THESIS

THE OCCURRENCE AND REMOVAL OF CYANOBACTERIAL METABOLITES MICROYCSTIN-LR AND GEOSMIN FROM SOURCE WATERS WITH POWDERED ACTIVATED CARBON.

Submitted by

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ABSTRACT

THE OCCURRENCE AND REMOVAL OF CYANOBACTERIAL METABOLITES MICROYCSTIN-LR AND GEOSMIN FROM SOURCE WATERS WITH POWDERED ACTIVATED CARBON.

Cyanobacteria blooms may result in the release of problematic algal metabolites, such as geosmin and microcystin-LR in source waters. The World Health Organization has set a guideline limit of 1 μg/L for the Microcystin-LR in drinking water to prevent adverse health effects. Microcystin-LR is the most common and potent cyanotoxin which can cause severe gastro-enteritis and hepatoritis. Unlike microcystin-LR, geosmin is not known to be harmful however, it imparts an unpleasant earthy off-flavor to drinking water detectable to humans at 2-10 ng/L.

Understanding the occurrence of these metabolites is the first step in mitigating waters contaminated with these algal metabolites. To understand their occurrence in Northern Colorado, environmental sampling was performed in local rivers, lakes and municipal waters. Results of environmental sampling in the Northern Colorado area revealed that microcystins frequently was detected with geosmin however; geosmin alone, without microcystin-LR, was more frequently detected. This common co-occurrence of both compounds may be helpful in the surveillance, prevention and elimination of geosmin and microcystin-LR from drinking water sources.

Since neither of the metabolites can be sufficiently removed by most conventional water treatment processes. This study also investigated the concurrent removal of microcystin-LR and geosmin from spiked raw Horsetooth Reservoir water, in Fort Collins CO, by powdered activated carbon (PAC). Water samples were spiked with microcystin-LR and geosmin to achieve various concentrations from 2 to 10 μg/L and 10 to 50 ng/L respectively, with PAC

concentrations ranging from 10 to 30 mg/L. Jar testing was employed for the experiments with 30 min mixing and 30 min settling. Geosmin was quantified by solid phase micro-extraction and gas chromatography/mass spectrometry. Microcystin-LR was quantified by liquid chromatography/mass spectrometry coupled with electrospray ionization. A PAC dose of 30 mg/L removed microcystin-LR concentrations up to 6 µg/L below WHO guidelines of 1 µg/L. Geosmin concentrations up to 50 ng/L were removed below human detection threshold (5 ng/L) with 20 mg/L of PAC. Competitive absorption by PAC was observed between microcystin-LR and geosmin where the removal efficiency of both metabolites; because of its smaller size geosmin was slightly better absorbed by PAC than microcystin-LR. PAC is a viable method to remove both metabolites.

To evaluate the toxicity of microcystin-LR, H4IIE, rat liver cells were cultured and exposed to microcystin-LR *in-vitro*. Cell viability and histological observations concluded that the toxin induced cellular apoptosis and cell viability is cyanotoxin concentration dependent.

ACKNOWLEDGEMENTS

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Introduction

Cyanobacteria are small photosynthetic bacteria that can be found in practically all waters of the world. They can grow in masses called blooms due to an abundance of nutrients and warmer temperatures. Cyanobacteria blooms have been occurring more frequently around the world due to climate change and poor nutrient management. This can be problematic as cyanobacteria may release a host of detrimental metabolites that can affect our drinking waters' quality (Chorus, 2001). Two types of algal metabolites were studied in this research project. The first is geosmin, which is one of the most common taste and odor compounds produced by cyanobacteria. Geosmin imparts an earthy odor that can be detected by the human nose at as low as 2 ng/L (Omur-Ozbek and Dietrich, 2005). The second metabolite is microcystin-LR, which is one of the commonly produced cyanotoxins that inhibits essential processes in the liver responsible for tumor suppression and may result in massive hepatic necrosis (Chorus and Bartman, 1999).

There is a need for methods to remove these detrimental metabolites from drinking water sources worldwide in a cost effective, practical and sustainable way. As geosmin and microcystin-LR are often found together (Graham et al., 2010) the prevention, management and removal of these detrimental metabolites will require understanding the relationship between the two metabolites, their behavior, possible removal techniques and obstacles that may be a result of treating water containing the metabolites.

The research conducted for this thesis contains three parts: First the occurrence of the metabolites was evaluated through environmental surveillance for both metabolites at various locations in Northern Colorado including lakes, rivers and municipal areas. Detection of geosmin

was performed following procedures laid out by Omur-Ozbek and Dietrich (2005). Microcystin-LR detection was performed following procedures laid out by Triantis et al.(2010). Second, the removal of the metabolites was experimented on source water spiked with known concentrations of both geosmin and microcystin-LR was investigated. Horsetooth Reservoir water was selected as the raw water source to perform the experiments. Horsetooth Reservoir provides water for municipal, agricultural and industrial uses in Northern Colorado (USBR, 2012). Powdered activated carbon was selected as the treatment method to remove the metabolites for its versatility and ability to effectively remove organic compounds (Westrick and Szlag, 2010). The third part of this research observed the toxicity of microcystin-LR *in-vitro* experiments with H4IIE rat liver cells to better understand its toxicity. Cells were grown under conditions laid out by Ding et al. (2001) and exposed to various concentrations of microcystin-LR for a period of 24 hours. Histological observations were performed on stained cells and the cell viability was observed through SRB laid out by Fricker (1994).

This thesis consists of three chapters to disseminate the experimental procedures and findings. The first chapter provides background information on cyanobacteria, their metabolites including microcystins and geosmin, detection methods of the metabolites, and treatment options to remove the metabolites from source waters. The second chapter is prepared in a manuscript format for an academic journal submission on the occurrence and removal of cyanobacterial metabolites. The third and final chapter, also in manuscript format, addresses the specific toxicity of the cyanobacterial metabolite, microcystin-LR in *in-vitro* studies. Both manuscripts include details regarding their respective experiments including, materials and methods, results, and conclusions for the related research conducted. The raw data is provided in the appendices at the end of this thesis.

1.0 Literature Review

1.1 Cyanobacteria

Cyanobacteria, also known as blue-green algae, are photosynthetic unicellular bacteria found throughout almost every environment in the world. They can be found in the lush bayous of Louisiana to the most barren areas of Antarctica where they have even been observed to form colonies in snow and ice (Chorus, 2001). Some nitrogen fixing cyanobacteria are photoautotrophs, only requiring light energy, CO_2, N_2, water and a few minerals allowing them to live in a wide variety of environments (Svrcek and Smith, 2004).

Cyanobacteria are as single celled organisms varying in sizes between 3-10 μm and can aggregate into larger clumps. They can appear as scum layers or be dispersed causing a pea soup appearance in lakes, ponds and other slow moving water bodies. Although they are referred as blue-green, cyanobacteria appear in shades of blue, green, red or brown. Cyanobacteria are among the most important group of bacteria in the world. They are thought to have been responsible for creating the Earth's oxygen rich environment over 2.5 billion years ago. Cyanobacteria are an essential part of the aquatic food chain and are a source of food for phytoplankton which converts gaseous nitrogen into a form that can be taken up by plants (Bean et al., 2002; Davis and Cornwell, 2008).

1.1.1 Cause of cyanobacterial blooms

When periods of mass cyanobacterial growth occur, they are often referred to as blooms (seen in Figure 1-1). The frequency of these blooms has increased throughout the world due a combination of warmer climates and an abundance of available nutrients such as nitrogen and

phosphorous due to agricultural and industrial runoff. Certain cyanobacteria species can have mean doubling times as fast as 21 hours to 14.7 days. Stagnant water conditions leading to stratified water bodies and neutral to alkaline pHs also known to be favorable to cyanobacterial growth (Svrcek and Smith, 2004).

Figure 1-1 Bloom of the cyanobacteria *Microcystis aeruginosa* in Lake Erie in Oct 2011. (Lester, 2012)

1.1.2 Impacts of cyanobacteria

Blooms can abruptly interfere with an ecosystem's balance by reducing light penetration, out-competing other native organisms and depleting dissolved oxygen levels when the cyanobacteria die off and decompose (Svrcek and Smith, 2004; Miller, 2010). In addition, throughout their life cycle, cyanobacteria can release various metabolites which can contaminate

drinking water sources. These metabolites can be sources of nuisances or even dangers in the form of taste and odor compounds and toxins. (Chorus, 1999; Omur-Ozbek and Dietrich, 2005).

1.1.2.1 Surface Water Ecology

Some studies have shown that cyanobacterial toxins can inhibit aquatic invertebrate grazers which are key elements to fresh water eco-systems. The cyanotoxins have the ability to disrupt and destroy the digestive systems of daphnia and mosquito larvae. In a recent study by Rohrlack et al. (2005), daphnia were fed cyanobacteria of the *Microcystis* species, which are known to contain intracellular toxins. After 9 hours of ingestion, there was clear indication of the loss of cell to cell epithelial gut cell junctions and lowered physical activity. After 32-41 hours of exposure, convulsions occurred and finally death. However, it should be noted that not all zooplankton avoid cyanobacteria as some can actually be the dominating species benefiting from blooms (Oberholster et al., 2006).

1.1.2.2 Taste & Odor

Consumer acceptance of drinking water is a critical factor to the success of the drinking water industry. Taste and odor compounds released by cyanobacteria can cause significant problems for drinking water suppliers (Cook and Cook and Newcombe, 2002). Source waters contaminated with taste and odor compounds can result in customer dis-satisfaction and lead to perceptions of unsafe tap water (Omur-Ozbek and Dietrich, 2005; Zoschke et al., 2011).

1.1.2.3 Toxins

Cyanobacteria and their toxins have been found all across the world including Colorado (Oberholster et al., 2006). One of the earliest documentations of poisonings comes from China, approximately 1,000 years ago; General Zhu Ge Ling reported losing troops whom drank from a

green colored river that they crossed during a military campaign (WHO, 2003). Today, emerging concerns regarding to hepatotoxins and neurotoxins produced by certain species of cyanobacteria are making the headlines.

Cyanotoxin poisonings have been reported in 50 countries, including over 35 states within the US in 2011 (Graham et al., 2009). Many of these poisonings are a result of accidental ingestion by humans or animals from water bodies experiencing blooms. A recent incident of exposure occurred in August 2011 at Lost Creek Lake in Oregon, where a man was swimming in and occasionally gulping in the lake trying to fetch his boat out of the water. He later suffered from intense vomiting, diarrhea, vertigo, nausea and abdominal pains (Freeman, 2011). Due to adverse health effects of toxin exposure, the World Health Organization has set a drinking water guideline limit of 1 ppb (µg/L) for the cyanotoxin microcystin-LR (Chorus, 1999).

Photo Courtesy of Gretchen Bensen

Figure 1-2. Exposure may result in skin irritations, weakness, nausea and diarrhea (Svrcek and Smith, 2004; Lynne, 2011)

The most common pathway of human exposure to cyanotoxins is from accidental ingestion or contact with water containing cyanobacterial cells through recreational activities. Animals on the other hand, are more commonly affected by the cyanotoxin poisonings as humans tend to stay away or avoid water bodies with floating mats of cyanobacteria (Svrcek and Smith, 2004; Graham et al., 2009). Incidents of dog poisonings are common due to people taking them along during their recreational activities. In the state of Oregon, several reports of dog deaths related to cyanotoxins have been reported every year, including four from 2009 in the US. Wildlife are also not immune from the effects of cyanotoxins either, the mysterious deaths involving 11 sea otters in the Monterey Bay area in Northern California were determined to be microcystin toxicity related (Miller, 2010). The most severe documented case of cyanotoxin exposure in humans occurred in 1996 where renal dialysis patients in a hemodialysis center in Caruaru, Brazil were inadvertently provided with a dialysate using water drawn from a reservoir experiencing a bloom. All 126 patients became ill and 60 eventually died from complications of liver necrosis (Chorus and Bartman, 1999).

Photo Courtesy of Laura Elkomy

Figure 1-3. Pet symptoms can include, weakness, drooling, convulsions, vomiting and even death (Lynne, 2011).

