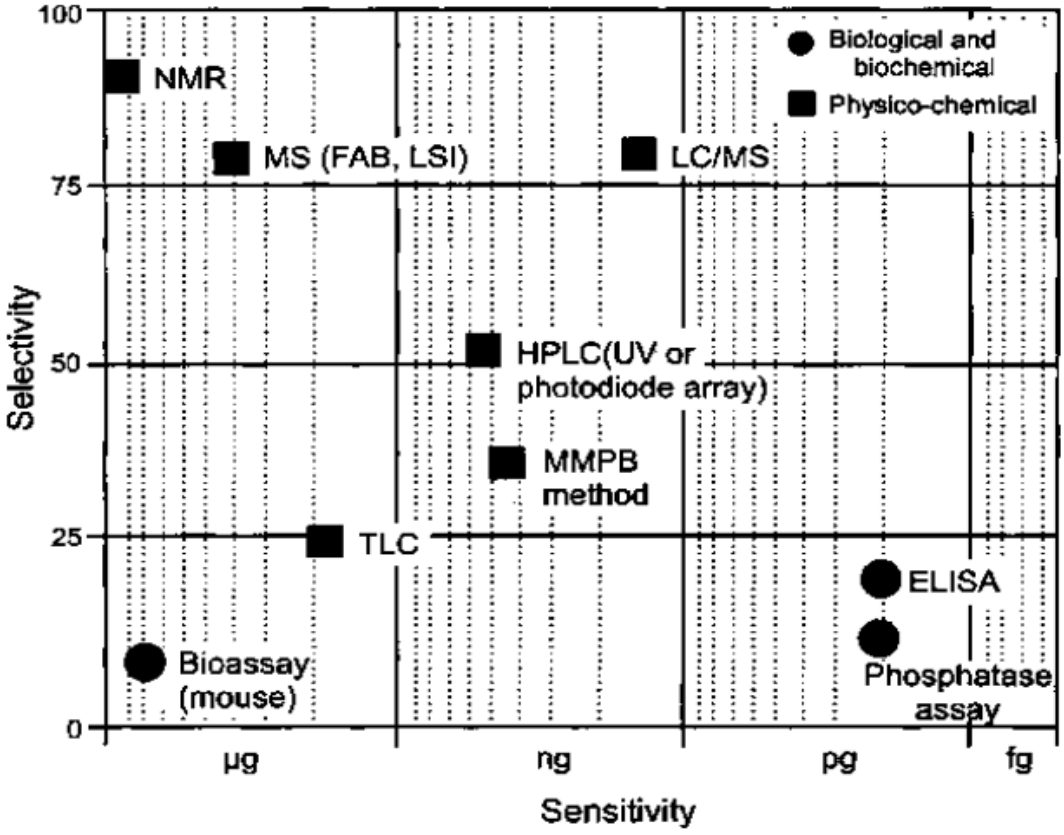

Figure 3: Comparison of Selectivity vs Sensitivity for different analytical methods of microcystins where selectivity is the ability to differentiate between molecules, and sensitivity refers to detection limits (as presented by Harada et al. 1999)

Analysis by LC/MS is popular because several classes of toxin can be identified using a single test as well as differentiate between individual toxins within a given class, meaning different microcystin congeners can be identified in a single sample (Gaget et al. 2017). Minimal preparation of samples is needed for LC/MS analysis, so samples from different sources can be used even if the collectors did not preserve samples properly (Shoemaker et al. 2015; Sklenar et al. 2016). The downside is that the equipment used in

this detection method is very expensive and requires extensive training to use properly. The testing equipment is not portable, so samples must be collected on site and sent to a lab for testing, which can slow down the testing process (Pelander et al. 2000; Carpenter and Khiari 2015). Finally, the detection limit ranges from between 1-10 μg/L, so concentrations that are harmful to human health in natural waterways may not register during testing due to any breakdown of the toxins before testing can occur (Carpenter and Khiari 2015).

The other common detection for cyanotoxins uses ELISA test kits. These test kits are portable and can be run in under six hours without much training, so they can be used to test samples in the field for the presence of cyanotoxins (Aranda-Rodriguez and Zhiyun 2011; Carpenter and Khiari 2015). ELISA kits can also run multiple samples simultaneously, reducing the total cost of each test (Gaget et al. 2017). ELISA kits are also very sensitive, able to detect toxin concentrations as low as 0.15 μg/L, and allow for very precise estimations of toxin concentrations in sample waters (Carpenter and Khiari 2015). The disadvantages of ELISA testing are that the kits cannot differentiate between different toxins within the same family, so multiple microcystin congeners would add to the total concentration in a single test kit. The ELISA kits are specific to toxin families however, so separate kits are needed to test for anatoxin and microcystins (Carpenter and Khiari 2015; Sklenar et al. 2016).

Treatment Options

Cyanotoxins in drinking water are difficult to treat for a variety of reasons: irregularity of HABs, many different toxins, different concentrations, and different half-

lives. Intensive monitoring of source waters must be undertaken to identify which toxins are present and at what concentrations. Cyanotoxins are produced in the cells of cyanobacteria so special care must be taken to not lyse, or break apart, intact cells causing more cyanotoxins to be released into the water during treatment. Finally, different cyanotoxins respond differently to different treatment methods, so multiple methods of treatment may be required (U.S. EPA 2014). Some of the most common treatment methods are adsorbtion with activated carbon, oxidation with chlorine or ozone, and irradiation with UV light. Physical removal of large molecule toxins such as microcystins through membrane filtration has shown success in some studies, though the success is highly dependent on a membrane's pore size and distribution (U.S. EPA 2014). Unless membranes are already being used in water treatment, such as in desalination plants, the use of membranes to remove cyanotoxins in drinking water is not recommended due to the short-term duration of HAB events (Sklenar et al. 2016). The first step of treatment is to remove as many cyanobacterial cells as possible from the water without breaking them apart, usually through flocculation or filtration, to avoid releasing further cyanotoxins during treatment (Hitzfeld et al. 2000; Ohio 2015).

Activated carbon

Two forms of activated carbon are utilized in the drinking water treatment process: powdered (PAC) and granulated (GAC). Both PAC and GAC rely on adsorption to remove cyanotoxins from water, so the amount of surface area coming into contact with the water body is directly related to how effectively they remove toxins from the water column. Both methods require 30-60 minutes contact time with the contaminated

water to remove toxins (Hitzfeld et al. 2000; Ohio 2015). PAC is generally more efficient at adsorbing cyanotoxins than GAC for a few reasons. Since GAC is used as a layer in filtration medium, it develops a biofilm in much of its pore space (Hitzfeld et al. 2000). The biolayer does not break down cyanotoxins, and instead interferes with adsorption of toxic compounds. In contrast, PAC is added as needed, so there is no time for the biofilm to develop, leaving much more surface area available for adsorption (Hitzfeld et al. 2000). This also helps keep costs down since PAC can be added to the treatment train after a HAB has been identified. Also, PAC can be added at a variety of points in the treatment train, making it easier to achieve the long contact times needed to remove toxins (U.S. EPA 2014). There are different types of PAC, depending on the source materials (wood, peat, etc.), which have shown varying levels of effectiveness in cyanotoxin removal. For this reason, jar testing is recommended to find a suitable type of activated carbon for each treatment plant (U.S. EPA 2014; Ohio 2015). The biggest drawbacks to the use of activated carbon are that the incoming concentrations of toxin must be known to apply a high enough dose of activated carbon, different PAC or GAC source materials have varying levels of effectiveness, and once contaminated water has been treated, the activated carbon then becomes hazardous material themselves requiring proper disposal. Finally, the cyanotoxins in the water compete with other organic matter in the water for the adsorption sites (U.S. EPA 2016). These drawbacks add cost and complexity to water treatment that may reasonably be surmounted by community-scale water treatment plants, but may prove too challenging for people with a small-scale water treatment system.

Chlorine

Chlorine has played an important role in the disinfection of drinking water for over 100 years, especially as a means of maintaining disinfection in distribution systems once treated water has left a treatment plant (Sklenar 2016). Free chlorine has shown some effectiveness in treating microcystins as long as the pH is between 6 and 8, but it has not shown to be effective at treating Anatoxin-a (Sklenar 2016; U.S EPA 2016). The CT (the product of contact time and concentration of disinfectant) needed for chlorine to treat microcystins varies widely depending on the water temperature and pH, posing a challenge when calculating a CT to treat a known concentration of cyanotoxin (Ohio 2015). Chlorine also tends to lyse cyanobacteria cells, which may result in an increased concentration of cyanotoxins in the water if it is applied before filtration has occurred (Sklenar 2016). Finally, when chlorine is used as a disinfectant, trihalomethanes and haloacetic acids, disinfection by-products, which are harmful to human health can be formed (Hitzfeld et al. 2000; Ding et al. 2010). For these reasons, chlorine is not a suggested method of treatment for cyanotoxins for either community-scale or small-scale water treatment systems.

Ozonation

Ozone (O_3) has been used in drinking water treatment for both the disinfection of pathogens such as bacteria and viruses, and the removal of color and odor issues from source waters (U.S. EPA 2016). Two pathways are described in ozonation reactions: direct attack by molecular ozone, and indirect attack by resulting free radicals such as Hydroxyl radicals (•OH) (Hitzfeld et al. 2000; Sklenar et al. 2016). •OH radicals are

considered the most reactive oxidizing agents in water treatment and are used for treating organic and inorganic compounds, as well as disinfection processes (Koivunen and Heinonen-Tanksi 2005). Ozone has also been shown to be very effective at degrading a wide variety of cyanotoxins, including MC-LR and A-a (U.S. EPA 2014). Of all the treatment methods tested to date, ozone is the fastest acting and effective against the widest variety of cyanotoxins (Hitzfeld et al. 2000; U.S EPA 2014; Ohio 2015). Ozone degrades MC-LR at a second order reaction rate, fully degrading MC-LR from concentrations of 500 to 0 μg/L within 10 seconds and 4 minutes (Hitzfeld et al. 2000; Ding et al. 2010). While ozone is effective at degradation, calculating the proper dose to use is difficult because so many factors can affect its effectiveness including pH, temperature, and most importantly competition from other organic material in the water (Hitzfeld et al. 2000; Ding et al. 2010). Similar to chlorine, if there are cyanobacterial cells still present when ozone is added, the ozone can lyse the cells, releasing any intracellular cyanotoxins into the water, resulting in greater concentrations of toxins than the source water (Hitzfeld et al. 2000; Sklenar et al. 2016). Another factor is that ozone is a very reactive compound, so it must be generated as needed on site, which is a complicated and energy intensive process (Hitzfeld et al. 2000). For these reasons, ozone is not a recommended treatment option for small-scale water treatment systems.

Ultraviolet irradiation

UV light is the portion of the light spectrum with wavelengths found between visible light and X-rays. UV light is further broken down into four ranges: UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm), and Vacuum UV (100-200 nm)

(U.S. EPA 2006) (Figure 4). While most disinfection occurs in the UV-C and Vacuum UV regions of the spectrum, Vacuum UV light dissipates quickly in water, and consequently is not appropriate for water treatment (U.S. EPA 2006). UV light is generally produced in a treatment system by applying a voltage to a gas mixture that includes mercury gas (U.S. EPA 2006). UV-LEDs that do not use mercury gas are a new and promising technology due to the variety of wavelengths that can be produced and their overall lower energy needs (Nyangaresi 2018). The most commonly used UV lamps are low-pressure (LP) UV lamps, which are monochromatic and emit light at 254 nm, and medium-pressure (MP) UV lamps which produce light across the UV-C spectrum, from 200-300 nm (Wright 2001; U.S. EPA 2006).