With so many emerging incidents of cyanotoxin exposures, municipalities have begun to be more aware of cyanobacteria blooms and follow WHO guidelines to protect the public from

cyanotoxins. Higher levels of microcystins in source waters may be associated with cases of gastroenteritis and liver toxicity (Hitzfeld, et al., 2000; Haddix et al., 2007). Today over eight countries, including Australia, Brazil, Finland and the US, have established water quality and programs to address cyanotoxins levels (Svrcek and Smith, 2004). Since any freshwater body with the right conditions can harbor cyanobacteria, the distribution of states with reports or active programs to address cyanotoxins is widespread across the country. Cyanotoxins are currently on the EPA Contaminant Candidate List 3, which is a list of contaminants that are known to exist in public water systems, in which research is underway about potential national regulation (USEPA, 2011). Currently, there are over 9 states in the US, which have statewide and local monitoring programs for cyanotoxins in freshwaters. An additional 13 states have event based response procedures or public education materials posted on their websites (Graham et al., 2009). Cyanobacteria produce a variety of toxins including microcystins, nodularins, anatoxins, saxitoxins, cylindrospermopsin, and dermatoxic alkaloids (Svrcek & Smith, 2004).

1.1.2.4 Water Treatment & Disinfection By-products

Cyanobacteria can interfere with certain water treatment processes generally causing filter clogging (Shehata et al., 2008). Additionally, odor compounds and cyanotoxins may be released as the cyanobacterial cells are lysed, due to the addition of oxidative compounds such as chlorine during treatment, thus worsening the water quality (Matsushita et al., 2008). Disinfection byproducts (DBPs) are formed when organic material that is not removed during water treatment comes in contact with a disinfectant. Trihalomethanes (THMs) and haloacetic acids (HAAs) are common DBPs created in the water treatment process which pose regulatory and health concerns (Bruce et al., 2002). The US EPA (1998) set maximum contaminant levels (MCLs) for total THMs at 0.08 mg/L and HAAs at 0.06 mg/L, based on an annual average.

THMs and HAAs are environmental pollutants and many forms are carcinogenic. The release of algal organic matter (AOM), extracellular or intracellular, produces chloramines and halo-acetaldehydes in drinking water systems as the AOM ‘s are not removed by conventional treatment techniques (Fang et al., 2010). Hence organic metabolites released by the cyanobacteria may lead to increased disinfection by-product levels in the finished water.

1.2 Cyanobacterial metabolites

1.2.1 Geosmin

1.2.1.1 Occurrence

Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalhol) is one of the most common odorous algal metabolites which causes an earthy odor that leads to an unpleasant flavor in drinking water. Geosmin, shown in Figure 1-3, is not known to be harmful to humans, it is the primary odor compound found in sugar beets and can be produced by other microorganisms such as *streptomyces*, actinomycetes and myxobacteria (Giglio et al., 2008). Geosmin production by cyanobacteria is suspected to be a secondary metabolite of cellular growth. Through experiments conducted by Giglio et al. (2011), it is shown that geosmin production could be linked to isoprenoid production. Isoprenoids can range in function from production and processing of pigments, vitamins and other cellular building blocks (Giglio et al., 2011). Typical bloom concentrations of geosmin can range from 1-2 ng/L to up to 7,500 ng/L (Li et al., 2010).

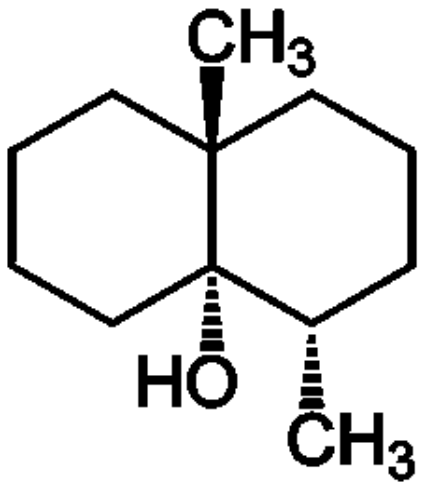

Figure 1-4. Chemical structure of geosmin

1.2.1.2 Detection Limit

The human odor detection threshold for geosmin is determined as 2 ng/L. The presence of geosmin in drinking water can adversely affect consumers' confidence and acceptance of their drinking water source and may drive customers to find alternative sources (Omur-Ozbek and Dietrich, 2005; Giglio et al., 2008). For these reasons billions of dollars are spent each year to remove the odorants from finished water. In reaction to customer complaints, South Korea and Japan has set guideline limits at 10 ng/L for geosmin in tap water (McGuire, 1995).

1.2.1.3 Properties

Geosmin is a tertiary alcohol, fairly water soluble and has a Henry's law constant (at 20 °C) of 0.0023; molecular weight of 182.31 g/mol; and water solubility of 150.2 mg/L (at 20 °C) (Bruce et al., 2002; Omur-Ozbek and Dietrich, 2005). Natural degradation of geosmin is relatively slow, around 3 days; the main path for removal is through microbial degradation

(Lawton et al., 2003). If water containing geosmin is stored with no headspace in dark and at 4 ^{o}C, geosmin can remain stable for up to a year (Brownlee et al., 2007).

1.2.2 Microcystin-LR

1.2.2.1 Occurrence

Microcystins are a common type of cyanotoxins that specifically targets liver cells. Microcystin-LR is among the most frequently detected and most toxic of the microcystin variants. Typical bloom concentrations in source waters range from 2 to 10 μg/L of microcystin-LR (Ho, 2011). Microcystin-LR was detected in all 33 Northeastern and Southeastern US water supplies studied during the summer of 2003 (Haddix et al, 2007). It has also been found to occur with other cyanotoxins so, in studying microcystins, other toxins may be addressed in the future studies. (Chorus, 1999; Triantis et al., 2010).

1.2.2.2 Properties

Since microcystins, see in Figure 1-4, are cyclic peptides, they are very stable and resistant to temperatures up to 300^{o}C, chemical hydrolysis or oxidation around neutral pH. They can range in molecular weights from 900 to 1100 da (Svrcek and Smith, 2004). There are over 60 known variants of microcystins. Microcystins may persist for months to years in a stable environment (Chorus, 1999). However, in natural waters microcystins can naturally breakdown due to biodegradation and photolysis with a half-life around one week (WHO, 2003). Cyanobacteria carrying the *mcy* gene have the potential to produce microcystins. It is believed that cyanotoxins may be produced in order to reduce predation from zooplankton (Oberholster et al, 2006; Rohrlack et al., 2005; Hawkins et al., 2005; Li et al., 2010; Dixon et al, 2010).

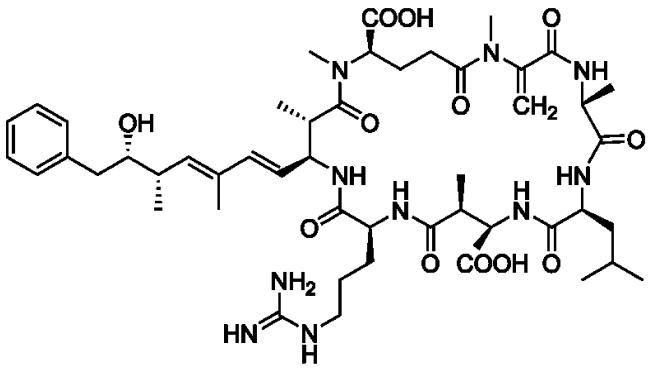

Figure 1-5. Chemical structure of Microcystin-LR

1.2.3 Geosmin and Microcystin-LR in cyanobacteria

Recently it was shown that toxins such as microcystins co-occur with taste-and-odor compounds such as geosmin (Graham et al., 2010). Cyanobacteria in the genera *Anabaena, Aphanizomenon, Lyngbya, Microcystis, Oscillatoria, Phormidium, Schizothrix* and *Symploca* (Fig 6), are all known geosmin producers. All, with the exception of *Symploca*, are known to be capable of producing cyanotoxins (Chorus, 1999). Since geosmin can be easily detected by the human nose, the surveillance of harmful toxins such as microcystin-LR may be easily performed due to the likely co-occurrences of the two types of metabolites.

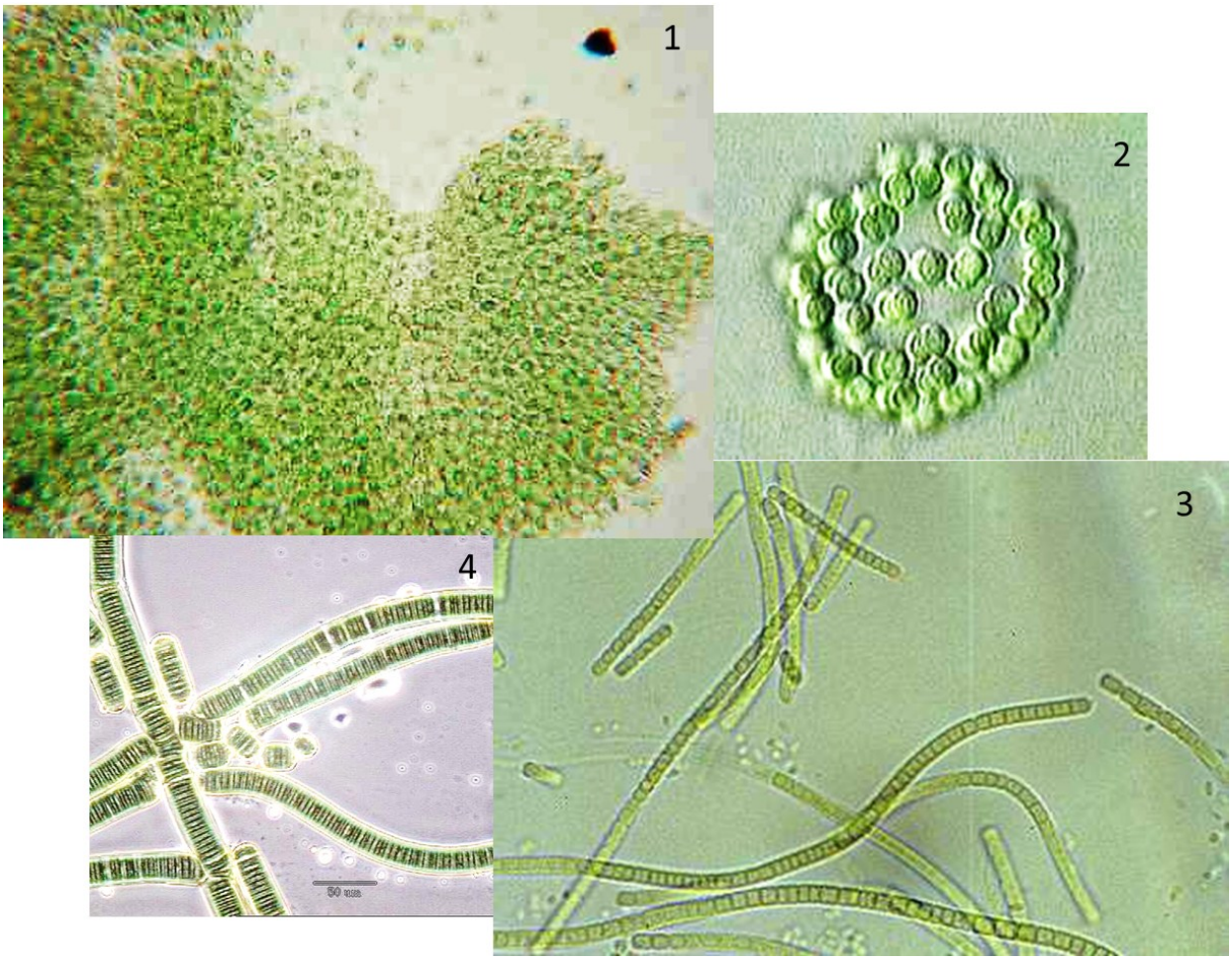

Figure 1-6. (1) *Microcystis spp.* (2) *Anabaena spp.* (3) *Phormidium spp.*(4) *Lyngbya spp*. (Schneegurt, 2001)

1.3 Detection methods of cyanobacterial metabolites

Since cyanobacterial metabolites can be detrimental at very low concentrations, advanced detection methods are required. In order to detect and quantify at such low concentrations the methods used must be specific to either physical separation and/or explicitly selective to the compound of interest.

1.3.1 Geosmin

1.3.1.1 Solid Phase Microextraction

The extraction of geosmin for detection and quantification first starts with solid phase micro-extraction (SPME) of the compound from either the headspace or from the liquid matrix of a sample. There are three phases involved in headspace sampling, fiber coating, headspace and sample matrix. Geosmin is diffused from the sample matrix to the headspace then finally through equilibrium diffusion into the fiber coating. Manual assemblies of SPME with extraction fiber coated with polydimethylsiloxane (PDMS, 100 um), Carboxen (Car)/PDMS 75 um, StableFlex divinylbenzen.

The conditions of diffusion during the SPME must be standardized to successfully predict the gas phase concentration of geosmin in the samples using Henry's law constants at the selected standardized conditions (Omur-Ozbek and Dietrich, 2005). Several parameters such as fiber coating, agitation and extraction temperature and time had to be standardized. Extraction of geosmin can be increased 1.2x by a salting out effect. Extraction efficiency is low at low pH but constant at pH of 4-8 (Saito et al., 2008).

1.3.1.2 Gas Chromatography/Mass Spectrometry

Gas chromatography is a highly selective test which can isolate volatilized geosmin in a mixture of gas through a silica capillary column of cross-linked DB-5. Injection and detector temperature is set at 280^{o}C and a column temperature at 190^{o}C for 2 minutes, increased to 270^{o}C at 10^{o}C/min. Inlet helium carrier gas flow rate at 1.43 ml/Min. In gas chromatography geosmin that is extracted from the SPME procedure mentioned above can be desorbed and detected through subjecting the fiber carrying the absorbed geosmin in a gas inlet of the GC column.

Ionization voltage is set at 70eV and ion fragments detect for geosmin occurred at 112 m/z (Matsushita et al., 2008; Saito et al, 2008).

1.3.2 Microcystin

1.3.2.1 Liquid Chromatography/Mass Spectrometry

Liquid chromatography (LC) is a highly selective test that separates the compound of interest in the liquid phase through a specialized column. Liquid chromatography was the detection method of choice when it comes to accuracy and sensitivity by Triantis et al, 2010, Hawkins et al, 2005, and Agrawal et al, 2010 among others. The stationary phase is composed of octadecyl carbon chains bonded silica column (C18), which was chosen depending on the polarity of the compound to allow microcystin to be retained longer for a better resolution (Figure 1-7). The column temperature is set at 30 ^{o}C. For microcystin-LR, a mixture of high purity water which is the polar solvent and acetonitrile which is a non-polar solvent, both containing 0.5% acetic acid are used as the mobile phases. A gradient program starts at 75% of the polar component in the mixture then to 30% of polar in 9.5 minutes then 10 for the polar component holding for one minute (Triantis et al., 2010).

Microcystin-LR is detected from multiple reaction ion monitoring using the two most intense and characteristic precursor/product-ion transitions obtained from the MS-MS procedure. The effluent from the LC is then ionized and the liquid phase is vaporized. The vapor is accelerated into fragmentor and a mass filter which selects for certain target masses at 213 m/z to detect the amount of microcystin-LR that has gone through. Additionally, to confirm microcystin, microcystin-LR is fragmented and mass filtered again at 135 m/z to give two peaks, this is called a daughter fragment (Hawkins et al., 2005).

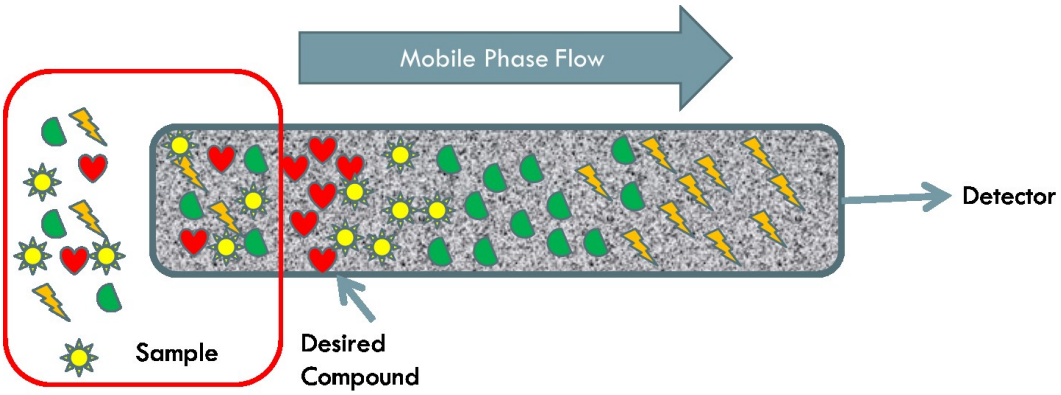

Figure 1-7. How liquid chromatography works.

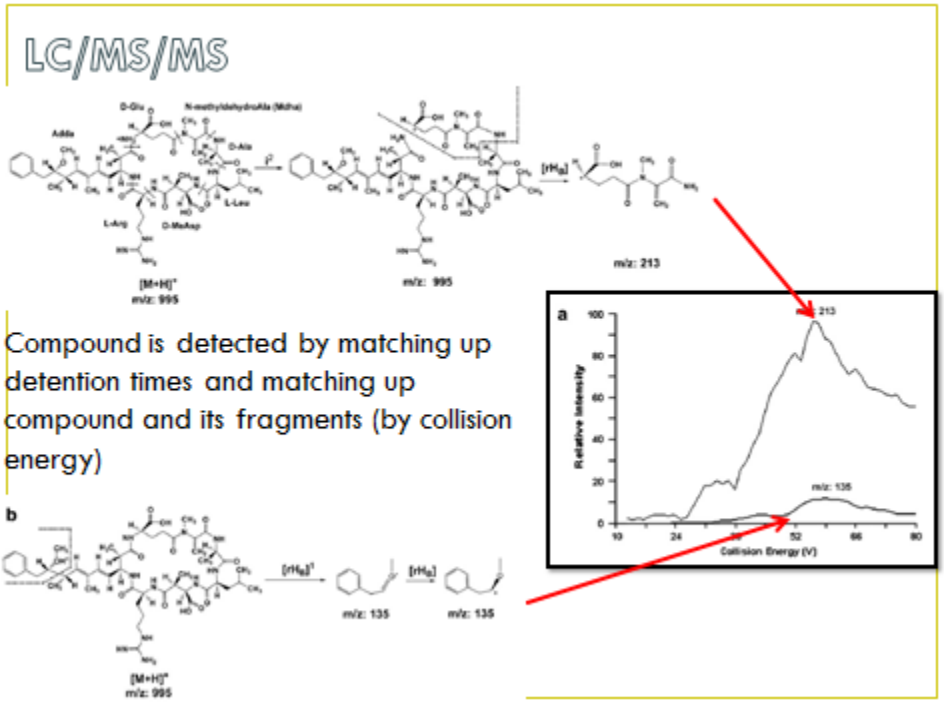

Figure 1-8. LC/MS/MS

1.3.2.2 Liquid Chromatography/ UV Photodiode Array

Microcystin has to be purified by solid phase extraction before subjected to detection using the photodiode array. Once microcystin is separated by the LC at a known time, a UV source is set to emit at the compounds' maximum absorbance frequency. The detection experiences a drop in UV radiation which is proportional to the concentration of microcystin-LR present. The photodiode array detector is set at 238 nm in a thermostated column. Acetonitrile

and water containing 0.05% trifluoroacetic acid are used as the mobile phases (Triantis et al., 2010).

1.3.2.3 Enzyme Linked Immunosorbant Assay

The enzyme linked immunosorbant assay (ELISA) is a low cost, rugged, and simple way to detect for microcystins for both environmental and laboratory applications. The ELISA testing kit is a good, quick and inexpensive way to screen many samples at once for microcystin-LR before confirmation and quantification with more advanced methods. The assay requires very little processing of samples only requiring simple filtration or dilutions if concentrations are suspected to be beyond the specific kit's detection limit. ELISA is a good way to screen environmental samples for the presence of microcystins due to its specificity for the compound type, low cost and fast turnaround time (Hawkins et al., 2005; Triantis et al., 2010).

As seen in the Figure series 1-9 the well plates are lined with anti-rabbit IgG polyclonal antibodies that are raised to bind microcystins and a microcystin-enzyme conjugate. There are the same number of antibody binding sites in each well allowing the same number of microcystin-enzyme conjugate enzyme molecules to bind to the antibodies. Once the conjugate binds and the reaction proceeds, a blue color appears. Conversely, if there is a high concentration of microcystins, there will be fewer microcystin-enzyme conjugate molecules bound to the antibodies thus a lighter blue color indicating the presence of microcystins. Cross reactivity could occur if variants of microcystins and nodularins are present. Nodularins are another type of cyanotoxin. The detection limit of the assay is 0.1 μg/L of Microcystin-LR.

There are however a few limitations on the assay in the form of accuracy and false positives. Low concentrations of microcystins (< 0.1 μg/L) can be difficult for the ELISA assay

to accurately measure concentrations. False positives may arise due to cross reactivity between similar toxin variants or structures similar to the toxin of interest. In a study done by Triantis et al (2010), by evaluating ELISA kits available to detect and quantify microcystins, 17% of samples reported false positives. There can also be high variability between selected ELISA kits for microcystin detection/quantification. Recovery rates ranged from 73%-189% between two kits tested by Triantis et al. (2010). Commercial ELISA kits can successfully detect microcystins in both raw and finished water even though microcystin concentrations may be low (Haddix et al., 2007).

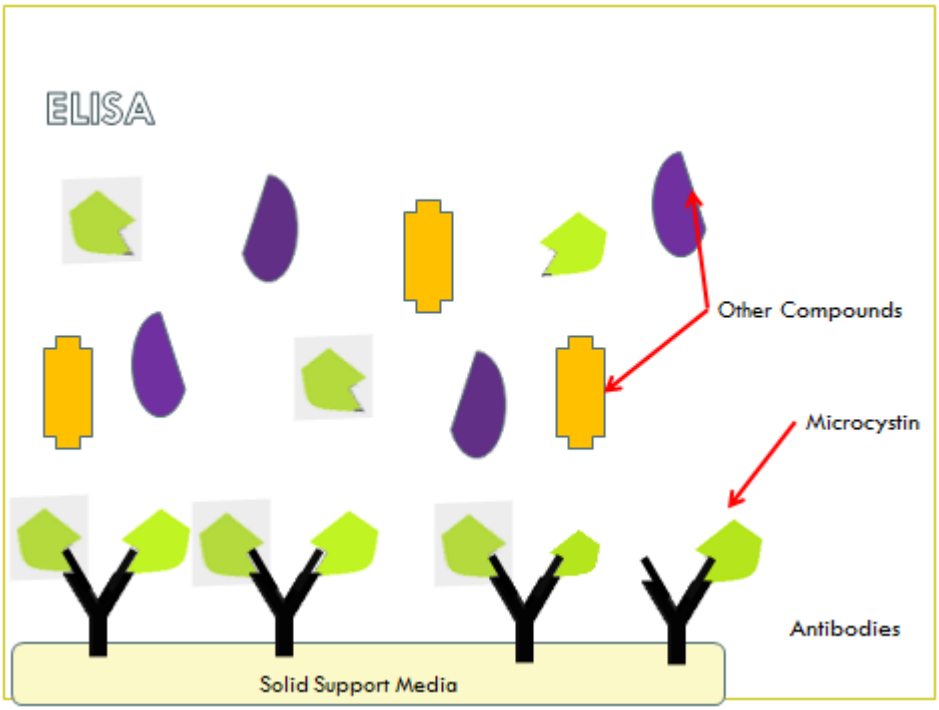

Figure 1-9a. Antibodies in media are specific only to microcystin compounds

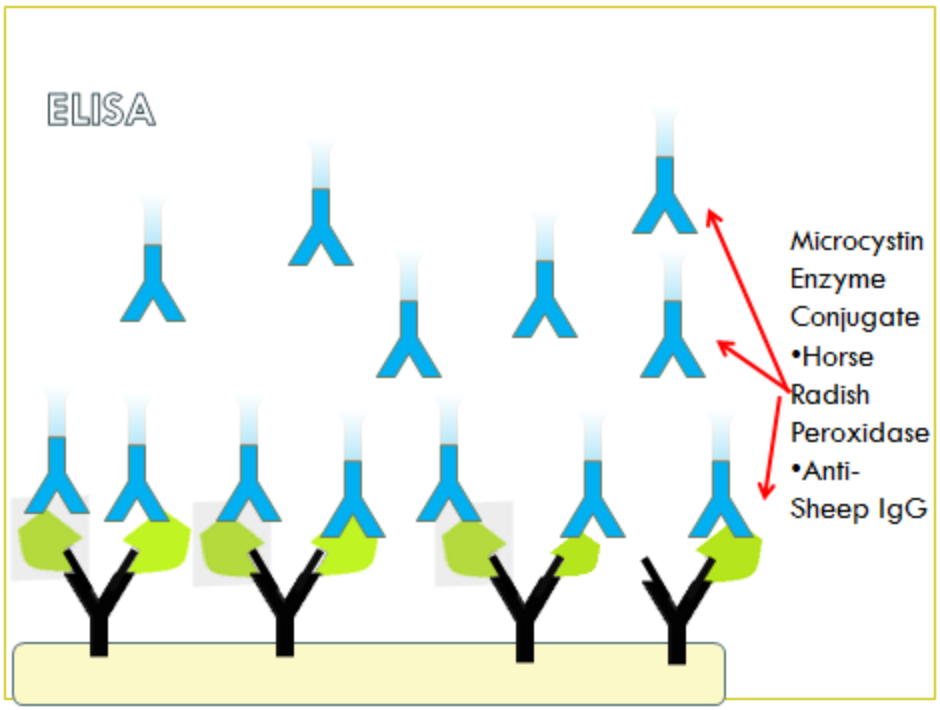

Figure 1-9b. A conjugate to microcystin is then attached to prepare for an indicator substrate.