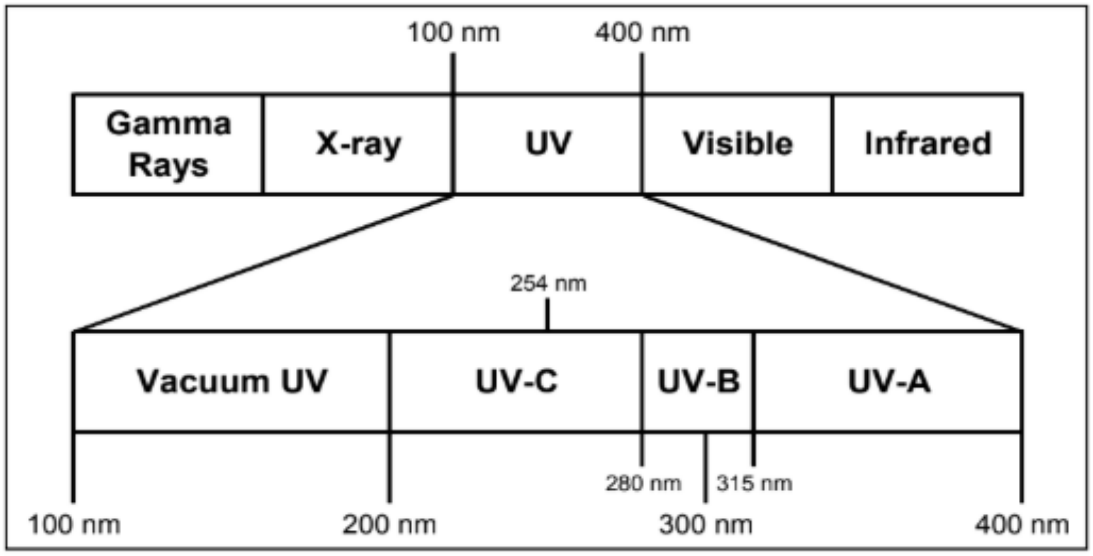

Figure 4: Breakdown of the UV light spectrum (as presented by U.S. EPA 2006)

The first use of UV for disinfection on a large scale was in Marseilles, France in 1910 and has been used in both drinking water and wastewater treatment (U.S. EPA

2006). Use of UV light in water treatment has increased in popularity in the 21st century due to the wide variety of water-borne pathogens it can remove, the speed with which it acts, and because UV treatment does not generally result in harmful disinfection by-products, unlike chlorine and ozone (Alkan et al. 2006). UV light functions as a disinfectant by shining high-energy light through a cell membrane and degrading the DNA of whatever pathogens the light contacts by breaking the chemical bonds, preventing those cells from replicating (Alkan et al. 2006; U.S. EPA 2006). DNA has a peak absorbance at 254 nm, so the LP lamps are the most commonly used in water treatment since they produce nearly monochromatic light at that wavelength (U.S. EPA 2006; Wright and Hargreaves 2018).

Since UV disinfection works by breaking the bonds in DNA, it is effective against a wide variety of pathogens found in source waters (Wang et al. 2006, Wright and Hargreaves 2001). UV disinfection is generally a fast process; however, the speed of disinfection is dependent on the intensity of the light and the transmittance of the water. UV transmittance is a measure of the percentage of UV light applied that reaches a target pathogen. The transmittance is affected largely by suspended solids in the water which may affect the turbidity (Alkan et al. 2006). The dose applied by UV light is a function of the intensity of light measured in mW/cm^2 and the amount of exposure time, measured in seconds, with the total dose applied measured in mJ/cm^2 (Blume and Neis 2003; Koivunen and Heinonen-Tanksi 2005). The intensity of the light decreases with the square of the distance from the light source, so an average value across the water column is used to measure the applied dose (Qualls and Johnson 1982). The doses applied for

disinfection of pathogens range from between 10-40 mJ/cm^2 and can be applied very quickly, over a matter of seconds (Alkan et al. 2006; Sklenar et al. 2016). While UV light has also shown to be effective at degrading a wide variety of cyanotoxins, they require a dose between 1 and 3 orders of magnitude higher than those used for disinfection (Ding et al. 2010; Sklenar et al. 2016).

There are several water quality factors that affect the UV dose needed for disinfection including hardness, turbidity, and particle size of suspended solids (Alkan et al. 2006; Wang et al. 2006). The most important aspect is the particle size of suspended solids since they can block the UV light from making contact with pathogens in the water, allowing them to pass by the lamp without receiving any treatment (Wang et al. 2006). Studies have shown that suspended particles with a diameter greater than 50 μm interfere with UV light and decrease the treatment efficiency or efficacy (Blume and Neis 2003; Alkan et al. 2006). To avoid interference from suspended particles, UV treatment is usually applied near the end of the treatment train, after settling and filtration have occurred (U.S. EPA 2006).

Due to the high doses required by UV light alone to degrade cyanotoxins, many studies of its use have considered the combined use of UV light and advanced oxidation techniques such as the addition of Hydrogen Peroxide (H_2O_2) (Senogles et al. 2001; Afzal et al. 2010; He et al. 2012). The addition of an oxidant to the contaminated water helps decrease the dose of UV light needed to degrade a given cyanotoxin. One study found that a dose of 1285 mJ/cm^2 of UV light alone degraded A-a by 50%-88% depending on the starting concentration. However, when UV light was coupled with an addition of 30

mg/L of H_2O_2, a dose of 200 mJ/cm^2 was all that was needed to degrade 70% of the A-a dissolved in solution (Afzal et al. 2010). MC-LR showed a similar response in degradation rates when treated with UV light alone and a combination of UV light and H_2O_2, where the combined treatment resulted in much lower doses of UV light required to achieve the same level of degradation, however the amount of degradation achieved was not quantified (He et al. 2012). The addition of an oxidant such as H_2O_2 adds another layer of complexity to treatment that may be a barrier to use by a layperson operating a private, small-scale treatment system. The ease of use of UV light, and the wide variety of pathogens and toxins it can treat, may make UV light an ideal treatment method for small-scale drinking water treatment systems, assuming that there is an available source of power.

METHODS

Materials

- 1 mg dry powder microcystin-LR (Cayman Chemicals)
- 1 mg dry powder anatoxin-a (Cayman Chemicals)
- 35-Watt UV light and ballast (CureUV)
- 4 mL amber sample vials (Fisher Scientific #02991215)
- 2 mm I.D. silicon peristaltic tubing (Fisher Scientific #14179126)
- 1.5 mm I.D. quartz tube (Wilmad Labglass)
- Peristaltic pump P-1 (GE Healthcare)
- Microcystin ELISA (ENZO Life Sciences)
- Anatoxin-a ELISA (Abraxis Inc.)
- pH meter (Accumet AB150)
- Dissolved Oxygen Meter (YSI 5100)
- Turbidimeter (Hach 2100P)
- Thermo Scientific Barnstead Genpure UV/UF xCAD water purifier

Experimental Set Up

Figure 5 shows the ultraviolet (UV) reactor used in this experiment which consisted of a metal housing, 204 mm long, and lined on the inside with reflective aluminum foil. The housing contained a movable metal sheath, also 204 mm long, that covered a 457 mm long and 1.5 mm inner diameter quartz tube through which the

samples flowed while being exposed to UV light. A quartz tube was used in the reactor since quartz does not interfere with UV light (Qualls and Johnson 1982). The samples traveled through silicon peristaltic tubing before entering and after leaving the quartz tube. Each sample's flow rate was controlled by a peristaltic pump with variable speed settings. The UV light was provided by a 35-Watt bulb that emitted monochromatic light at a wavelength of 254 nm. The light drew 39 Watts when turned on, which the ballast lowered to 35 Watts to power the light. The UV bulb was located 1.5 inches from the quartz tube. After treatment, samples were collected in individual amber vials to prevent further exposure to UV light, and stored in a refrigerator at 4° C until testing with the ELISA test kits occurred. Samples were stored for no more than 72 hours.

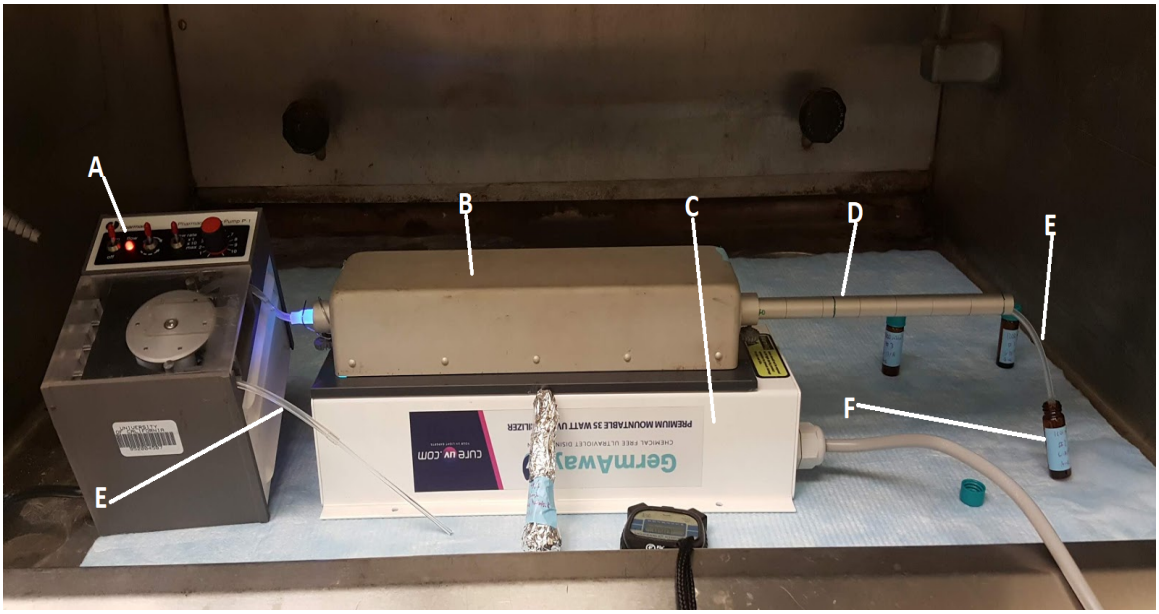

Figure 5: Test apparatus: A) Peristaltic pump B) Metal housing for quartz tube C) Low-pressure UV light D) Metal sheath E) Peristaltic tubing F) Amber collection vials

Sample Preparation

The test sample concentrations of microcystin-LR (MC-LR) and anatoxin-a (A-a) were calculated to be identical on a mass/volume basis and were subjected to the same doses of UV light. Two different water types were used for these tests, Nanopure water and water collected from the Klamath River watershed that was pre-treated by a slow-sand filter drinking water treatment system. Nanopure water is ultrapure water, also known as Type 1+, that is filtered until the resistivity is no higher than 18.2 mΩ. While Nanopure water will have no dissolved solids left in solution, the water treated by a slow sand filter will have some dissolved solids still present.

The concentrations used in this study reflect the guidance/action levels of microcystins in recreational waters set by the World Health Organization (WHO) and utilized by the U.S. EPA (WHO 2003; U.S. EPA 2017) (Table A1). The lowest concentration used in our study was the recommended maximum allowable concentration suggested by WHO for drinking water, 1 µg/L, followed with sample concentrations of 10 µg/L, 100 µg/L, 1000 µg/L, and a maximum concentration of 5000 µg/L (WHO 1998).

Concentrations of MC-LR and A-a were diluted from an original stock solution, created by dissolving 1 mg of dry powdered toxin in 1 mL of either Nanopure or sand-filtered water. Sample concentrations were diluted through serial dilutions starting with the stock solution and mixed with the appropriate water type to create a 10 mL solution of each desired concentrations (Table B1). The vials containing the stock solution of each toxin were used to start the serial dilutions for each of the three trials.

UV Dose/Exposure

A 35-watt low-pressure UV lamp obtained from CureUV was used to apply monochromatic UV light at 254 nm to each test sample (Figure A1). The dosage of UV light is a function of the UV intensity (I) and the exposure time (t) (Equation 1). The same UV light was used to treat each sample from the same distance throughout this experiment so the intensity was constant for all samples. The light intensity decreases as distance from the light source increases, so the intensity was calculated by interpolating from the distance and intensity values given by the light manufacturer (Equation 2). In this experiment the UV light intensity was calculated as 17.13 mW/cm^2 (Figure A2). This calculation is a rough estimate based of the interpolated values and so the doses of UV light applied in this experiment are approximate values. The exposure time was controlled using two different components of the UV reactor to apply each UV dose: adjusting the flow rate of the sample through the reactor and changing the length of quartz tube exposed to UV light (Equation 3) (Table 1). The exposure length was adjusted by moving a metal sheath along the quartz tube through which the treatment water was flowing, exposing only certain length of the quartz to the UV light.

$$D = I * t \qquad \text{(eq. 1)}$$

Where:
D = UV dose (mJ/cm^2)
I = UV Intensity (mW/cm^2)
t = exposure time (seconds)

$$I = 0.0663x^2 - 2.6837x + 26.389 \quad \text{(eq. 2)}$$

Where:
I = UV Intensity (mW/cm^2)
x = Distance from light (cm)

$$t = (l * A)/Q \quad \text{(eq. 3)}$$

Where:
$l = length\ of\ quartz\ sheath\ (mm)$
$A = cross\ sectional\ area\ of\ quartz\ sheath\ (mm^2)$
$Q = flow\ rate\ (\frac{mm^3}{s})$

Table 1: Exposure times and length of quartz tubed exposed to UV light to achieve each dose

Dose (mJ/cm^2)	**60**	**750**	**1500**	**4000**
Exposure Length (mm)	198.0	205.0	170.0	221.0
Flow Rate (mm^3/s)	97.0	8.0	3.5	1.5
Exposure Time (s)	3.5	43.5	87.0	232.0

Each sample concentration of each toxin was exposed to the following UV doses: 60, 750, 1500, and 4000 mJ/cm^2. The range of UV doses represent typical values used in drinking water treatment systems on the low end to the highest value that could be reasonably applied by the experimental set-up (AWWA 2016). All exposure doses were confirmed as an effective treatment range by a preliminary test using this experimental set-up. The UV reactor reached a maximum temperature of 40° C. MC-LR is stable in boiling water, so it is assumed no degradation occurred due to temperature affects (Metcalf and Codd 2000). However, A-a has shown accelerated degradation at

temperatures above 40° C, but those affects are not well studied and were not considered in this experiment (Kaminski et al. 2013). Each concentration of cyanotoxin, in the two different waters, was run in triplicate at each UV dose, for a total of 60 samples per cyanotoxin, per sample water type. After treatment with the UV light, each sample was stored in an amber vial and refrigerated at 4° C for no more than 72 hours until the final concentration was measured using ELISA testing kits. The final concentrations were then compared to their starting concentrations to determine the log removal obtained through each UV dose.