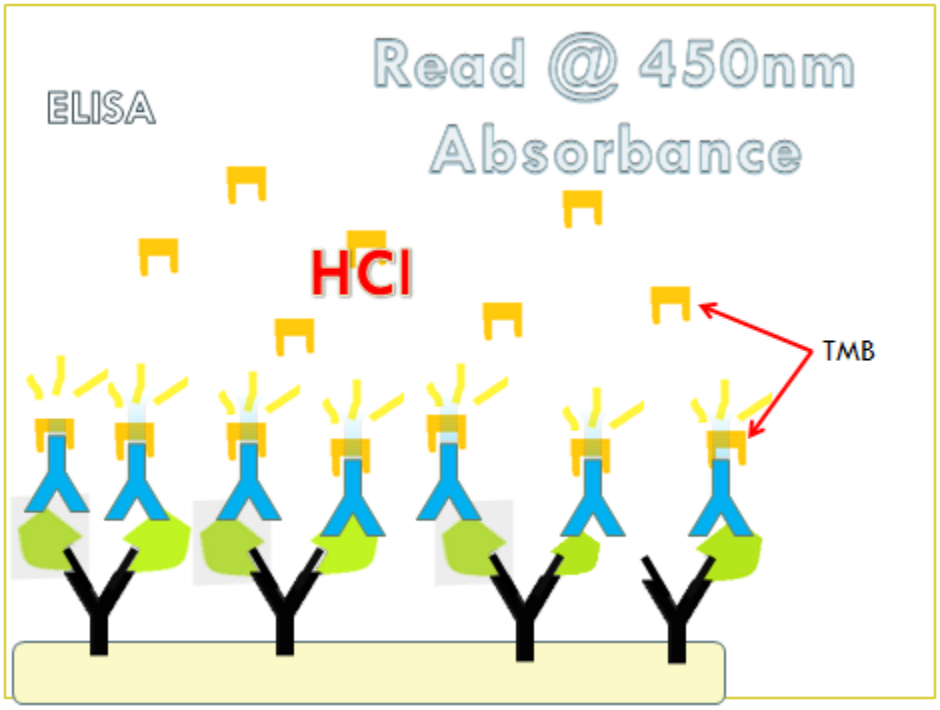

Figure 1-9c. An indicator substrate Tetramethylbenzidine is attached to allow adsorbance.

1.3.2.4 Protein Phosphatase Inhibition Assay

The protein phosphatase inhibition assay (PPIA) is a good indicator of hepatotoxicity and tumor promotion of compounds. Microcystins and other cyanotoxins such as nodularins inhibit serene and threonine phosphatase enzymes that are found in liver tissues. These enzymes can play a key role in tumor suppression in the liver and with their inhibition toxic effects can be observed such as apoptosis and necrosis. The assay can provide bioactivity of the cyanotoxins since detection is based on the functional activity of the toxins rather than the recognition of their chemical structure.

Like ELISA, the PPIA can be done in micro-wells but with protein phosphatase enzymes as the reactive protein. The active substrate is either P-nitrophenyl phosphate or the microcystin compound. Without the presence of microcystins, the P-nitrophenyl phosphate substrate binds to the enzyme to carry out a hydrolysis reaction, which is indicated by a color development. Conversely, if microcystin is present, the enzyme is inhibited and there is less or no color development.

The PPIA is fast, cost effective and easy to use. However an extensive amount of work for reagent preparation is required as commercial kits are not available. PPIA also cannot provide accurate quantitative results and not all microcystin variants react with protein phosphatase enzymes to a similar extent so the assay is strictly only for detection of microcystins (Triantis et al., 2010).

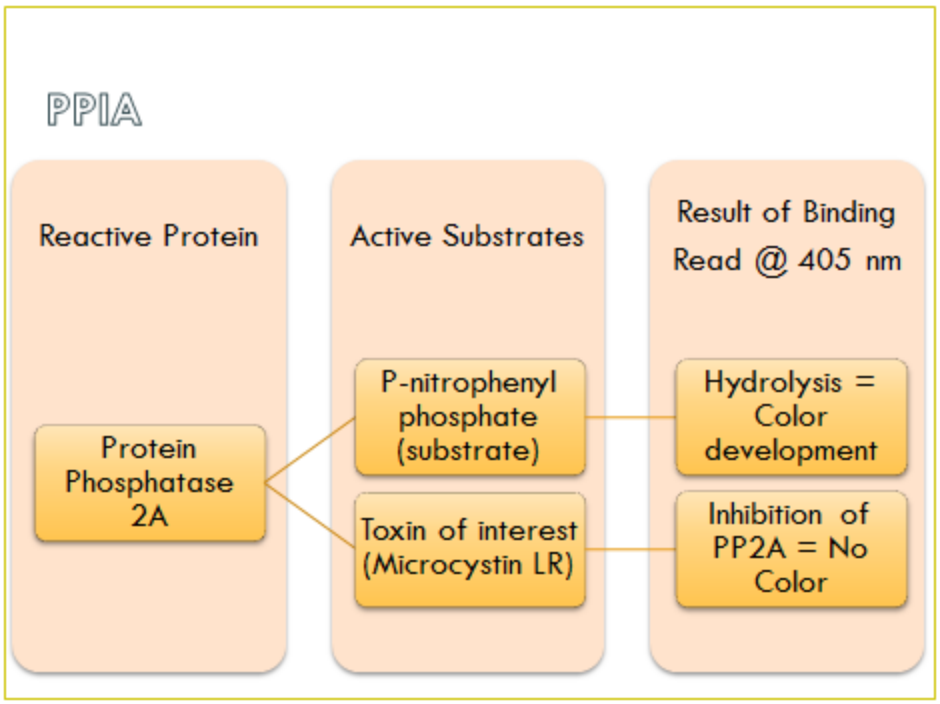

Figure 1-10. PPIA Assay

1.3.3 Integrated method analysis

In order to detect and quantify the microcystin-LR in surface waters, an integrated multi-method laboratory system is necessary to optimize reliability, efficiency and cost. Research by Hawkins et al. (2005) and Triantis et al. (2010) developed an analytical strategy to provide cost effective and validated results. Both studies proposed using ELISA method to first screen environmental samples for microcystins followed by a confirmation step using another detection method. The ELISA test was determined to be most rugged as it required the least amount of sample processing which is why the study used it in all of their sample matrices. However, the ELISA method had the largest range of percent recovery which is related to its cross reactivity of similar compounds as seen in Figure 1-11. The costs associated with the detection and quantification of microcystins can be significant and sometimes very high depending on the

types of tests ran. The following are the average cost rankings per sample for microcystins: PPIA<ELISA<LCMS<HPLC. Proper uses of different toxin analysis techniques was laid out by Triantis et al, 2010 to maximize cost effectiveness and accuracy depending on the circumstance and sample type.

Analytical Process	Detection Limit	% Recovery Range
ELISA Kit A	0.147 ug/L	73-93%
ELISA Kit B	0.087 ug/L	133-189%
PPIA	> 1 ug/L (0.2 ug/L)	N/A
HPLC/PDA	0.25 ug/L	71-102%
LC/MS/MS	0.002-0.017 ug/L	69-113%

Figure 1-11. Method result characteristics.

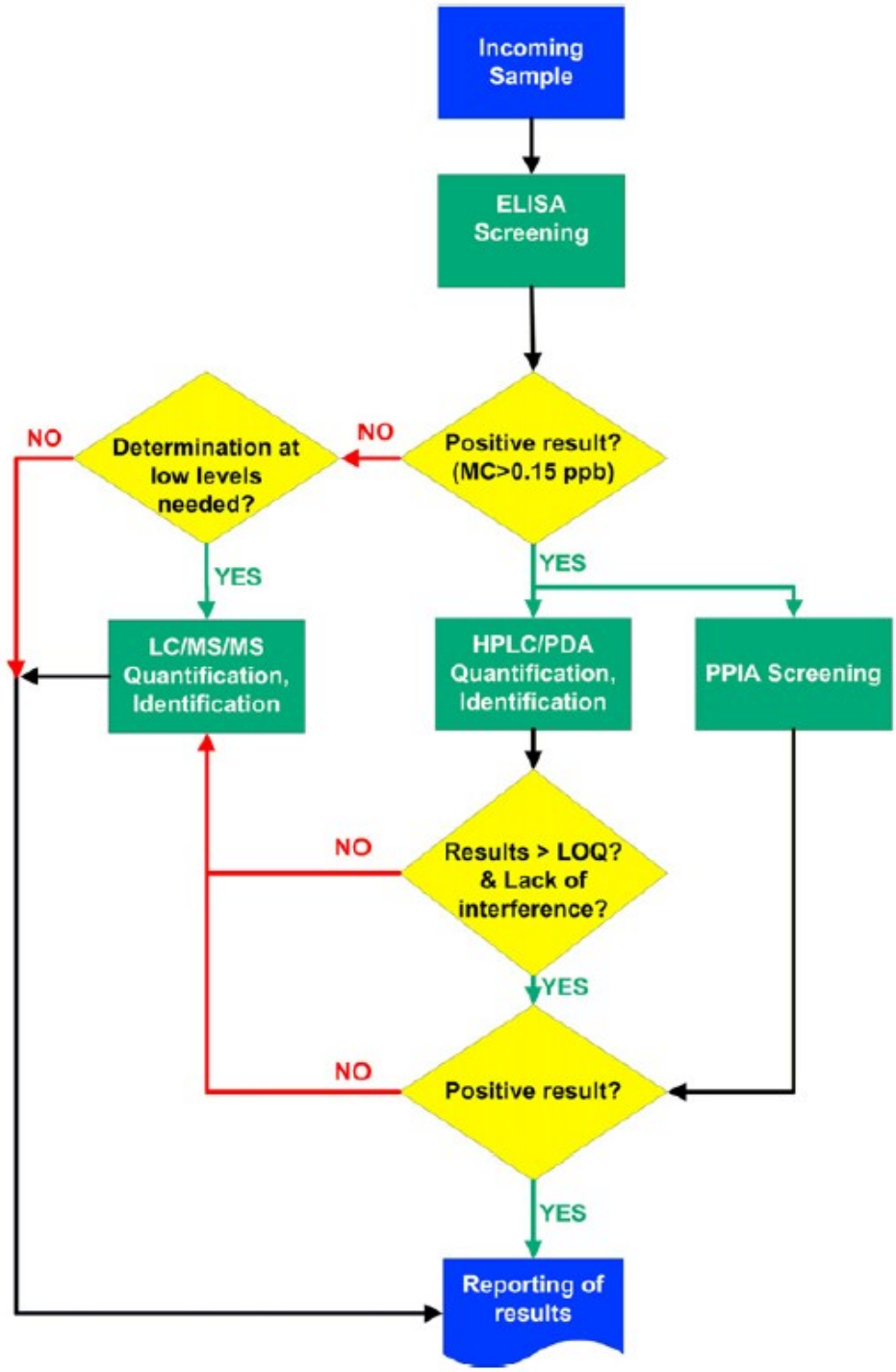

Figure 1-12. Analytical Protocol for analyzing microcystins developed by Triantis et al. 2010.