Experimental Procedure

Each sample of cyanotoxin was passed through the UV reactor beginning with the lowest concentration of 1 µg/L and moving to increasingly higher concentrations, ending with the 5000 µg/L concentration. Each individual concentration was exposed to every UV dose before moving to the next higher concentration starting with the highest dose of 4000 mJ/cm^2 and moving to progressively lower UV doses. Samples were treated in this order to avoid higher concentrations contaminating lower concentration samples. After treatment, samples were collected and stored in amber vials to protect them from further exposure to light before testing the concentrations. The amber vials containing treated samples were stored in a cardboard box in a refrigerator at 4° C until testing with the ELISA test kits. Samples were stored in the fridge for no more than 3 days before ELISA testing started.

ELISA Testing

The concentration of each treated sample was determined using enzyme-linked immunosorbent assay (ELISA) testing kits. ELISA test kits are one of the most commonly used methods for quantifying cyanotoxin concentrations because they have a short run time, have a high sensitivity, are relatively simple to use, and if all the sample wells are filled, then the price per samples is relatively inexpensive (Sanseverino et al. 2017). Separate ELISA microtiter plate kits specific to microcystins and anatoxins were used in this study.

Each ELISA kit had space for up to 96 samples, including 16 samples of known toxin concentration from which a calibration curve could be calculated. Four different ELISA kits were used in total: one for each toxin type dissolved in each of the two different source waters. The ELISA kits were stored at 4° C until ready for use and the testing protocol for each kit was followed (Figures B1 and B2).

Each ELISA kit comes with a set of standards with known concentrations of cyanotoxin ranging between 0.15 µg/L and 5.0 µg/L from which a calibration curve is calculated (Figures B3-B6). A regression equation derived from the calibration standards was used to calculate the final concentration, corrected for dilution, of the sample solutions through interpolation. Given that the sample concentrations were outside of the calibration curve range, samples were diluted with either the Nanopure water or sand-filtered water in which the toxins were originally dissolved. The resulting concentrations, determined from the calibration curve, were then multiplied by each sample's respective dilution factor to find the sample's final concentration (Table B2). Samples with a

starting concentration of 1 μg/L did not need to be diluted further since they already fell within the calibration curve. Tables of the samples' diluted concentrations (D1-D6) and undiluted concentrations (D7-D12) are found in the appendix.

Water Quality Parameters

The pH, turbidity, total suspended solids, and 5-Day Biological Oxygen Demand (BOD_5) of both the Nanopure water and sand-filtered water were measured and compared. The UV transmittance of each water type could not be measured, but due to the small cross-sectional area inside the quartz tube it was assumed that the transmittance for each water type was 100%. The pH and turbidity of each water type was tested three times, where an average value was calculated, with an Accumet AB150 pH meter and Hach 2100P Turbidimeter, respectively. The procedure for determining the total suspended solids and BOD_5 concentrations followed *Standard Methods for the Examination of Water and Wastewater* procedures 2540D and 5210B, respectively (Eaton 2005). Dissolved oxygen measurements were taken with a YSI 5100 dissolved oxygen meter.

RESULTS AND DISCUSSION

This section presents the water quality parameters tested for both the Nanopure and sand-filtered water which may impact the effectiveness of UV treatment, followed by the degradation of the cyanotoxins microcystin-LR (MC-LR) and anatoxin-a (A-a) in each water type. The degradation results are reported over the dose of UV light applied. Finally, the log removal of each cyanotoxin is reported over the dose of UV light applied. Log removal values are a measure of how much a toxin was degraded with each dose, and are calculated by taking the logarithm of the ratio of the starting concentration over the final concentration (Sklenar et al. 2016). The values shown are the average of three different runs completed for each starting concentration and each UV light dose. Lines connecting each value are not intended to imply a functional relationship between each point, but allow trends in the data to be viewed more easily. The error bars shown above and below each value represent the standard deviation calculated with each average value.

Water Quality Parameters

The pH, turbidity, total suspended solids, and 5-Day Biological Oxygen Demand (BOD_5) were all measured in the Nanopure and sand-filtered waters. Table 2 shows the pH, turbidity, and total suspended solids for both Nanopure water and the sand-filtered water used in this experiment. The pH of each water type was important to measure because A-a and MC-LR have both been shown to degrade more quickly in alkaline conditions with a pH above 8 (Newcombe and Nicholson 2004; He et al. 2012).

The pH for both water types was below 8, so it is unlikely any degradation seen in this experiment was due to pH levels.

Table 2: Average pH, turbidity, and total suspended solids for Nanopure and sand-filtered water

Parameter	Nanopure H_2O	Nanopure St. Dev.	Sand-filtered H_2O	Sand-filtered St. Dev.
pH	7.63	-	7.80	-
Turbidity (NTU)	0.00	0.00	0.84	0.18
Total Suspended Solids (g/L)	0.00	0.00	0.00	0.00

Suspended solids and turbidity are both factors that can affect UV treatment due to interference of the light by large molecules in the water (Alkan et al. 2006; Wang et al. 2006). Solids with a diameter greater than 50 µm can shade cyanotoxins dissolved in the water from the UV light, allowing the toxins to pass through the light chamber without contacting any UV light and decreasing the effectiveness of treatment (Wang et al. 2006). Since no suspended solids were detected in either water type, and the turbidity was below 1 NTU, their impact on UV treatment was considered negligible.

The standard deviation was calculated from the values collected for each parameter in Table 2. Nanopure water is very consistent so there was no standard deviation for the turbidity or total suspended solids, and the pH was only measured once. The sand-filtered water used in this experiment was all collected one time from a single

site, so the measurements recorded were also very consistent. Only the turbidity showed a measurable standard deviation with a value of 0.18.

In each water type the BOD_5 measured a total depletion of dissolved oxygen less than 2 mg/L, meaning there was no measurable biological activity in either water source. The BOD_5 can influence the half-life of cyanotoxins due to biodegradation that may occur in solution. Biodegradation of cyanotoxins has been recorded, but not well studied. Most studies on the subject have looked at the biodegradation of microcystins only, so more information on how A-a responds is needed before any conclusions can be reached on its effects (Nybom 2013). However, since there was no measurable oxygen depletion in the BOD_5 test, it is unlikely any degradation of the cyanotoxins seen in this experiment was due to biodegradation.

Degradation

Figure 6 shows the final concentrations of MC-LR in Nanopure water with increasing UV dose while Figure 7 shows the final concentrations of MC-LR in sand-filtered water. In both water types there is a general trend of decreasing toxin concentration with increasing UV light dose. While degradation of MC-LR occurred in each water type, the final concentrations at each UV dose were lower in all instances in sand-filtered water. Final concentrations of MC-LR reached levels below the World Health Organization (WHO) recommended maximum allowable concentration of 1 µg/L when the starting concentration was 1 or 10 µg/L and treated with a minimum UV dose of 750 mJ/cm2, except for a starting concentration of 10 µg/L in Nanopure water which required a UV dose of 1500 mJ/cm^2. When starting concentrations were higher than 10

µg/L there was a marked reduction in cyanotoxin concentration with increased UV doses, but none were reduced below 1 µg/L, even with the highest UV dose of 4000 mJ/cm^2 applied.

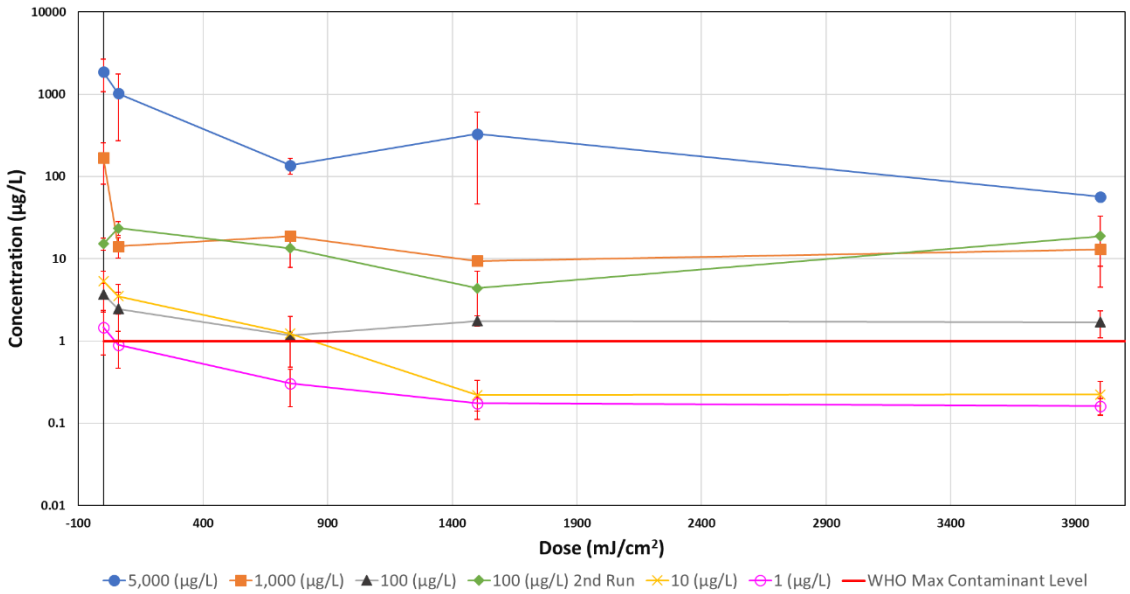

Figure 6: Plot of the final concentration of microcystin-LR in Nanopure water over the dose of UV light applied with the y-axis on a log scale

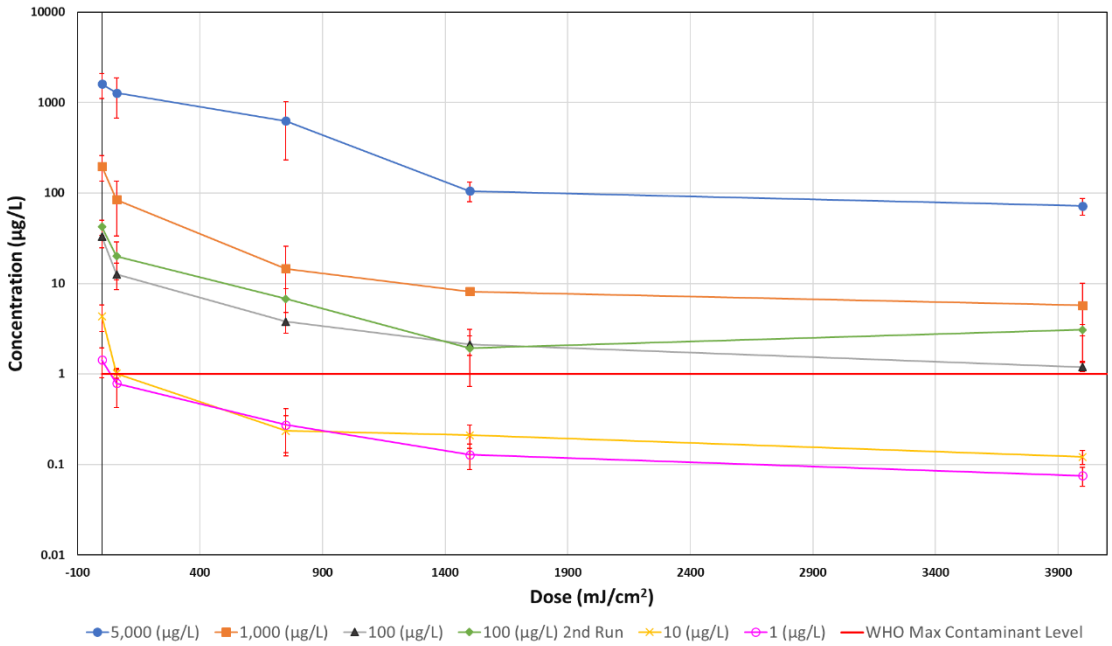

Figure 7: Plot of the final concentration of microcystin-LR in sand-filtered water over the dose of UV light applied with the y-axis on a log scale

Figure 8 shows the final concentrations of A-a in Nanopure water with increasing UV dose while Figure 9 shows the final concentrations of A-a in sand-filtered water. In both water types there is a general trend of decreasing toxin concentration with increasing UV light dose, similar to that shown in the degradation of MC-LR. Also similar to MC-LR, the final concentrations of A-a after treatment with UV light were lower in all instances when A-a was dissolved in sand-filtered water. However, A-a was unique in that a starting concentration of 10 µg/L dissolved in Nanopure water required the maximum applied dose of 4000 mJ/cm^2 to reach the WHO recommended maximum allowable concentration of 1 µg/L, and did not fall below that concentration.