1.4 Treatment of cyanobacterial metabolites

Most cyanobacteria metabolites are stored intracellularly and are released when the cell is lysed (Giglio et al., 2008; Graham et al., 2010; Dixon et al., 2010). Mitigation methods involving destroying the cyanobacteria during full bloom conditions may lead to the release of these metabolites and hence result in peaked concentrations of cyanotoxins and taste-and-odor compounds in the water. There were many instances when the efforts of controlling blooms with copper sulfate to kill off the cyanobacteria resulted in elevated fish and human related poisonings in Australia and Brazil (Chorus, 1999).

1.4.1 Conventional treatment

Traditional conventional water treatment methods including coagulation/flocculation with chemicals or polymers and rapid/slow sand filtration has been proven to be only effective in removing cyanobacterial metabolites stored inside cyanobacterial cells themselves (Hargesheimer and Watson, 1996). Cyanotoxins on the other hand are ineffectively removed by these methods. The exception to this would be slow sand filters which develop a biofilm specifically targeted for cyanotoxin treatment (Holst, 2003). Removal rates up to 90% could be achieved through laboratory studies but the methods were ultimately unfeasible as cyanotoxin release events are short lived and do not provide enough time for biofilm layers to optimize for cyanotoxin removal (Svrcek and Smith, 2004). The occurrence of bacteria with the capacity to degrade microcystin is strictly limited in freshwaters, especially with waters which have had no previous experience with microcystins (Holst et al., 2003).

Heavy chlorination using aqueous chlorine and calcium hypochlorite has been proven to remove around 95% of microcystins with a 30 minute full strength contact. However, this

treatment method is highly variable depending on pH and cyanotoxin concentrations. High removal rates were only achieved through very high concentrations of microcystin-LR in mg/L concentrations (Svrcek and Smith, 2004). Chlorination may also result in harmful chlorination by-products (Hitzfeld et al., 2000).

1.4.2 Powdered Activated Carbon

PAC has been commonly used for the removal of organic compounds. PAC treatment methods can significantly reduce toxicity and odors by actually removing the detrimental cyanobacterial metabolites from the water source (Hitzfeld et al., 2000; Matsushita et al., 2008; Li et al., 2010). Activated carbon is a highly porous adsorptive medium with a complex structure which houses a high pore volume giving it its large surface area for contaminants to sorb onto (Norit Americas, 2009). Activated carbons for drinking water treatment are typically made from coal, coconut or wood sources. Different sources of carbon determine the pore structure distribution among the activated carbons (seen in Figure 1-13). Each type of activated carbon has different adsorptive capabilities that can sometimes be customized to be somewhat selective for specific contaminants (Cook and Newcombe, 2002).

Raw material	Activation	Pore Structure		
		Micro	Meso	Macro
Bituminous Coal	Steam	High	Low	Medium
Lignite	Steam	Medium	High	High
Peat	Steam	High	Medium	High
Wood	Chemical	High	Very High	High

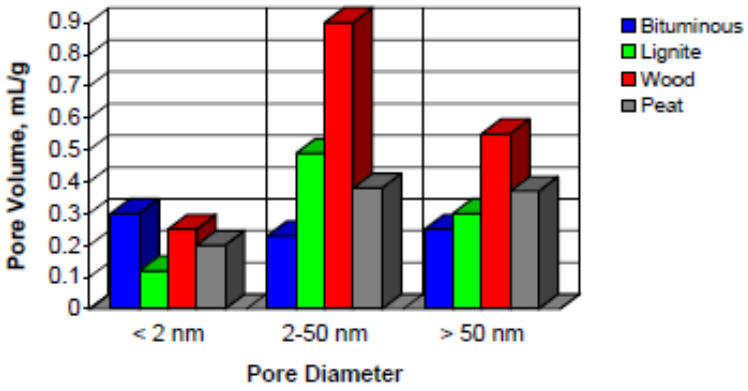

Figure 1-13. Pore Volume distribution from Norit Americas (2009).

Adsorption onto the activated carbon surface can be driven by physical forces such as van der Waals or chemical sources of polarity. Since the metabolites of interest are comprised of various peptides, it is suggested that adsorption will be due to hydrophobic, electrostatic and dispersion forces (Pendleton, 2001). Diffusion effects, pore size, surface chemistry, surface area volume, pH, temperature and concentration can also affect the efficiency of adsorption. Generally, non-polar organic species can be efficiently absorbed by activated carbon (Norit Americas 2009).

Figure 1-14. Powdered Activated Carbon

The advantage to using PAC is that it can be easily incorporated into existing plant operations and can be applied only when there is need to treat for organic compounds such as cyanotoxins and odors (Cook and Newcombe, 2002). Typically water treatment plants can apply up to 50 mg/L of PAC without affecting downstream processes (Ho et al., 2011). The dosing variability is highly dependent on the ratio of micro, meso, macro pore structure of the activated carbon (Westrick and Szlag, 2010).

In a recent study by Ho et al. (2011) it was determined that negligible differences in removal rates occur while using PAC contact times above 30 minutes to remove microcystin-LR from reservoir water. This suggests the removal of microcystins to be more PAC dosage dependent than contact time dependent because the kinetics of adsorption are relatively rapid. It was concluded that over 96% removal of microcystin was achievable with 50 mg/L of PAC after 30 minutes of contact time. Another study reported that up to 98% microcystin removal can be achieved with wood based activated carbon with a 30 minute contact time. Only 60% removal of microcystin is observed when coconut based activated carbon is used (Svreck and Smith, 2004). Compared to microcystins, geosmin is a flatter and smaller molecule which allows it to enter smaller pores by diffusion. It was found that adsorbed amount of geosmin increased after 24 hours compared to the 30 minute studies (Ho et al. 2011). Geosmin is relatively resistant to

higher natural organic matter (NOM) concentrations when laboratory studies were compared with source water studies indicating that typical conventional treatment only affects larger and less adsorbable NOM components which are irrelevant for competitive adsorption against geosmin (Zoschke et al., 2011). Li et al.(2010) suggested that that a PAC dosage of 80 mg/L could treat a bloom containing 473 ng/L of geosmin below 10 ng/L. This was observed when the waterworks at Qinhuangdao city had to control a severe release of geosmin in the drinking water. In a laboratory study done by Park et al. (2010) 85% of geosmin was removed by PAC within 60 minutes.

Hydrodarco ® B from Norit Americas was the selected PAC for this research project because it is commercially available to remove taste and odor compounds such as geosmin, as well as other organics from potable water sources. Hydrodarco ® B is produced by steam activation of lignite coal under carefully controlled conditions. It is finely milled to have a high degree of suspension and high capacity for adsorption.

1.4.3 Membranes

Advanced treatment methods involving membranes through ultrafiltration and nano-filtration can be effective in removing up to 95 to 100% of microcystins and taste and odor compounds (Alvarez et al., 2010; Omur-Ozbek and Dietrich, 2005). Membranes utilize low molecular weight cut off pore sizes to only allow water and other small molecules to pass through while retaining larger molecules (Hitzfeld et al., 2000). Low pressure membranes capable of microfiltration and ultrafiltration are successful in removing cyanobacterial cells. High pressure membranes involving reverse osmosis and nanofiltration can effectively remove up to 99% of cyanotoxins.

A few of the disadvantages of membranes involve dealing with the cyanobacteria cells themselves. Pretreatment is often required and filtration through membranes can rupture cyanobacteria cells thus releasing intracellular metabolites. It was found that under high pressure, intracellular geosmin was not released in static conditions but was released in dynamic conditions (Matsushita, 2008). Backwashing of membranes for removal of cyanobacteria can be problematic as live cells may be trapped inside the pores causing the flux of the membrane to be reduced. Membranes have a high capital cost and require highly trained operators to ensure the integrity of the membrane operations making it difficult to adopt this method for algal metabolite removal (Svrcek and Smith, 2004).

1.4.4 UV

Ultraviolet photolysis is a widely used pathogen reducing process. The ultraviolet energy can also cause one or more bonds to break in a molecule without the addition of chemicals. In experiments by Tsuji et al. (1995), ultraviolet radiation dosages of 1530 mJ/cm2 to 20,000 mj/cm^2 could degrade microcystin-LR to a similar degree of photolysis in nature. These ultraviolet dosages however are several orders of magnitude higher than what is practical for water treatment plants (Svrcek and Smith, 2004).

1.4.5 Ozone

Ozonation has been one of the predominant methods for disinfection or the removal of organic compounds responsible for color, taste and odor problems. Ozonation contact times are relatively short compared to chlorination and activated carbon with average times around 10 to 20 minutes (Hitzfeld et al., 2000). Ozone has been found to completely remove up to 5 mg/L of

microcystin-LR at an ozone dosage of 2 mg/L (Al Momani and Jarrah, 2010). However other studies reported removal rates that were highly variable from 0% to 100%. This is due to ozonation conditions including temperature, pH, ozone dosage and most importantly the presence of organic matter. Like chlorination, ozonation relies on the oxidation of the compound (Alvarez, 2010). If oxidation of microcystin-LR is not complete, the free and intact toxic side chain of the microcystin-LR molecule can become more toxic. The side chain, Adda side chain, is responsible for the toxicity associated with cyanotoxins. Only complete cleavage of the Adda side chain results in reduced or absence of toxicity (Al Momani and Jarrah, 2010; Svrcek and Smith, 2004; Lawton and Robertson, 1999). Another issue is the conversion of odorous compounds into a displeasing "plastic-like" odor which can be as undesirable as the original odor compound (Hargesheimer and Watson, 1996).

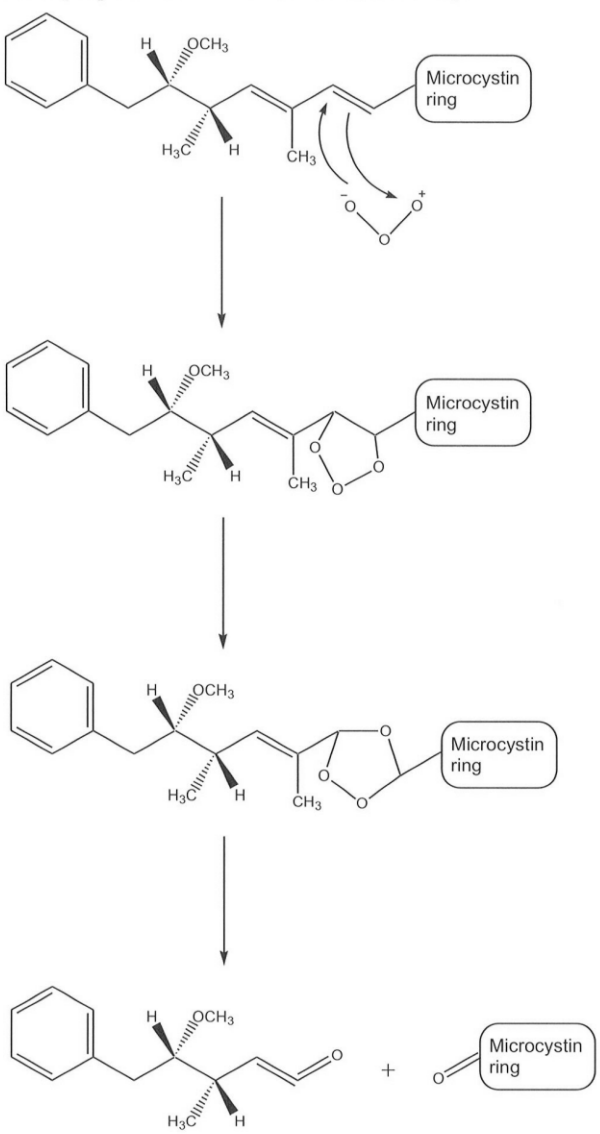

Figure 1-15. Ozone decomposition pathway.

1.4.6 Moringa Oleifera Coagulant

The water purifying attributes of the *moringa oleifera* tree seed (seen in Figure 1-16) have already been established as an effective and low cost method to treat water in many economically disadvantaged countries. The *moringa oleifera* tree is native to western and sub-Himalayan tracts, India, Pakistan, Asia Minor, African and Arabia. It is now cultivated in

Southeast Asia, Central America, North and South Americas and the Caribbean Islands (Farooq et al., 2006). The crushed seeds are a viable replacement for synthetic chemicals used in a water treatment process called coagulation and flocculation.

Figure 1-16. *Moringa oleifera* seeds in their husks

During coagulation and flocculation, contaminants are neutralized and mobilized so they are able to adhere to each other to form larger and denser particles that can be settled or filtered out (Davis, 2008). The aqueous solution of the *moringa oleifera* seed is a heterogeneous mixture of various functional groups, mainly comprised of low molecular weight amino acids. These amino acids generate a negatively charged atmosphere which is key in the coagulation process. The proteins have an approximate molecular weight of 1300 Da and an iso-electric point between 10 and 11 (Anwar et al., 2007). Coagulation mechanisms involved in this case include adsorption and sweep coagulation (Fig. 1.17). The moringa coagulant is aided by dissolved cations, in this case calcium cations, to form a net structure to induce sweep coagulation and flocculation (Okuda et al., 2001).

The coagulant is a low cost and low technology way for developing countries to treat their source waters during algal bloom events. The moringa oleifera coagulant has been more efficient in removing algal cells compared to alum. Up to 97% of algal cells in all algal groups

observed from raw Nile river water without pre-chlorination in a study done by Shehata et al. (2008).

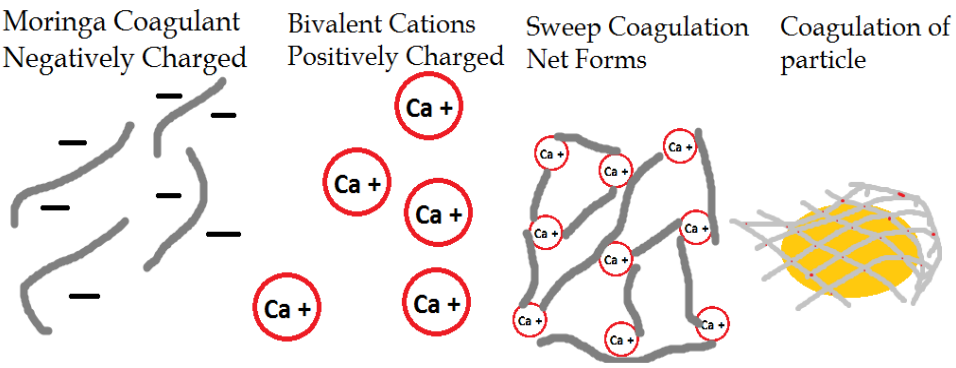

Figure 1-17. Coagulation mechanism of the moringa coagulant (Adopted from Okuda et al. 2001)

The crushed moringa seeds also have anti-microbial and anti-cyanobacterial properties. Dosages of 20 to 160 mg/L of the seed can inhibit replication of bacteriophages by damaging bacterial membranes. This activity is attributed with the compound 4(α-L-rhamnosyloxy) benzyl isothiocynate (Anwar, 2007; Lurling, 2010). Optimal dosage for microbial inhibition was determined as 40 mg/L of the moringa oleifera seed.

1.5 Toxicity Analysis

1.5.1 Toxicity of microcystin-LR

Microcystin-LR is a highly toxic compound with an oral LD_{50} of 5,000 µg/kg in mice (WHO, 2003). Microcytins are primarily hepatotoxins that can cause severe liver damage characterized by liver cell cytoskeleton damage, a loss of sinusoidal structure, intrahepatic hemorrhaging, haemodynamic shock, formation of hepatic tumors, apoptosis, vacuolization and

progressive liver necrosis. Higher doses can lead to death occurring in as little as a few hours to a few days of ingestion (Chorus, 1999; 2003; Svrcek and Smith, 2004; Agrawal et al., 2006).

1.5.1.1 Acute exposure

Effects of acute exposure to cyanotoxins on humans have been recorded around the world, including developed countries such as the US and Australia where cyanobacterial toxins were ingested through contaminated water supplies. These supplies were not monitored properly for cyanobacteria blooms and resulted in public poisonings. Children are particularly at risk because they can drink a higher volume of water in proportion to their body weight than adults (WHO, 1999). Symptoms of poisoning through ingestion included cases of severe gastro-enteritis and hepatoitis. Histomicrographs of mouse livers exposed with bloom extracts demonstrated tissues engorged with blood and grossly distended cells, indicating apoptosis (Agrawal et al., 2006). An epidemiologic study found that patients in India who frequently bathed in waters infested with cyanobacteria developed acute rhinosporidiosis, which is an infection of the mucous membranes (Agrawal et al., 2006).

A significant cyanobacteria related acute exposure case occurred in 1988 in the Paulo Afonso region of the Bahia State in Brazil. 2,000 gastro-enteritis cases followed over a 42 day period, of which 88 resulted in death. It was later found that cyanobacteria of the *Anabaena* and *Microcystis* genera were present in the Itaparica Dam reservoir at the time of the poisonings and concluded to be the source of the epidemic (Chorus, 1999).

Figure 1-18. Dying fish in a reservoir experiencing a bloom in Loveland, CO.

1.5.1.2 Chronic exposure

Chronic lower dose exposure to cyanobacterial toxins may result in carcinogenesis and tumor growth promotion. China has one of the highest incidents of hepatocellular carcinoma, which may be caused by cyanotoxin exposure. In Quidong county in China, residents who have been drinking surface water with average microcystin-LR concentrations of 0.1 μg/L reported higher frequencies of liver cancer compared to residents drinking from well water (Alvarez et al., 2010). Another study conducted in Florida reported that there was an increased risk for primary hepatocelluar carcinoma for residents living in the service area of water treatment plants drawing from surface waters with a history of blooms compared to groundwater users (Bean et al., 2002). After ingestion of microcystin-LR, the toxin is transported across the ileum into the bloodstream to the liver where it is taken up by hepatocytes. It is generally up-taken through the adenosine triphosphate transport system of the cell (Hitzfield et al., 2000). Microcystin-LR is a potent inhibitor of eukaryotic protein serine/threonine phosphates 1 and 2A. Protein phosphatases serve as enzymes that play an important regulatory role in maintaining homeostasis in the cell (Chorus, 1999; Chorus and Bartman 2003). Protein phosphatases can regulate cellular processes such as

cell cycle progression, proliferation, protein synthesis, muscle contraction, carbohydrate metabolism, transcription, cytokinesis and neuronal signaling. If any of these processes malfunction, tumor growth and even cellular death may occur through apoptosis (Ayllon, 2001).

1.5.1.3 Mechanism of action

The effects of microcystins are strictly organ and cell specific, targeting primary hepatocytes. The cell specificity is not due to metabolic activation in the liver cells but to the specific pathway of uptake. Microcystins have been shown to be taken up through the multi-specific transport system for bile acids, which is also an entrance pathway for several xenobiotic substances. The in-vitro effects of microcystins on hepatocytes can be inhibited by low concentrations of bile acids or bile acid transport inhibitors. Only primary hepatocytes and intestinal cells are equipped with this bile acid transport system (Chorus, 1999).

The Adda side chain, which is a unique amino acid only to cyanotoxins, is suspected to be responsible for inhibiting protein phosphatases (Hitzfield, 2000). The individual amino acids of microcystin-LR may be seen in Figure 1-20

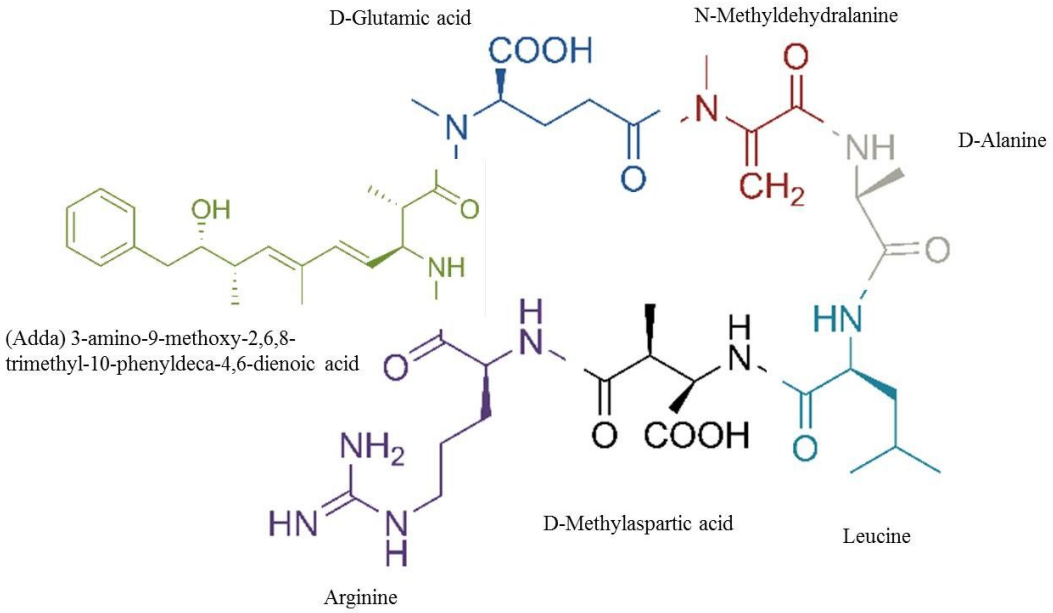

Figure 1-19. Amino acids that make up microcystin-LR (Adopted from Schneegurt 2001)

Apoptosis is characterized by cell shrinkage, chromatin condensation, plasma membrane blebbling, vacuolization, oligonuclesomal DNA fragmentation, and breakdown of the cell into smaller pieces (Chen et al., 2005). Even at smaller doses of microcystin-LR, apoptosis occurs through the BID-BAX-BCL-2 regulatory protein pathway (Chen et al., 2005). Proteins in the BCL-2 gene family are potent regulators of apoptosis that can influence the permeability of the outer mitochondrial membrane. This can protect various cell types from death induced by growth factor deprivation, heat shock and viral agents (Ayllon, 2001). BCL-2 proteins contain both pro and antipoptotic members that elicit opposing effects on the mitochondrial membrane including the anti-apoptotic protein BCL-2 and pro-apoptotic proteins BAX and BID. Essentially the BID-BAX-BCL-2 pathways are responsible for homeostasis regulation of the cells (Akcali et al., 2004).

The up-regulation of BCL-2 is a host response to toxin exposure, protecting the host cell from destruction by apoptosis. Upon apoptosis, as seen in Figure 1-20, BAX oligomerizes with

BAK into large complexes that form pores in the lipid bilayer and facilitate the release of cytochrome C and other factors. BAK is another pro-apoptic member of the BCL-2 gene family. Thus, the up-regulation of BAX at relatively low doses of microcystin-LR could initiate the consequent apoptoic events with BID serving as an initiator of BAX.