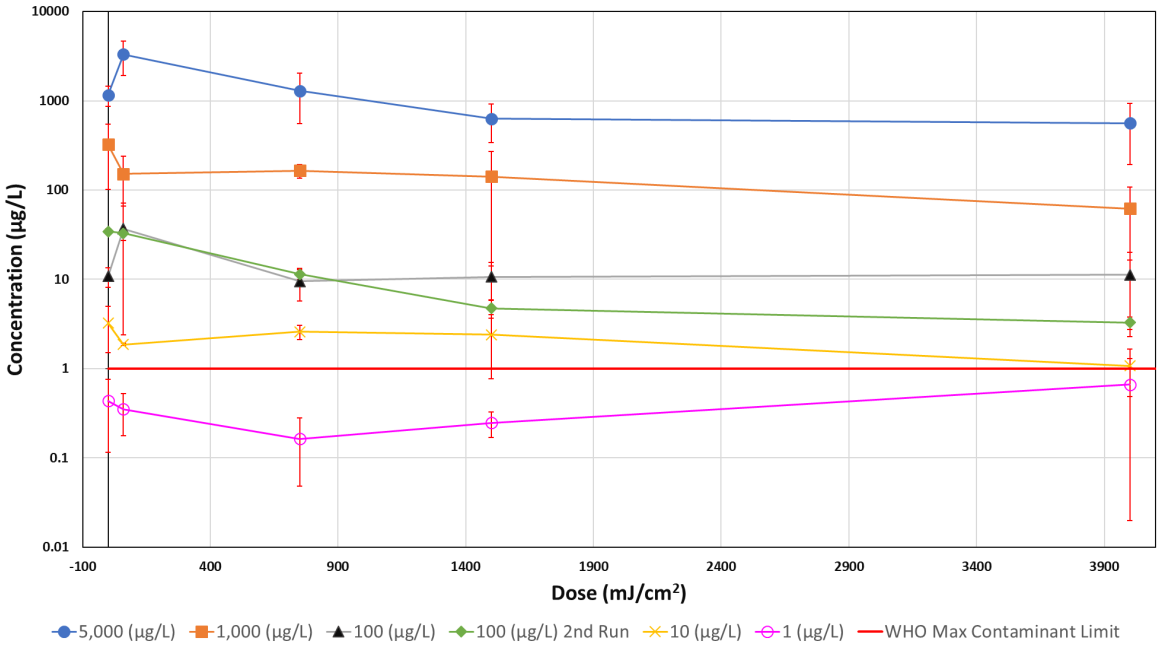

Figure 8: Plot of the final concentration of anatoxin-a in Nanopure water over the dose of UV light applied with the y-axis on a log scale

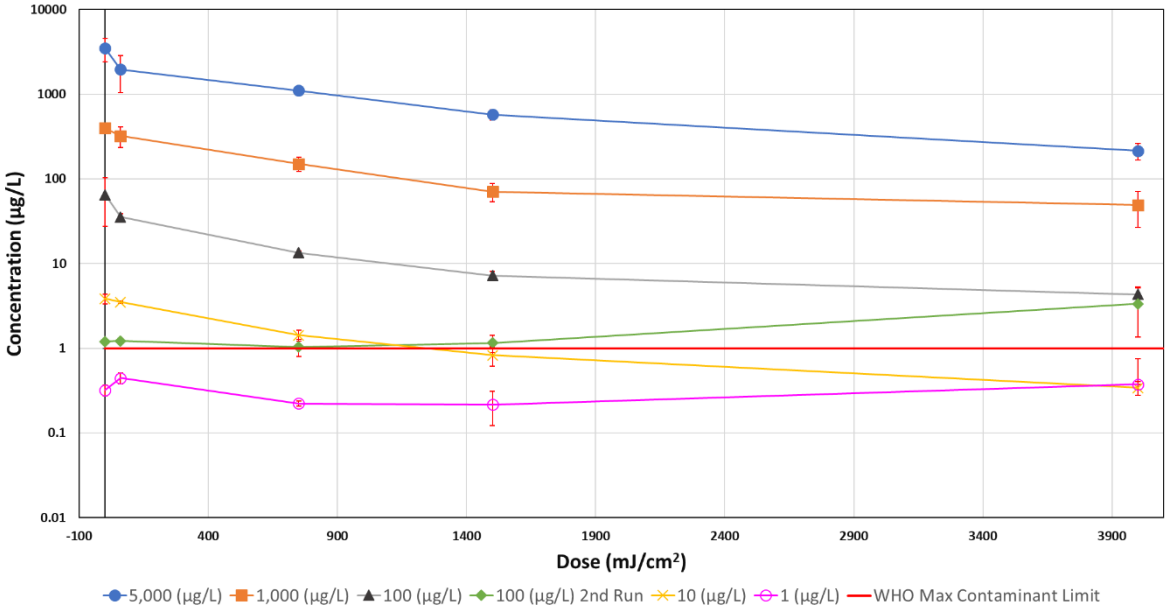

Figure 9: Plot of the final concentration of anatoxin-a in sand-filtered water over the dose of UV light applied with the y-axis on a log scale

Both MC-LR and A-a showed diminishing returns in degradation with increased doses of UV light. The difference in degradation between 750 and 1500 mJ/cm^2 for both toxins was greater than the difference in degradation between treatment with 1500 and 4000 mJ/cm^2. With a high enough dose of UV light, even the highest concentrations of each toxin could be fully degraded. Complete degradation of MC-LR at a concentration of 10 mg/L by sunlight alone was achieved after 29 days of exposure (Tsuji et al. 1994). However, the diminishing returns found in this experiment are evidence that the optimal UV dose for treating cyanotoxins is likely between 750 mJ/cm^2 and 1500 mJ/cm^2.

MC-LR dissolved in sand-filtered water (Figure 7) was degraded the most effectively, with the lowest concentrations of 1 µg/L and 10 µg/L reaching the WHO recommended maximum allowable concentration after treatment with 60 mJ/cm^2 which is in the same order of magnitude as that used for disinfection in water treatment systems, and falling well below the limit with higher doses. A-a in Nanopure water (Figure 8) was the most difficult to degrade, not reaching the MCL until treated with 4000 mJ/cm^2 of UV light. A-a is known to break down under UV light, where MC-LR is considered a more stable molecule, so these results are unexpected, and require further research to explain completely.

The highest concentration of each toxin would require a 4-log removal to be reduced below WHO's recommended maximum allowable concentration of 1 µg/L. The highest log removal value achieved was for MC-LR dissolved in Nanopure water, with a maximum of 1.5-log removal, with the lowest value of 0.5-log removal achieved by A-a dissolved in Nanopure water (Figure 10).

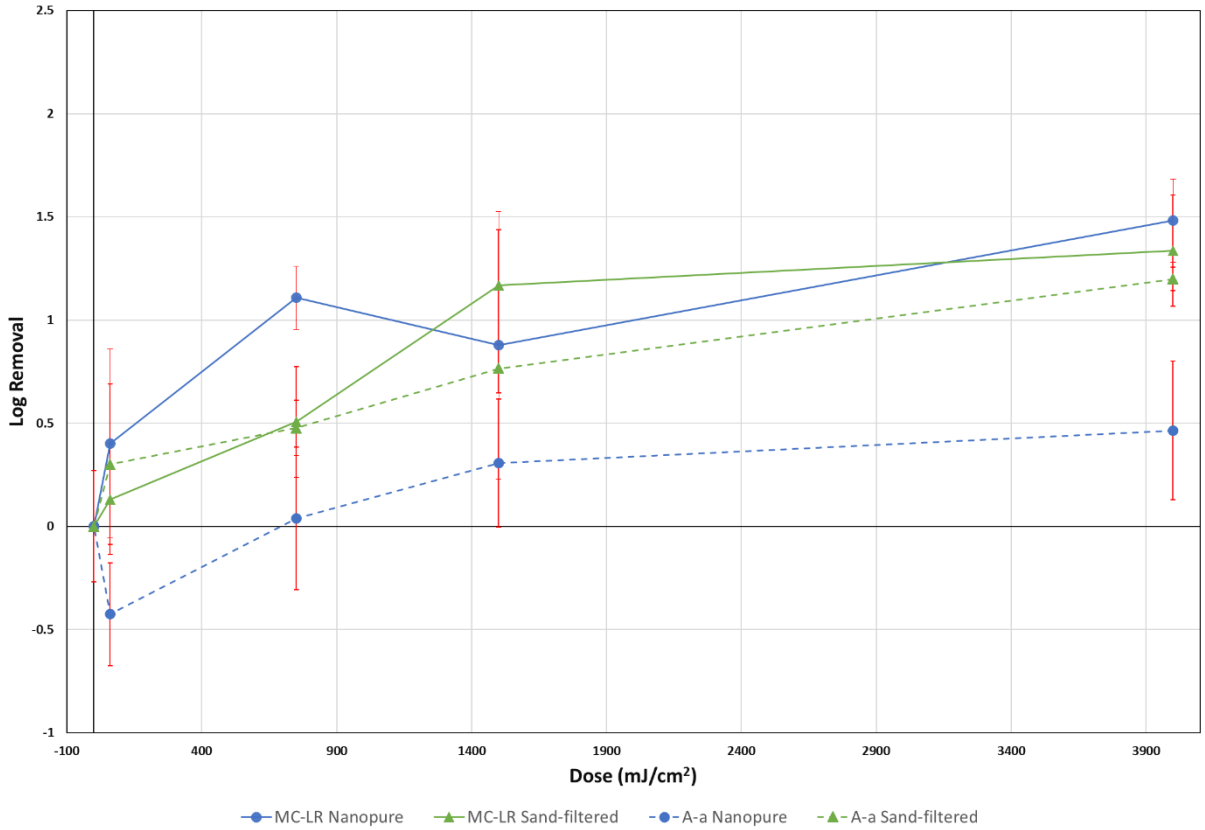

Figure 10: Plot of log removal values for each microcystin-LR and anatoxin-a dissolved in Nanopure water and sand-filtered water with a starting concentration of 5000 (µg/L)

As Figure 10 shows, MC-LR achieved greater log removal values in both water types at the highest starting concentration of 5000 µg/L than A-a dissolved in either water type at the same starting concentration. While the greatest log removal value shown in Figure 10 is achieved with MC-LR in Nanopure water, comparing the log removal values for every sample shows that both toxins were generally more easily degraded when dissolved in sand-filtered water than when dissolved in Nanopure water (Figures C1-C4). This behavior is unexpected because the Nanopure water does not have any dissolved solids or other material in the water that would interfere with the UV light, while the

sand-filtered water contains dissolved solids or other compounds in the water that could interfere with treatment. One possibility for the increased degradation seen in sand-filtered water is presence of dissolved organic material (DOM) that would not be present in Nanopure water. When DOM is irradiated with UV light, photo-oxidants may be formed (Lester et al. 2013). Since oxidation is a known effective treatment method for cyanotoxins, the presence of DOM may actually lead to increased degradation of the cyanotoxins also dissolved in solution. Other experiments have shown that coupling UV light with an oxidant such as Hydrogen Peroxide (H_2O_2) can decrease the dose of UV light needed to degrade both MC-LR and A-a (Afzal et al. 2010; He et al. 2012). While the greater success of treatment in sand-filtered water was not expected, it is a welcome success since slow sand filters are a common filtration method for small-scale drinking water treatment systems.