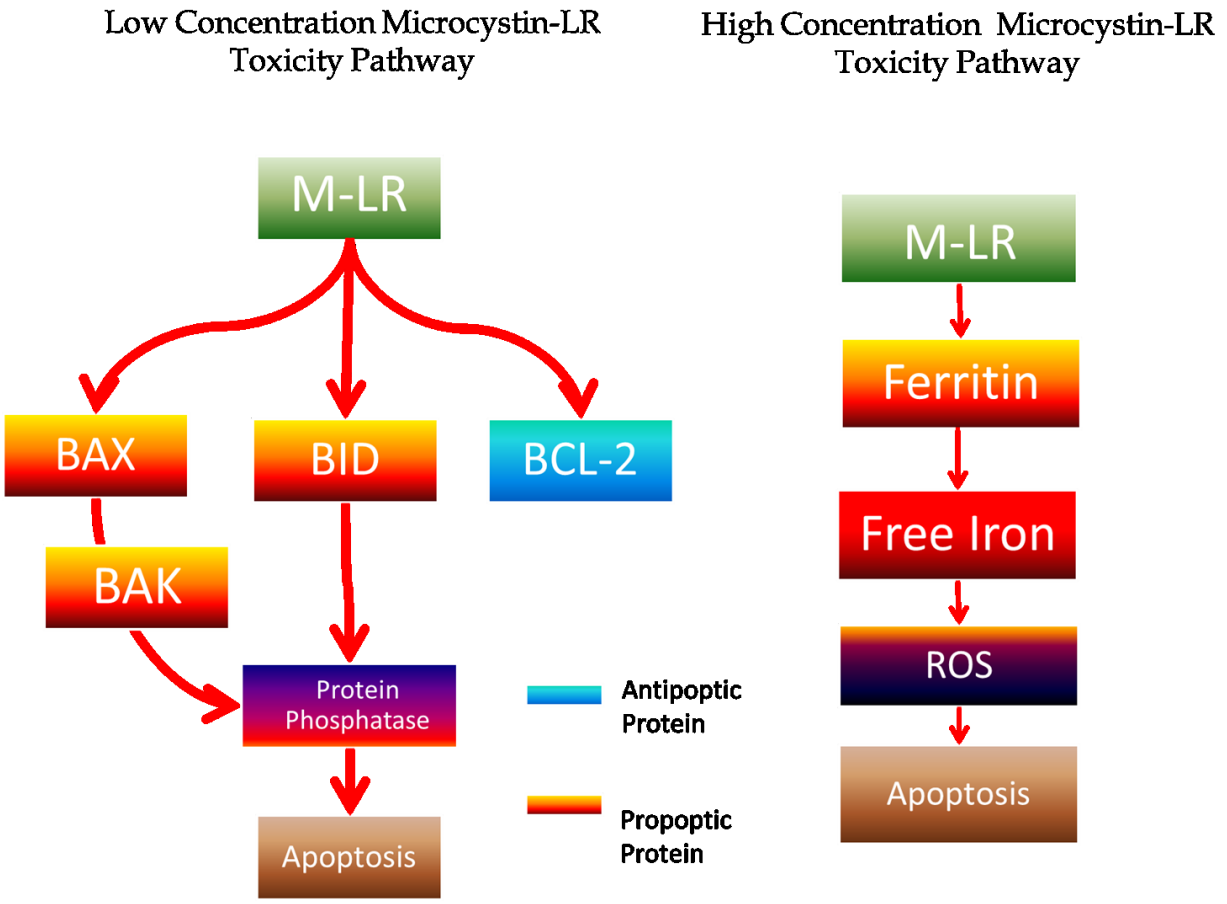

Figure 1-20. Microcystin-LR toxicity pathways (Adopted from Chen 2005).

At high concentrations of microcystin-LR, the expression of BAX can remain high but the expression of BID and BCL-2 will decrease. The main promoter and inhibitor of mitochondrial apoptosis pathway becomes disabled causing difficulty for the BCL-2 family to control the process. The reactive oxygen species pathway then dominates apoptosis during high

concentrations of microcystin-LR. In the reactive oxygen species pathway, the protein ferritin is the major iron binding protein limiting the catalytic availability of iron for participation in oxygen radical generation. Ferritin functions as the primary storage for intracelluar iron. The up-regulation of ferritin can be induced by microcystin-LR thus, allowing a higher concentration of iron to be present.

The increased concentrations of cellular iron can catalyze redox reactions, enabling iron to generate radical species from a Fenton reaction, $Fe^{2+} + H_2O_2 => Fe^{3+} + HO* + OH^-$. Reactive oxygen species causes an increase in cytosolic calcium and has been observed in cells undergoing oxidative stress. This leads to mitochondrial membrane rupture, massive intrahepatic hemorrhaging and damage to the cytoskeleton (Ding et al, 2001; Chen et al., 2005). Carcinogenesis of tissue could occur from repeated damage, by chronic exposure to microcystin-LR, through apoptosis or reactive oxygen species (Bean et al., 2002). This damage can be characterized by damage to the cytoskeleton which is a major role in hepatotoxicity (Ding et al., 2001).

1.5.2 Cell culturing

The H4IIE rat hepatoma cell line is used in many *in vitro* tests for cytotoxicity screening and testing. Typically the cells are used to detect and semi-quantify specific contaminants and classes of contaminants from the environment. H4IIE cells can be sensitive to environmental and experimental conditions so it is important to regulate and standardize growth factors and growth conditions. Use of H4IIE cells can have a powerful predictive ability in terms of risks to organisms namely liver toxicity (Whyte and Tillitt, 2004). Cells can be plated into plates or flasks in 10% fetal bovine serum. Unattached dead cells can be washed with a saline buffer such as HEPES or PBS (Ding et al, 2001).

1.5.3 Quantification

Cytotoxicity assays can be quantified through the sulforhodamine B (SRB) colorimetric assay. The assay is commonly used to predict pharmacokinetic behavior, target organ toxicity, or specific effects such as genotoxicity (Fricker, 1994). The assay is based on the measurement of cellular protein content. This is a robust assay with a stable colorimetric end point which gives relatively linear results in respect to cell counts over a range of 5×10^3 to 10^5 cells. The assay requires simple equipment and inexpensive reagents (Vichai and Kirtikara, 2006).

The SRB assay involves fixing living cells with trichloroacetic acid solution (TCA) for 30 minutes and washing under tap water 5x to remove dead cells. 0.1% sulforhodamine in acetic acid is used as the stain which is applied for 15 minutes. Cells are then washed 4x with acetic acid. Once stained the, cells are then solubilized with Tris base and read through a plate reader at 540 nm adsorbance. The plates can be stored for several weeks at room temperature before adsorbance is measured (Fricker, 1994, Swennen et al., 2010).

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2.0 The occurrence and removal of cyanobacterial metabolites Microcystin-LR and Geosmin from source waters by Powdered Activated Carbon.

2.1 Introduction

The occurrence of cyanobacteria blooms has become more frequent throughout the world due a combination of warmer climates and an abundance of available nutrients such as nitrogen and phosphorous. Cyanobacteria can produce several metabolites that can be causes of nuisances (taste and odor compounds) or cause health concerns (toxins). The most commonly produced and detected metabolites in surface waters are microcystin-LR (a hepatotoxin) and geosmin (taste and odorant) (Chorus and Bartman, 1999; Omur-Ozbek and Dietrich, 2005; Haddix et al., 2007; Dixon et al., 2010). In the environment, most of the cyanobacterial metabolites are stored intracellularly and are released when the cells lyse at the end of their life-cycle or by other means (e.g. algicides) (Giglio et al, 2008; Graham et al, 2010; Dixon et al, 2010).

Multiple studies have linked chronic microcystin-LR exposure to an increased risk of hepatocellular carcinoma by comparing users of surface water containing the toxin and ground waters in which the toxin is absent (Alvarez et al., 2010, Bean et al. 2002). The most common pathway of human exposure to cyanotoxins is from accidental ingestion or contact with water containing cyanobacterial cells through recreational activities. Recreation victims have reported symptoms including intense vomiting, diarrhea, vertigo, nausea and abdominal pains (Freeman, 2011).

Due to adverse health effects of toxin exposure, the World Health Organization(WHO) has set a drinking water guideline limit of one ppb (μg/L) for the cyanotoxin microcystin-LR,

which is the most potent of the microcystin variants (Chorus, 1999; WHO, 2003). Cyanotoxins are also on the EPA Contaminant Candidate List 3 (USEPA, 2011).

Unlike microcystin-LR, geosmin has no known detrimental effects on humans, nor is its presence in drinking water regulated in the US (Omur-Ozbek and Dietrich, 2005). Geosmin is an earthy smelling compound commonly produced by cyanobacteria, actinomycetes and myoxbacteria (Giglio et al., 2008). At concentrations as low as 2 ng/L, geosmin can impart an unpleasant odor and flavor to drinking water (Omur-Ozbek and Dietrich, 2005; Giglio et al., 2011). Geosmin can therefore adversely affect consumer confidence and acceptance of their drinking water. For these reasons, billions of dollars are spent each year to remove the odorants from finished water. In response to customer complaints, South Korea and Japan have set guideline limits at 10 ng/L for geosmin in tap water (McGuire, 1995).

Mitigation methods involving destroying the cyanobacteria during full bloom conditions may lead to the release of these metabolites and hence can result in increased concentrations of cyanotoxins and taste-and-odor compounds in the water body (Chorus and Bartman, 1999; Dixon et al 2011). Conventional water treatment methods including coagulation/flocculation and sand filtration have been proven only effective in removing cyanobacterial cells (Hargesheimer and Watson, 1996). The dissolved metabolites, microcystin-LR and geosmin on the other hand, are not effectively removed by these methods (Svrcek and Smith, 2004; Lawton and Robertson, 2003). Slow sand filtration has shown some success in removing cyanobacterial metabolites through developing a biofilm layer specifically to treat for the metabolites. Unfortunately, metabolite release events are short-lived and do not provide enough time for the biofilm layers to optimize and target cyanobacterial metabolites (Holst et al., 2003, Svrcek and Smith, 2004).

Advanced treatment practices can be very effective in removing both microcystin-LR and geosmin however; there are also disadvantages associated with operations, feasibility or cost related to them. Ozonation and chlorination can remove up to 100% of both microcystin-LR and geosmin. However, removal rates are highly variable depending on optimal conditions regarding pH, temperature, oxidant dosage and most importantly the presence of organic matter (Alvarez, 2010). In addition, only complete cleavage of the microcystin-LR molecule can result in reduced or absence of toxicity, otherwise toxicity still is present or can even be magnified (Lawton 1999, Svrcek and Smith, 2004, Al Momani and Jarrah, 2010). Similarly, regarding geosmin, ozonation can convert odorous compounds into a displeasing "plastic-like" odor which can be as undesirable as the original odor compound (Hargesheimer and Watson, 1996).

Powdered activated carbon (PAC) is commonly used for the removal of organic compounds in water treatment processes. PAC treatment methods can significantly reduce toxicity and odors by removing the detrimental cyanobacterial metabolites from the source waters (Hitzfeld et al., 2000; Matsushita et al., 2008, Dixon et al., 2010). PAC can be both cost effective and feasible to be incorporated into many existing water treatment processes without significant modifications to plant operations. PAC can also be applied whenever a metabolite release event occurs (Westrick and Szlag, 2010).

Understanding the occurrence of these metabolites is the first step in mitigating contaminated waters. To understand their occurrence in Northern Colorado, environmental sampling was investigated on local rivers, lakes and municipal waters. The next objective of this study focused on effectiveness of PAC treatment on co-removal of microcystin-LR and geosmin at different concentrations below WHO standards and human detection limits through jar testing.

2.2 Materials and Methods

2.2.1 Reagents and Supplies.

The high purity toxin microcystin-LR (CAS 101043-37-2) was purchased from Cayman Chemicals (Ann Arbor, MI). Geosmin (CAS 16423-19-1), ammonium formate in 0.01% (v/v) formic acid(A) and acetonitrile 0.01% (v/v) formic acid in LC grade water was purchased from Sigma Aldrich (Pittsburg, PA). Optima grade methanol (CAS 67-56-1), 2,4,6-trichloroanisole, and sodium chloride was obtained from Fisher Scientific (Hampton, New Hampshire). The microcystin plate kit was obtained by Beacon Analytical Systems Inc. Cat# 20-0068(Saco, ME). Standard solutions of microcystin-LR were prepared in methanol at 20 and 5 mg/L, 40, 20, 10, 5, 1, 0.5 and 0.2 µg/L. Standard solutions of geosmin were prepared in methanol at 4 and 0.04 mg/L, 25, 10, 5, and 1 ng/L. The lignite coal based Hydrodarco-B (PAC) was supplied by Norit America Inc. (Marshall, TX). The PAC solution was prepared using nanopure® water at 2 g/L. The PAC dosages tested were 10, 20 and 30 mg/L, 1-L Pyrex beakers, Whatman glass fiber filters and 40 ml amber glass (VOA) vials were purchased from Fisher Scientific Inc. Solid-phase microextraction (SPME) fibers (65µm PDMS/DVB cross-linked) and holders were purchased from Supelco (Bellefonte, PA).

2.2.2 Water Samples

Raw Horsetooth Reservoir water used in the experiments was collected from the Fort Collins Water Treatment Facility (FCWTF) located in Fort Collins, CO, in 4 liter amber glass jugs and stored at 4^{o}C until use. Water quality parameters such as pH, DO, conductivity, and TOC were given by FCWTF monitoring instruments at the time of sample collection. Environmental samples were collected from the Poudre River, the Big Thompson River, high mountain lakes in the Colorado Rocky Mountains, water bodies in the cities of Fort Collins and

Loveland, and areas east of interstate 25 in Northern Colorado. Grab samples were collected during the summer and fall of 2011 in 40 ml amber glass vials, headspace free and stored at 4^{o}C in dark until analysis.