In order for UV light to be a viable treatment method for cyanotoxins, it must be capable of up to a 4-log removal of toxins for the highest starting concentrations of 1000 and 5000 µg/L. In this experiment, the highest log-removal achieved was approximately 1.5. This is a sufficient level of degradation when the starting concentrations of toxins were below 10 µg/L, but did not provide adequate treatment at the doses of UV light applied for higher starting concentrations.

While there are general trends that can be found in the data, such as increased degradation of each toxin with increased exposure to UV light, there are also a number of anomalies. To help explain these anomalies, the standard error for each average value was calculated, and is represented by the error bars seen in each plot. In the plots of log

removal values for A-a (Figures C3 and C4), there are some negative values, which would mean treatment actually increased the concentration of toxin in the water. An increase in toxin concentration due to increased exposure to UV light is not a likely occurrence, however the negative values also have large error bars associated with them, meaning there was a high amount of variance between each of the sample concentrations. The anomalies in the data may be corrected with a greater number of data points, but this experiment was limited by the number of ELISA kits that could be procured.

Further Research

The results from this experiment raise a number of questions that can only be answered by further research. This experiment used two of the most common cyanotoxins, however there are a wide variety of cyanotoxins including nearly 80 other known congeners of microcystin that may also be degraded by UV light and merit further study. Also, both MC-LR and A-a did show some response to treatment with UV light, but had diminishing returns with increasing UV dose or exposure lengths. Further experiments focusing on doses of UV light between 500 and 1,500 mJ/cm^2 are needed to find the optimal dose. Future experiments should also look to quantify the effects of temperature or exposure time on degradation. The same dose of UV light provided across different exposure times (so with different intensities of light) may produce different rates of degradation. Also, the heat applied by the UV light may also change the degradation rate at each UV dose. Neither of these possibilities were addressed in this experiment, and should be tested explicitly in future experiments. Finally, this experiment only used UV light with a wavelength of 254 nm since that is the most common wavelength used in

water treatment, and so the most readily available in UV lamps. However, the max absorbance of MC-LR is 238 nm, and the max absorbance of A-a is at 227 nm, so 254 nm may not be the ideal wavelength for treating cyanotoxins (Harada et al. 1999; Afzal et al. 2010). Further experiments with wavelengths of UV light closer to the max absorbance of each cyanotoxin molecule could show very different and more promising results than those achieved here.

CONCLUSION

The results of this experiment show that the cyanotoxins microcystin-LR (MC-LR) and anatoxin-a (A-a), which are very structurally different molecules, degrade by similar amounts when exposed to the same doses of UV light, suggesting that UV light could treat a wide variety of cyanotoxins. However, the highest dose of UV light used in this experiment, 4000 mJ/cm^2, only achieved a maximum of 1.5 log removal for each toxin, so was only able to meet the World Health Organization's (WHO) recommended maximum allowable concentration of 1 μg/L when the starting toxin concentration was under 10 μg/L. The highest starting concentrations of toxins require a 4-log removal of cyanotoxins to reach the recommended maximum allowable concentration. Higher UV doses than those used in this experiment, or a higher energy wavelength closer to each toxin's max absorbance wavelength, may be able to reach the 4-log removal necessary to treat the highest concentrations of toxins of 5000 μg/L used in this experiment, but more testing needs to be done to confirm these possibilities. Consequently, degradation of cyanotoxins with UV light cannot be recommended as a viable treatment method in small-scale drinking water treatment systems, systems that serve 1-4 homes and are operated and maintained by non-professionals, on its own at the doses used in this experiment. However, UV light may work well as a polishing step after primary treatment such as the addition of powdered activated carbon (PAC) or an oxidant such as Hydrogen Peroxide (H_2O_2) has occurred earlier in the treatment train. While the use of PAC and oxidants such as H_2O_2 do come with their own set of challenges for use in a

small-scale drinking water treatment system, the added removal or degradation of cyanotoxins they provide may be necessary when the concentration of a toxin in the source water is above 10 μg/L.

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APPENDICES

Appendix A

Information on natural concentrations of cyanotoxins and their action levels, as well as information about the UV light used in this experiment are provided below.

Table A1: WHO 2003 Recreational Guidance/Action Levels for Cyanobacteria, Chlorophyll a, and Microcystin (U.S. EPA 2017) S

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Chlorophyll α (µg/L)	Estimated Microcystin Levels (µg/L)
Low	<20,000	<10	<10
Moderate	20,000-100,000	10-50	10-20
High	>100,000-10,000,000	50-5,000	20-2,000
Very High	>10,000,000	> 5,000	> 2,000

Specifications:

- Light wave: UV-C 253.7nm wavelength
- Bulb lifespan: 10,000 hours
- Voltage supply: 120V, 60Hz
- Output: 35 watts
- Dimensions: 10.7” x 5.1” x 3.8”
- Bulb Length: 8.86” (225mm)
- Reflector Material: Electronically brightened aluminum.
- UV Lamp: Item # 191208
- UV Safety Glasses: Item # 302055

Figure A1: UV light and specs used in experiment provided by the vendor GermawayUV

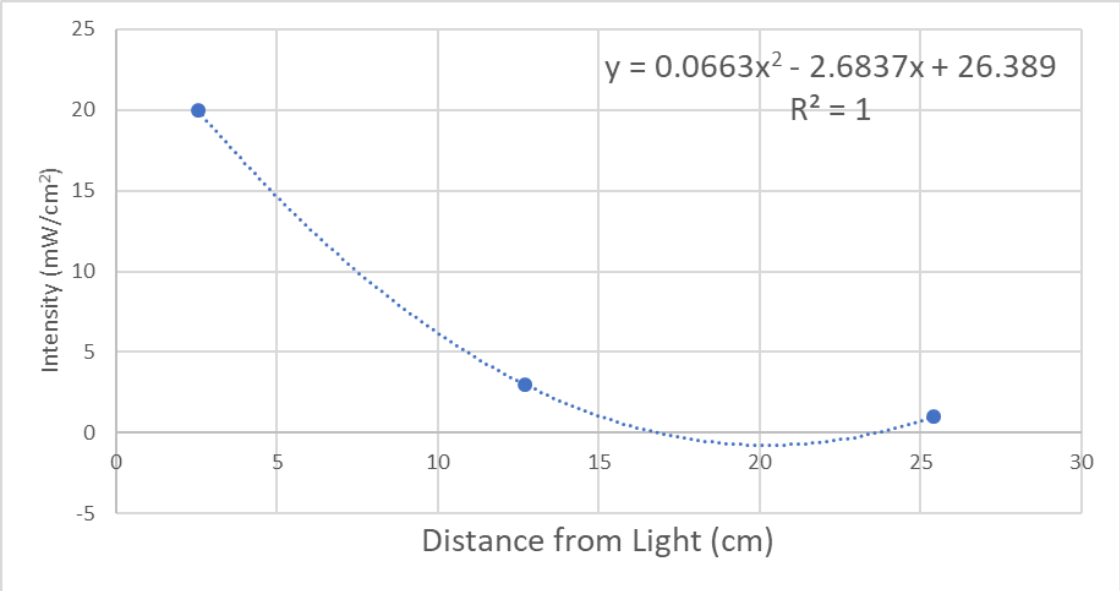


Figure A2: Interpolation of UV intensity by distance from light source

Appendix B

Information about ELISA kits and testing, including directions for their use, calibration curves, and serial dilution schemes used in this experiment.

ASSAY PROCEDURE

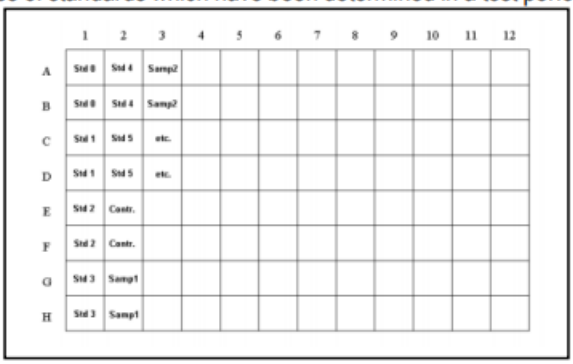
1. Add **50 µL of the standard solutions, control or samples** into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended.
2. Add **50 µL of the antibody solution** to the individual wells successively using a multi-channel pipette or a 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 and decant the contents of the wells in to a sink. Wash the strips **three times** using the 1X wash buffer solution. Please use at least a volume of **250 µL of wash buffer** for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
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 strips for **30 minutes** at room temperature.
5. Remove the covering and decant the contents of the wells into a sink. Wash the strips **three times** using the 1X wash buffer solution. Please use at least a volume of **250 µL of wash buffer** for each well and washing step. Remaining buffer in the wells should be removed by patting the plate dry in a stack of paper towels.
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 adsorbance at 450nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

Figure B1: Testing procedure for microcystin-LR ELISA test kit as provided by Enzo

E. Working Scheme

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.

Std 0-Std5: Standards
Contr.: Control
Samp1, Samp2, etc: Samples



F. Assay Procedure

1. Add **50 µL 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 **50 µL of the reconstituted enzyme conjugate solution** to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add **50 µL of the reconstituted 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 60 seconds. Be careful not to spill the contents. Incubate the strips for **60 minutes** at room temperature.
4. Remove the covering and decant the contents of the wells into a sink. 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. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
5. 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.
6. Add **100 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.
7. Read the absorbance at 450 nm using a microtiter plate ELISA photometer within 15 minutes after the addition of the stopping solution.

Figure B2: Testing procedure for anatoxin-a ELISA test kit as provided by Abraxis

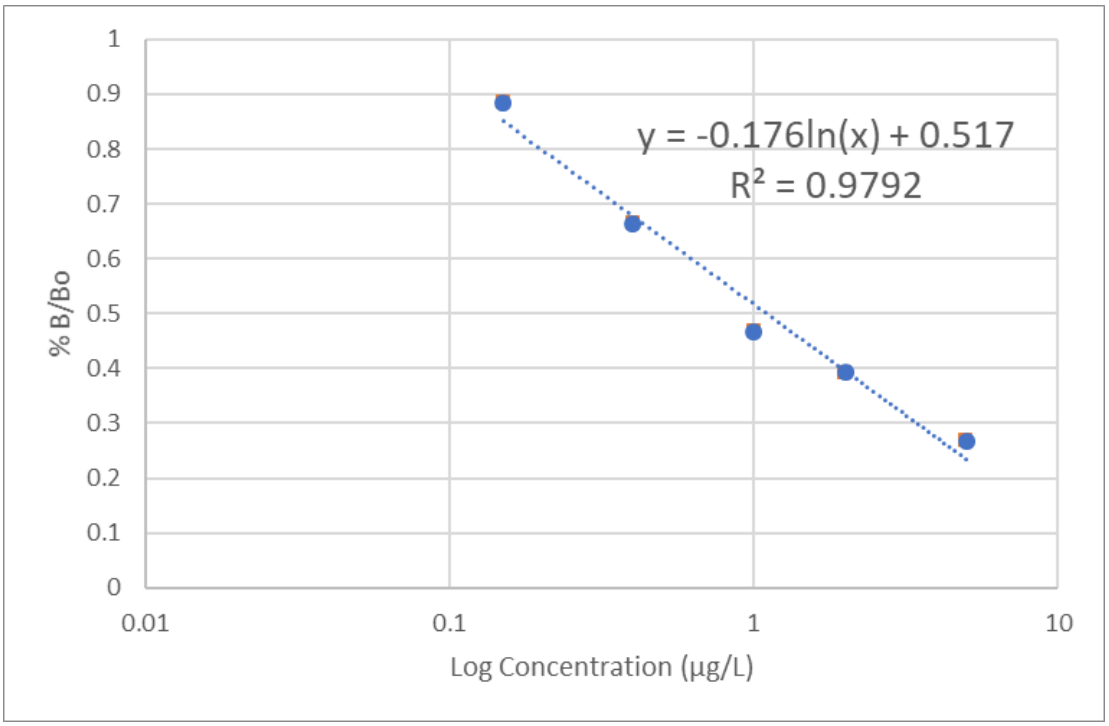


Figure B3: Calibration curve for ELISA standards used for microcystin-LR in Nanopure water

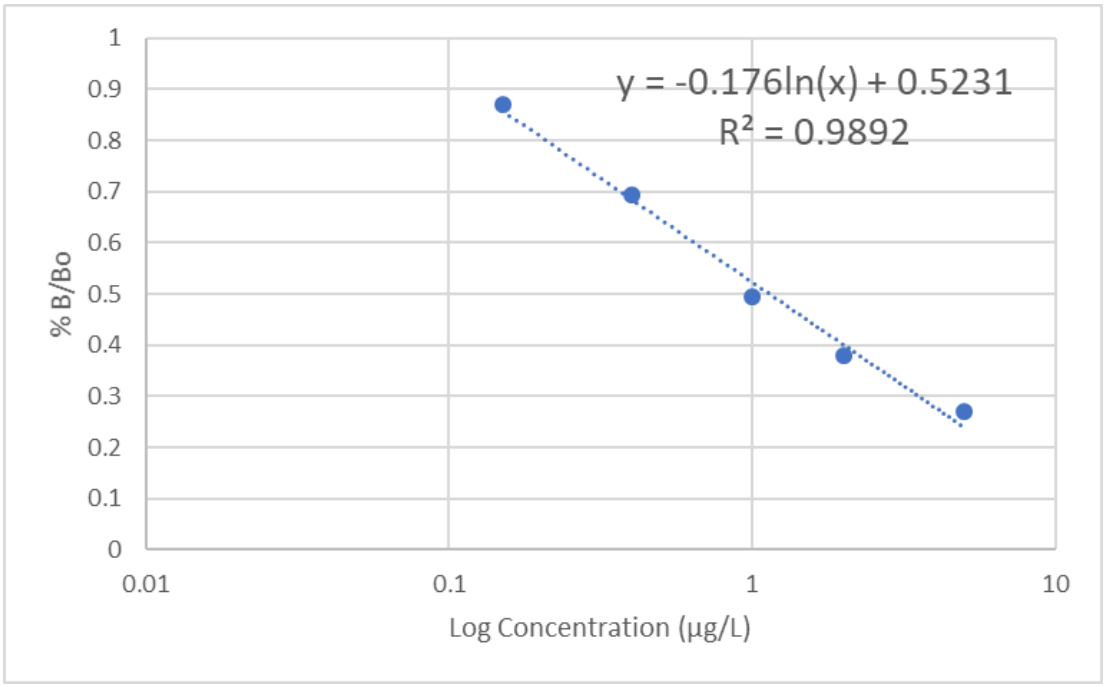


Figure B4: Calibration curve for ELISA standards used for microcystin-LR in sand-filtered water

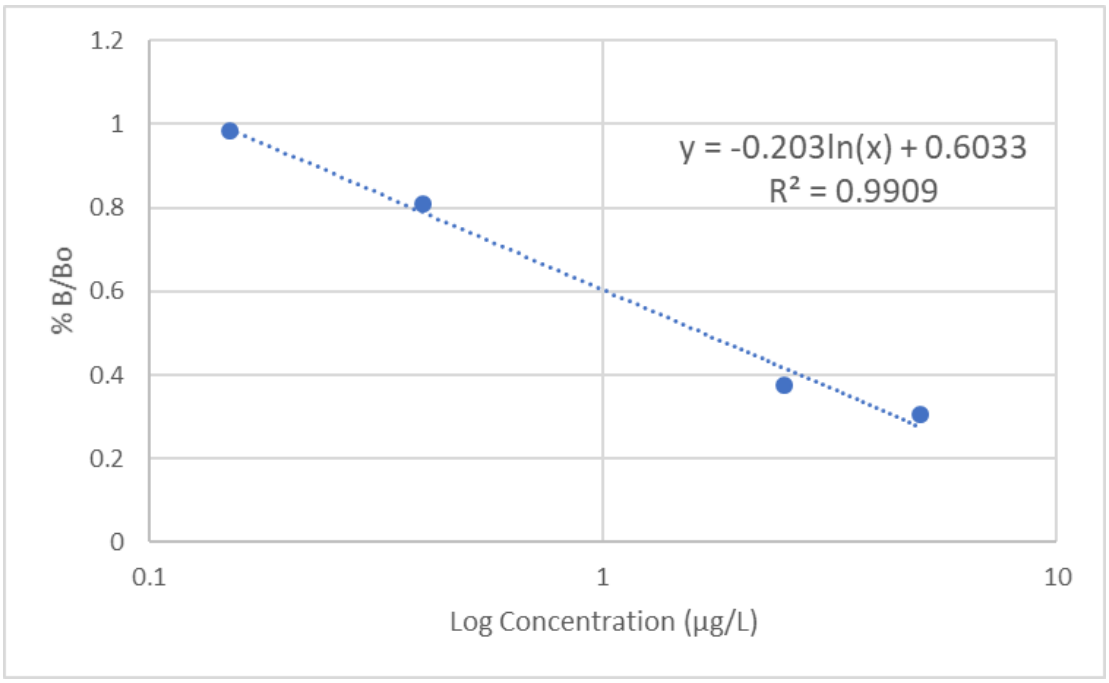


Figure B5: Calibration curve for ELISA standards used for anatoxin-a in Nanopure water

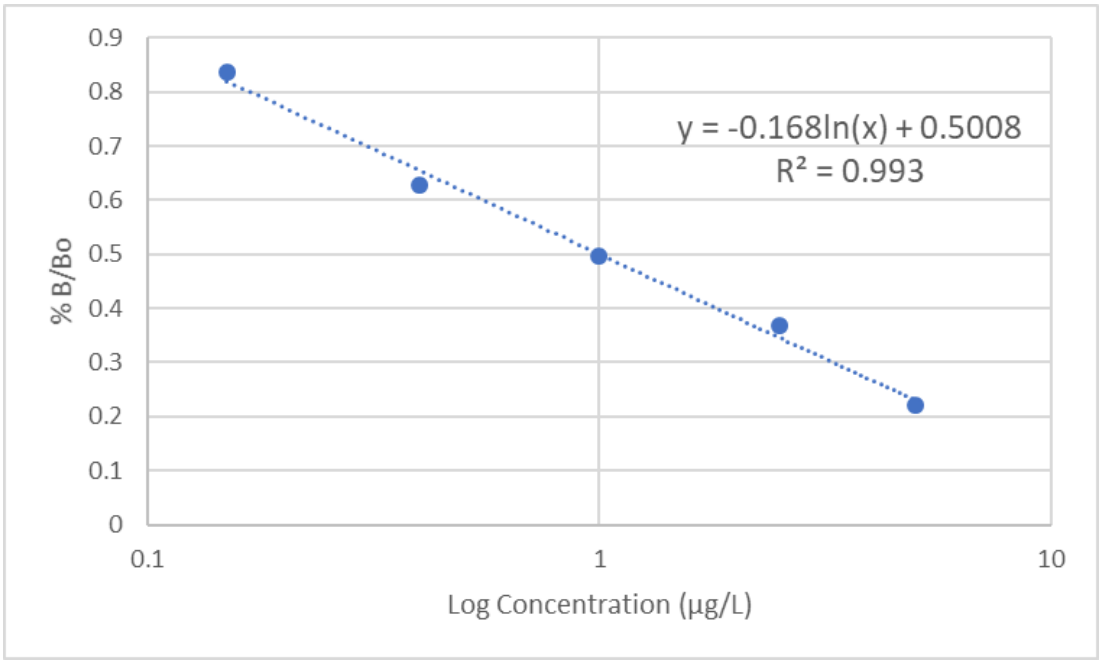


Figure B6: Calibration curve for ELISA standards used for anatoxin-a in sand-filtered water

Table B1: Volumes of stock solution and Nanopure or sand-filtered water used in serial dilutions to prepare desired toxin concentrations

Toxin Concentration (µg/L)	Volume of Stock Solution (mL)	Volume of Nanopure or sand-filtered water (mL)
5000	0.05	9.95
1000	2.0	8.0
100	1.0	9.0
10	1.0	9.0
1	1.0	9.0

Table B2: Volumes of concentrate and diluent used to dilute samples for ELISA testing, with associated dilution factors

Starting Concentration (µg/L)	Sample Volume (µL)	Diluent Volume (µL)	Dilution Factor	Final concentration (µg/L)
$1x10^6$	0.5	$1x10^5$	200,000	4.5
5000	1	999	1000	4.5
1000	4	996	250	4.5
100	22	478	22.72	4.5
10	67	83	2.23	4.5

Appendix C

Plots of all log-removal vs Dose measured during this experiment.

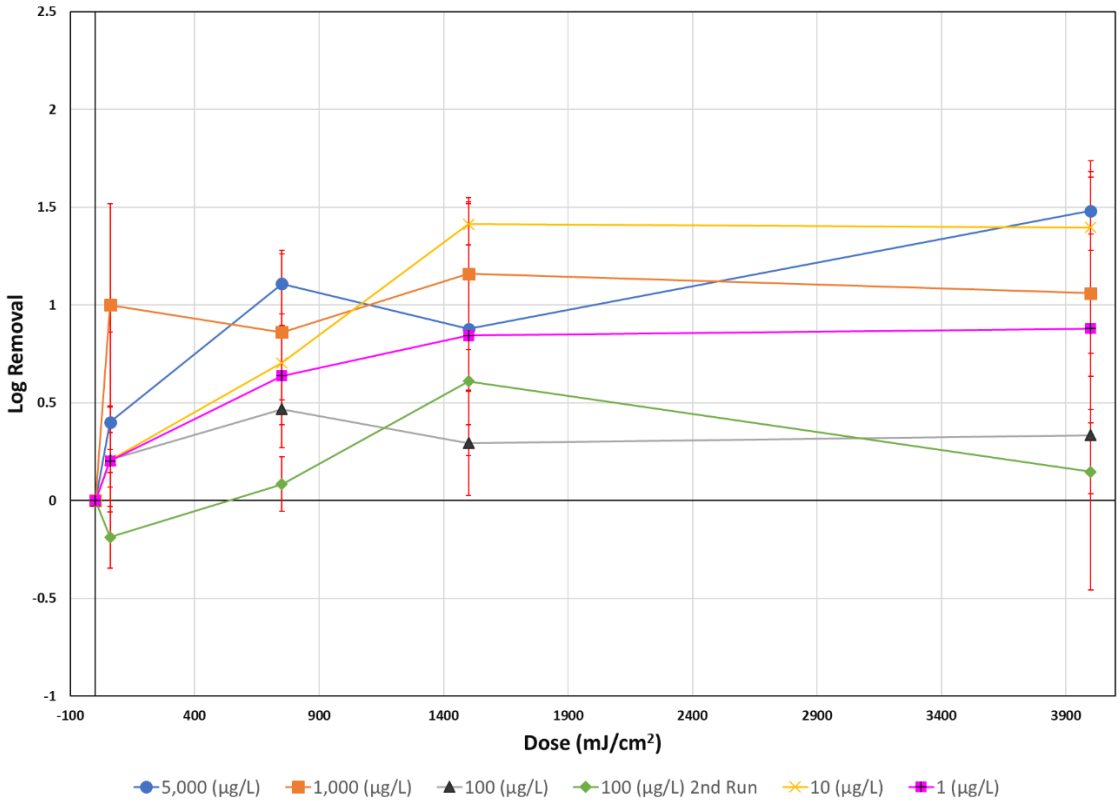


Figure C1: Log-removal of microcystin-LR in Nanopure water

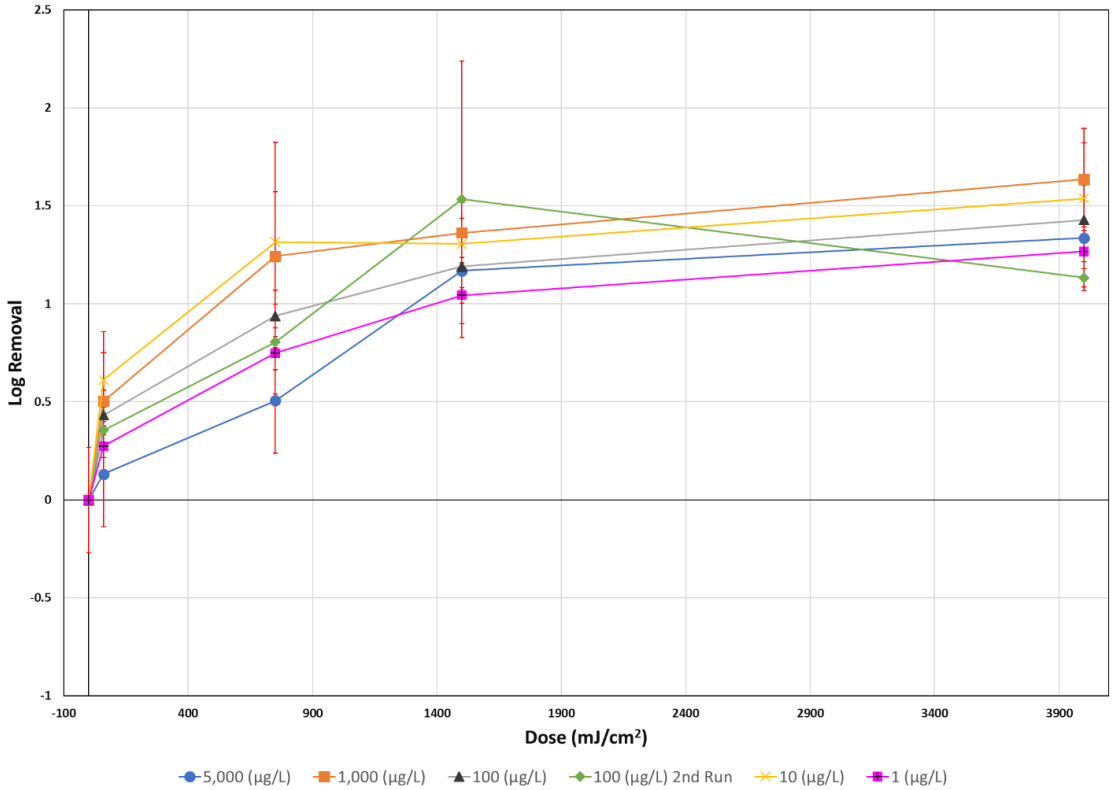


Figure C2: Log-removal of microcystin-LR in sand-filtered water

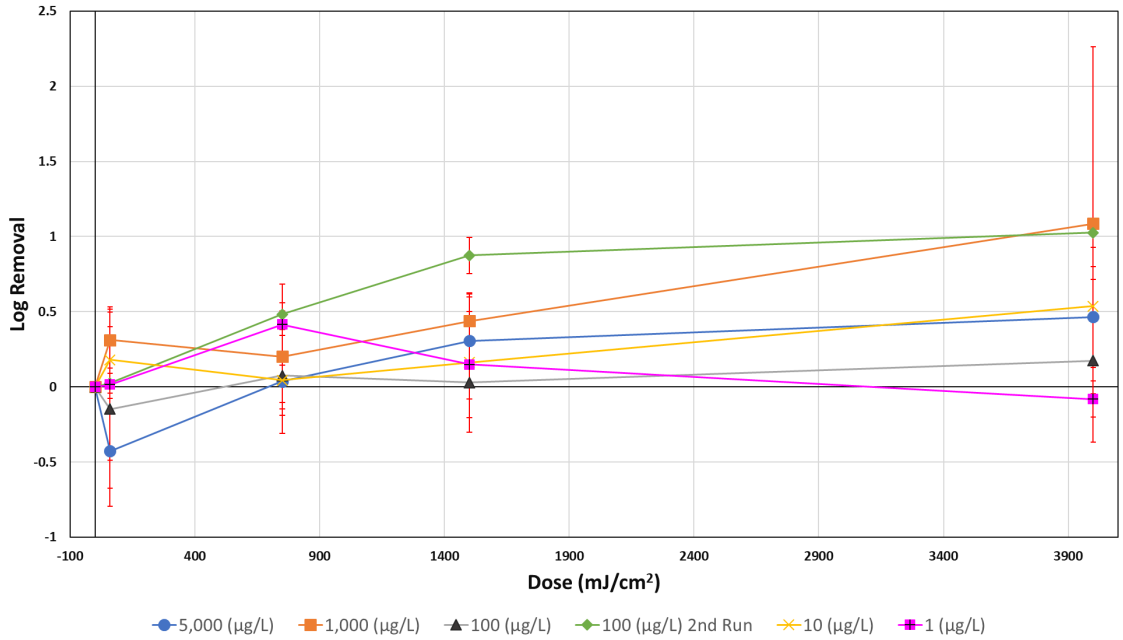


Figure C3: Log-removal of anatoxin-a in Nanopure water

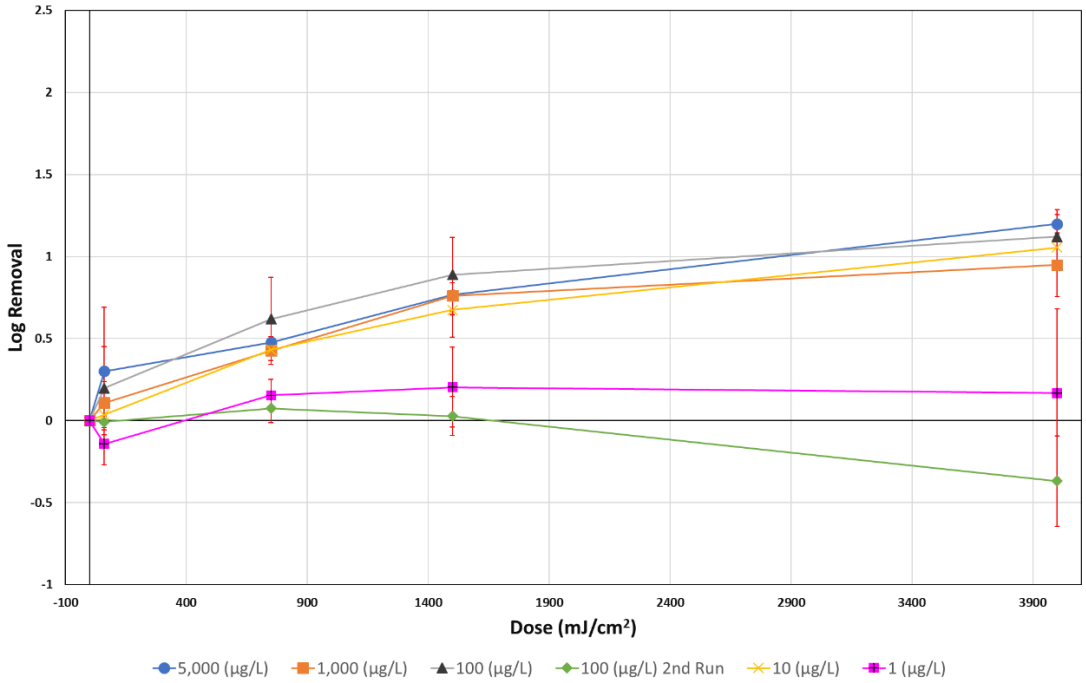


Figure C4: Log-removal of anatoxin-a in sand-filtered water

Appendix D

Tables of the diluted and undiluted values of concentrations measured with the ELISA test kits.

Table D1: Diluted concentrations of microcystin-LR samples in Nanopure water found by ELISA test kit

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	0.053256	0.725678	0.126555	0.176251	1.0861695	
2	5000	0.057899	0.155301	0.105461	1.993541	1.5657932	
3	5000	0.058372	0.103393	0.178075	0.891695	2.9422032	
1	1000	0.121019	0.06241	0.135632	0.128858	0.3253015	
2	1000	0.097475	0.063382	0.112813	0.067777	1.3422499	
3	1000	0.041886	0.062435	0.12714	0.085593	1.7056524	
1	100	0.061065	0.07003	0.037735	0.046527	0.0675433	
2	100	0.034225	0.050185	0.046548	0.162186	0.1504936	
3	100	0.088029	0.069087	0.041864	0.057829	0.1819994	
1	10	0.162534	0.065807	0.210437	0.749403	1.7015022	
2	10	0.068139	0.062495	0.437011	1.718108	2.0625859	
3	10	0.070684	0.16808	1.008846	2.229686	3.4183886	
1	1	0.213888	0.138813	0.191808	0.296012	0.456252	
2	1	0.129607	0.163907	0.209215	1.102187	1.5625695	
3	1	0.139586	0.218892	0.511136	1.276133	2.3614141	
1	0					0.0711268	Nanopure
2	0					0.0673053	Nanopure
3	0					0.0491187	Nanopure
1	5000					6.1624977	Vial of MC-LR from 6/11/2019 ($1x10^6$ µg/L)
2	5000					5.5995837	Vial of MC-LR from 6/11/2019 ($1x10^6$ µg/L)
3	5000					6.3402661	Vial of MC-LR from

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
							6/11/2019 ($1x10^6$ µg/L)
1	0.75					0.4395144	control
2	0.75					0.6492581	control

Table D2: Diluted concentrations of microcystin-LR samples in sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	0.078927	0.09664	0.15596	1.265848	0.922373	
2	5000	0.086061	0.079917	0.602447	0.546395	2.091413	
3	5000	0.050504	0.140428	1.124618	2.027216	1.805547	
1	1000	0.014093	0.048518	0.028527	0.095142	0.756965	
2	1000	0.021584	0.058901	0.201252	0.896371	1.474767	
3	1000	0.079292	0.05566	0.062812	0.700028	1.732825	
1	100	0.050387	0.052579	0.094919	0.247181	0.764385	
2	100	0.040362	0.078661	0.135435	0.532655	1.378805	
3	100	0.038804	0.097585	0.180521	0.583382	1.433307	
1	10	0.064545	0.079122	0.150213	0.38111	1.125874	
2	10	0.056064	0.132731	0.130125	0.495315	2.061212	
3	10	0.041909	0.07172	0.036088	0.480772	2.675889	
1	1	0.050184	0.081297	0.13278	0.486587	0.905692	
2	1	0.088749	0.12459	0.225526	0.579921	1.251267	
3	1	0.085917	0.177918	0.462698	1.300954	2.145621	
1	0					0.033318	Sand-filtered water
2	0					0.02632	Sand-filtered water
3	0					0.04204	Sand-filtered water
1	1000000					2.5418	Vial of MC-LR from 7/9/2019 ($1x10^6$ µg/L)
2	1000000					4.638663	Vial of MC-LR from 7/9/2019 ($1x10^6$ µg/L)
3	1000000					7.084074	Vial of MC-LR from 7/9/2019

Final [ ] (µg/L) (Diluted)				Dose (mJ/cm^2)			
							(1x10^6 µg/L)
1	0.75					0.58073	control
2	0.75					0.555564	control

Table D3: Diluted concentrations of microcystin-LR samples for second run with 100 µg/L in Nanopure and sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	100	0.777806	0.093052	0.428089	1.248466	0.534945	Nanopure
2	100	1.617663	0.127106	0.40235	0.776766	0.659855	Nanopure
3	100	0.082132	0.35726	0.932663	1.083198	0.810649	Nanopure
1	100	0.128851	0.123017	0.282052	1.438701	1.975387	Sand Filtered
2	100	0.161232	0.010117	0.199039	0.642256	2.221634	Sand Filtered
3	100	0.115678	0.121721	0.41301	0.560008	1.370568	Sand Filtered
1	1000000					2.94964	Vial of MC-LR from 7/18/2019 ($1x10^6$ µg/L)
1	0.75					0.707498	control
2	0.75					0.548089	control

Table D4: Diluted concentrations of anatoxin-a samples in Nanopure water found by ELISA test kit

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	0.099047	0.603571	2.206863	5.084791	0.857673	
2	5000	0.99638	0.28614	1.271221	3.134923	1.573725	
3	5000	0.586855	0.997755	0.395089	1.704353	1.063199	
1	1000	0.294813	0.168699	0.821303	0.224166	0.779497	
2	1000	0.446188	0.245605	0.561444	0.542276	0.557787	
3	1000	0.004632	1.290112	0.591466	1.055601	2.537594	
1	100	0.073474	0.339548	0.232955	3.671754	0.493112	
2	100	1.021779	0.304972	0.636689	0.065481	0.324532	
3	100	0.388826	0.768189	0.386704	1.119466	0.606834	
1	10	0.652194	0.51222	1.3694	0.869635	1.133186	
2	10	0.111098	0.608311	1.217267	0.836798	2.514321	
3	10	0.679954	2.112936	0.884755	0.80029	0.708902	
1	1	0.139488	0.302195	0.11467	0.59799	0.136548	
2	1	1.563423	0.30327	0.323086	0.232842	0.877783	
3	1	0.279488	0.135429	0.053055	0.224166	0.289219	
1	0					0.17641	Nanopure
2	0					0.147668	Nanopure
3	0					0.14923	Nanopure
1	1000000					1.544149	Starting Vial 6/25/2019 (diluted from $1x10^6$ µg/L)
2	1000000					0.545972	Starting Vial 6/25/2019 (diluted from $1x10^6$ µg/L)
3	1000000					0.984476	Starting Vial 6/25/2019 (diluted from

Final [ ] (µg/L) (Diluted)	Dose (mJ/cm^2)						
							1x10^6 µg/L)
1	0.75					0.489942	Control, +/- 0.185
2	0.75					0.64574	Control, +/- 0.185
1	1000000					7.975328	Old Vial from 6/13/2019 (diluted from 1x10^6 µg/L)

Table D5: Diluted concentrations of anatoxin-a samples in sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	0.185714	0.68111	1.067521	2.808574	3.2405157	
2	5000	0.177135	0.479343	1.133772	2.374085	2.323256	
3	5000	0.282602	0.56847	1.12691	0.692633	4.8953007	
1	1000	0.160471	0.317652	0.724674	1.624465	1.6030211	
2	1000	0.109907	0.188479	0.636745	1.441508	1.579797	
3	1000	0.31945	0.345143	0.448748	0.803138	1.5640208	
1	100	0.187336	0.270703	0.632708	1.464431	1.6295767	
2	100	0.144961	0.363106	0.588332	1.781139	1.7544845	
3	100	0.238343	0.322868	0.552452	1.448844	5.2094553	
1	10	0.129169	0.312519	0.739596	1.542127	1.9135966	
2	10	0.136356	0.298182	0.667459	1.647853	1.874676	
3	10	0.193802	0.505929	0.515126	1.546734	1.3987889	
1	1	0.090289	0.164465	0.207355	0.366662	0.3568244	
2	1	0.121938	0.137189	0.218934	0.442456	0.3530321	
3	1	0.913689	0.34885	0.243668	0.526347	0.2540689	
1	0					0.1374397	Sand-filtered Water
2	0					0.109552	Sand-filtered Water
3	0					0.0983759	Sand-filtered Water
1	1000000					5.4595233	Starting Vial from 7/10/2019 (diluted from $1x10^6$ µg/L)
2	1000000					6.4331768	Starting Vial from 7/10/2019 (diluted from $1x10^6$ µg/L)

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
3	1000000					8.8803664	Starting Vial from 7/10/2019 (diluted from $1x10^6$ µg/L)
1	0.75					0.6744112	Control, +/- 0.185
2	0.75					0.7045395	Control, +/- 0.185
1	1000000						Old Vial from 6/13/2019 (diluted from $1x10^6$ µg/L)

Table D6: Diluted concentrations of anatoxin-a samples for second run with 100 µg/L in Nanopure and sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Diluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	100	0.112581	0.235663	0.421284	1.40564	1.636833	Nanopure
2	100	0.15537	0.244393	0.508102	1.75792	1.357686	Nanopure
3	100	0.16485	0.143577	0.573993	1.173082	1.559609	Nanopure
1	100	0.108655	0.036344	0.059748	0.049318	0.054056	Sand Filtered
2	100	0.064333	0.051832	0.042785	0.057776	0.054981	Sand Filtered
3	100	0.268768	0.065136	0.034658	0.054784	0.049677	Sand Filtered
1	1000000					2.796969	Vial of A-a from 7/18/2019 ($1x10^6$ µg/L)
1	0.75					0.688504	control
2	0.75					0.697322	control

Table D7: Undiluted concentrations of microcystin-LR samples in Nanopure water found by ELISA test kit

Final [ ] (µg/L) (Undiluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	53.25619	725.6781	126.5546	176.2515	1086.1695	
2	5000	57.8994	155.3013	105.4614	1993.541	1565.7932	
3	5000	58.37157	103.3926	178.0748	891.6946	2942.2032	
1	1000	18.15282	9.361487	20.34479	19.3287	48.795226	
2	1000	14.62125	9.507365	16.92199	10.16659	201.33749	
3	1000	6.28285	9.365211	19.07097	12.83889	255.84785	
1	100	1.692731	1.941235	1.04602	1.28973	1.8722998	
2	100	0.948727	1.391123	1.290307	4.495803	4.1716817	
3	100	2.440168	1.915102	1.160471	1.603021	5.0450232	
1	10	0.362451	0.14675	0.469275	1.671169	3.79435	
2	10	0.151949	0.139364	0.974535	3.831381	4.5995665	
3	10	0.157624	0.374818	2.249726	4.9722	7.6230065	
1	1	0.213888	0.138813	0.191808	0.296012	0.456252	
2	1	0.129607	0.163907	0.209215	1.102187	1.5625695	
3	1	0.139586	0.218892	0.511136	1.276133	2.3614141	
1	0					0.0711268	Nanopure
2	0					0.0673053	Nanopure
3	0					0.0491187	Nanopure
1	1000000					1232499.5	Vial of MC-LR from 6/11/2019 ($1x10^6$ µg/L)
2	1000000					1119916.7	Vial of MC-LR from 6/11/2019 ($1x10^6$ µg/L)

Final [ ] (µg/L) (Undiluted)	Dose (mJ/cm^2)						
3	1000000					1268053.2	Vial of MC-LR from 6/11/2019 ($1x10^6$ µg/L)
1	0.75					0.4395144	control
2	0.75					0.6492581	control

Table D8: Undiluted concentrations of microcystin-LR samples in sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Undiluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	78.92743	96.64014	155.9598	1265.848	922.373	
2	5000	86.06112	79.91651	602.4468	546.3946	2091.413	
3	5000	50.50426	140.428	1124.618	2027.216	1805.547	
1	1000	2.113881	7.277716	4.2791	14.27131	113.5447	
2	1000	3.237584	8.835127	30.18774	134.4557	221.2151	
3	1000	11.8938	8.348986	9.421822	105.0041	259.9237	
1	100	1.396722	1.457501	2.631158	6.851849	21.18876	
2	100	1.118824	2.180492	3.75425	14.7652	38.22046	
3	100	1.075636	2.70505	5.004051	16.17134	39.73128	
1	10	0.143935	0.176443	0.334974	0.849875	2.5107	
2	10	0.125023	0.29599	0.290178	1.104552	4.596504	
3	10	0.093457	0.159936	0.080476	1.072123	5.967233	
1	1	0.050184	0.081297	0.13278	0.486587	0.905692	
2	1	0.088749	0.12459	0.225526	0.579921	1.251267	
3	1	0.085917	0.177918	0.462698	1.300954	2.145621	
1	0					0.033318	Sand-filtered water
2	0					0.02632	Sand-filtered water
3	0					0.04204	Sand-filtered water
1	1000000					508360	Vial of MC-LR from 7/9/2019 ($1x10^6$ µg/L)
2	1000000					927732.6	Vial of MC-LR from 7/9/2019 ($1x10^6$ µg/L)

Final [ ] (µg/L) (Undiluted)	Dose (mJ/cm^2)						
3	1000000					1416815	Vial of MC-LR from 7/9/2019 ($1x10^6$ µg/L)
1	0.75					0.58073	control
2	0.75					0.555564	control

Table D9: Undiluted concentrations of microcystin-LR samples for second run with 100 µg/L in Nanopure and sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Undiluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	100	17.67176	2.114136	9.726189	28.36516	12.15395	Nanopure
2	100	36.75331	2.887852	9.141391	17.64812	14.9919	Nanopure
3	100	1.86605	8.116956	21.19011	24.61025	18.41794	Nanopure
1	100	2.92749	2.794942	6.408214	32.68728	44.88078	Sand Filtered
2	100	3.663198	0.229854	4.522156	14.59206	50.47552	Sand Filtered
3	100	2.628201	2.765498	9.383595	12.72338	31.13931	Sand Filtered
1	1000000					589928	Vial of MC-LR from 7/18/2019 ($1x10^6$ µg/L)
1	0.75					0.707498	control
2	0.75					0.548089	control

Table D10: Undiluted concentrations of anatoxin-a samples in Nanopure water found by ELISA test kit

Final [ ] (µg/L) (Undiluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	99.04656	603.5713	2206.863	5084.791	857.6728	
2	5000	996.3805	286.1401	1271.221	3134.923	1573.725	
3	5000	586.8554	997.7547	395.0886	1704.353	1063.199	
1	1000	73.70326	42.17467	205.3259	56.04155	194.8743	
2	1000	111.5471	61.40137	140.361	135.569	139.4468	
3	1000	1.158049	322.5281	147.8666	263.9003	634.3985	
1	100	1.669335	7.714539	5.292729	83.42226	11.20351	
2	100	23.21483	6.928966	14.46557	1.487725	7.373378	
3	100	8.834133	17.45326	8.785924	25.43426	13.78726	
1	10	1.454392	1.14225	3.053762	1.939285	2.527004	
2	10	0.247748	1.356533	2.714505	1.86606	5.606937	
3	10	1.516298	4.711848	1.973003	1.784647	1.580852	
1	1	0.139488	0.302195	0.11467	0.59799	0.136548	
2	1	1.563423	0.30327	0.323086	0.232842	0.877783	
3	1	0.279488	0.135429	0.053055	0.224166	0.289219	
1	0					0.17641	Nanopure
2	0					0.147668	Nanopure
3	0					0.14923	Nanopure
1	1000000					308829.9	Starting Vial 6/25/2019 (diluted from $1x10^6$ µg/L)
2	1000000					109194.5	Starting Vial 6/25/2019 (diluted from $1x10^6$ µg/L)
3	1000000					196895.1	Starting Vial 6/25/2019 (diluted from $1x10^6$ µg/L)

Final [ ] (µg/L) (Undiluted)	Dose (mJ/cm^2)						
1	0.75					0.489942	Control, +/- 0.185
2	0.75					0.64574	Control, +/- 0.185
1	1000000					1595066	Old Vial 6/13/2019 (diluted from $1x10^6$ µg/L)

Table D11: Undiluted concentrations of anatoxin-a samples in sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Undiluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	5000	185.7144	681.1104	1067.521	2808.574	3240.5157	
2	5000	177.135	479.3435	1133.772	2374.085	2323.256	
3	5000	282.602	568.4696	1126.91	692.6333	4895.3007	
1	1000	40.11764	79.41297	181.1686	406.1162	400.75528	
2	1000	27.47667	47.11974	159.1862	360.3771	394.94926	
3	1000	79.86243	86.28571	112.1869	200.7844	391.0052	
1	100	4.256266	6.150363	14.37512	33.27187	37.023983	
2	100	3.293517	8.249779	13.36691	40.46748	39.861888	
3	100	5.415146	7.335565	12.55172	32.91774	118.35882	
1	10	0.288047	0.696917	1.649298	3.438943	4.2673204	
2	10	0.304073	0.664946	1.488433	3.674712	4.1805276	
3	10	0.432179	1.128221	1.148732	3.449217	3.1192993	
1	1	0.090289	0.164465	0.207355	0.366662	0.3568244	
2	1	0.121938	0.137189	0.218934	0.442456	0.3530321	
3	1	0.913689	0.34885	0.243668	0.526347	0.2540689	
1	0					0.1374397	Sand-filtered Water
2	0					0.109552	Sand-filtered Water
3	0					0.0983759	Sand-filtered Water
1	1000000					1091904.7	Starting Vial from 7/10/2019 (diluted from $1x10^6$ µg/L)
2	1000000					1286635.4	Starting Vial from 7/10/2019 (diluted from $1x10^6$ µg/L)
3	1000000					1776073.3	Starting Vial from 7/10/2019

Final [ ] (µg/L) (Undiluted)	Dose (mJ/cm^2)						
							(diluted from $1x10^6$ µg/L)
1	0.75					0.6744112	Control, +/- 0.185
2	0.75					0.7045395	Control, +/- 0.185
1	1000000					0	Old Vial 6/13/2019 (diluted from $1x10^6$ µg/L)

Table D12: Undiluted concentrations of anatoxin-a samples for second run with 100 µg/L in Nanopure and sand-filtered water found by ELISA test kit

Final [ ] (µg/L) (Undiluted)		Dose (mJ/cm^2)					
Trial Number	**Starting [ ] (µg/L)**	**4000**	**1500**	**750**	**60**	**0**	**Notes**
1	100	2.557836	5.354253	9.571563	31.93613	37.18884	Nanopure
2	100	3.53001	5.552612	11.54407	39.93994	30.84662	Nanopure
3	100	3.745385	3.262066	13.04113	26.65243	35.43432	Nanopure
1	100	2.468645	0.825728	1.357485	1.120516	1.228155	Sand Filtered
2	100	1.461637	1.17763	0.972075	1.31268	1.249167	Sand Filtered
3	100	6.10641	1.479883	0.787423	1.244703	1.128661	Sand Filtered
1	1000000					559393.8	Vial of A-a from 7/18/2019 ($1x10^6$ µg/L)
1	0.75					0.688504	control
2	0.75					0.697322	control