2.2.3 Jar Tests

1-liter glass beakers were filled with raw water and spiked with geosmin and microcystin-LR to achieve levels that correspond to common bloom events. Total microcystin-LR concentrations tested were 2, 4, 6 and 10 μg/L and total geosmin concentrations tested were 10, 20, 30 and 50 ng/L for the jar tests. A series of six jars were simultaneously run on a Phipps & Bird™ gang stirrer equipped with 3 inch x 1 inch stainless steel paddles. The solutions in the beakers spiked with geosmin and microcystin-LR, were mixed at 200 rpm for 15 seconds before the experiments began to ensure proper mixing of the algal metabolites. The PAC was then added to achieve a concentration of 10, 20, or 30 mg/L. Then the jars were mixed at 50 rpm for 30 minutes. After the mixing, the solutions were allowed to settle for 30 minutes. Samples were drawn from each jar at approximately 1.5 inches below the water surface and filtered through 0.45 μm glass filter into in 40 mL amber glass vials for storage at 4^{o}C for analysis. Gross water quality analyses were immediately conducted for the filtered samples. Temperature, conductivity and pH were measured by a Hach® sensIon 156 multi-parameter meter. Alkalinity and hardness were measured by Hach® Alkalinity AL-AP and 5B Hardness kits respectively. Microcystin-LR and geosmin analyses were performed within three days of sample collection.

2.2.4 Enzyme Linked Immunosorbant Assay (ELISA) for Microcystins

In order to detect and quantify microcystin-LR in surface waters, a two-step method was used as suggested by Hawkins et al. (2005) and Triantis et al. (2010). As a first step, the Enzyme Linked Immunosorbant Assay (ELISA) was used to screen environmental samples for

microcystins. Positive samples from the ELISA were later confirmed for microcystin-LR through liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS).

The ELISA test kit was used to screen environmental samples for the presence of microcystins for its specificity, low cost and fast turnaround time (Hawkins et al., 2005; Triantis et al., 2010). The detection limit of the assay is 0.1 µg/L of Microcystin-LR. Environmental samples were filtered through a 0.45 µm glass filter to measure dissolved cyanotoxin concentrations specifically. The experimental method outlined by the supplier was followed to perform the ELISA testing. After processing the samples according to the Beacon ELISA kit, the resulting plate was read on a FLUOstar Omega Plate Reader at 450 nm.

2.2.5 LC/MS/MS Analysis for Microcystin-LR

Microcystin-LR from the jar tests and environmental samples with positive ELISA results were detected and quantified by liquid chromatography-mass spectrometry-mass spectrometry (LC/MS/MS) following the method adopted from Triantis et al. (2010). The LC/MS/MS was performed on an Agilent 1290 UPLC coupled with an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization source in the positive mode (Agilent, Santa Clara, CA). Microcystin-LR was separated on a Zorbax Eclipse Plus C18 column (2.1 x 50mm, 1.8 um particle size) at 30^{o}C. A sample volume of 20 µl was injected and a binary mixture of ammonium formate in 0.01% (v/v) formic acid(A) and acetonitrile 0.01% (v/v) formic acid in LC grade water (Sigma, Pittsburg PA) (B) at a flow rate of 0.4 mL/min was passed through the column. The solvent gradient was 15% B at start, increased to 50% at 3 minutes and increased again to 90% at 5 minutes. The ionization source conditions used were as follows: nebulizer gas flow of 8 L/min at 20 psi. Sheath gas temperature was set at 400^{o}C and sheath gas flow was set to 10 L/min. The optimized fragmentor was set at 190 V with a cell

accelerator voltage of 7 V. The precursor ion was set at 995.6 m/z and product ions of 135.1 and 107.0 m/z per produced with a collision energy of 90 V. The collection and processing of chromatograms was performed by using the Agilent MassHunter software (v B.04.01). The detection limit of microcystin-LR was at 0.2 ug/L.

2.2.6 Solid Phase Microextraction coupled with GC/MS for Gesomin Analysis

The headspace solid phase micro-extraction (SPME) coupled with GC/MS was adopted from procedures laid out by Omur-Ozbek and Dietrich (2005) and Saito (2008) for geosmin analysis. For every sample 20 mL of experimental solution was placed in 40 mL amber glass vials. Standard curve solutions were prepared at 1, 5, 10 and 25 ng/L geosmin. Every vial received 4g of NaCl and were spiked with 2,4,6-trichloroanisole TCA to make up a concentration of 10 ng/L to serve as an internal standard. The vials were then heated to 75^{o}C and were equilibrated for 10 minutes before the SPME fiber was exposed to the headspace of the vials for 20 minutes. After the extraction the compounds on the fiber were desorbed at the GC inlet for 2.5 minutes. An Agilent 5890 gas chromatograph (GC) equipped with an Agilent DB-5 MS (30 m, 0.25 mm i.d., 0.25 um) column connected to an Agilent 5973 mass spectrometer (MS) were used for geosmin analysis. The mobile phase consisted of helium gas at 145 kPa and a flow rate of 1.6 ml/min. The GC inlet was set to 250^{o}C, pressure of 100 kPa (at 124^{o}C) in the GC oven. The mass spectrometer was set for selected ion monitoring at 112 and 125 m/z, to increase sensitivity of detection for geosmin. The internal standard, TCA was set to selected ion monitoring at 195 m/z. The detection limit of geosmin was at 0.5 ng/L.

2.3 Results and Discussion

Water quality parameters were measured before and after jar tests were conducted. Table 2-1 shows the raw Horsetooth Reservoir water characteristics. Temperature data were recorded right before experiments started. The data for post jar testing indicated no significant changes for hardness, alkalinity, pH, temperature and conductivity.

Table 2-1. Horsetooth Reservoir Water Quality Parameters

Parameter	Minimum	Maximum	Average	Standard Deviation
Hardness (mg/L $CaCO_3$)	34.2	51.3	36.2	6.14
Alkalinity (mg/L $CaCO_3$)	30.0	45.0	36.0	3.30
pH	7.10	7.69	7.39	0.170
Turbidity (NTU)	0.940	3.64	2.47	0.730
Dissolved Oxygen (mg/L)	3.39	10.6	7.52	2.43
Total Organic Carbon (mg/L)	3.30	4.00	3.73	0.180
Conductivity (μS/cm)	45.0	78.3	63.2	8.49
Temperature (^oC)	15.1	22.6	19.4	1.59

2.3.1 Geosmin and Microcystins in Samples from Surface Waters in Northern Colorado

A total of 52 sites were sampled in the Northern Colorado area from May through October of 2011. Each site was sampled at least twice, in May and August. Other samples in which microcystins or geosmin were found were sampled two more times in September and October. As seen in Figure 2-1, 56% of the samples contained detectable levels of geosmin or

microcystins. Of the detectable metabolites, geosmin was detected in 63% of the metabolite positive samples.

Geosmin levels ranged from 0.70 to 19.95 ng/L with the peak occurring in the month of June. The largest concentration of geosmin was detected on a slow moving section of the Poudre River near Interstate 25.

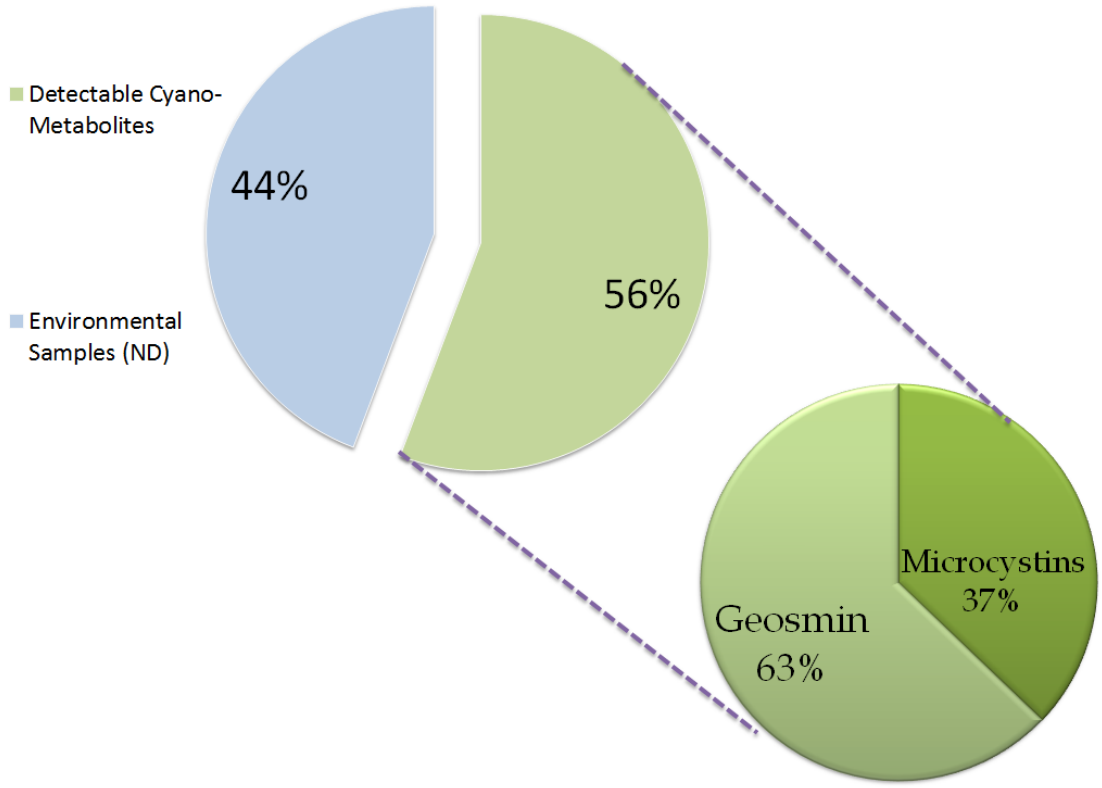

Figure 2-1. Environmental sampling results.

Microcystins detected ranged from 0.110 to 3.64 µg/L. However, the Barr Lake sample site was the only site to be confirmed to have detectable levels of specifically microcystin-LR and geosmin. Microcystin-LR concentrations were confirmed through using LC/MS/MS. Microcystin-LR levels ranged from 0.830 to 1.43 ug/L in Barr Lake, with the peak concentration occurring during June of 2011. Geosmin at 1.10 ng/L was also detected at Barr Lake during the microcystin-LR spike. Other sites with positive indications of microcystins did not have any

detectable levels of microcystin-LR through LC/MS/MS which may suggest the presences of other variations of microcystins the presence or nodularins which is another type of cyanotoxin.

85% (11 of 23) of the sites which microcystins were detected also had detectable levels of geosmin which supports the findings of Graham et al. (2010) where microcystins are commonly found with taste-and-odor compounds. However, sites with detectable levels of geosmin were found more frequently than sites with microcystins. This may be attributed to the lack of the sensitive detection of diverse cyanotoxins and taste and odor compounds in this study or the dependent on the particular species of cyanobacteria that thrive in the study area.

2.3.2 Removal of Geosmin and Microcystin by Powdered Activated Carbon

2.3.2.1 Microcystin-LR Removal

The removal of microcystin-LR by PAC, with a contact time of 30 minutes and 30 minutes of settling time, resulted in average removal rates ranging from 71% to 94% as seen in Figure 2-2. The highest average removal rates of microcystin-LR was achieved by 20 mg/L and 30 mg/L dosages of PAC which had relatively similar removal rates. The smallest average percent removal of microcystin-LR of 74% was observed with a dosage of 10 mg/L of PAC to treat 2 µg/L of microcystin-LR and a 94% removal was observed with 20 mg/L of PAC to treat 4 µg/L of microcystin-LR. Up to 100% removal of was achieved when observing individual experiments contributing to removal averages. The removal rates are comparable to previous studies done by Donati et al. (1994) and Ho et al. (2011) where up to 98% of microcystin was removed by 25 mg/L of a wood based PAC and 96% removal was achieved with a 50 mg/L dose of PAC with a contact time of 30 minutes for both cases.

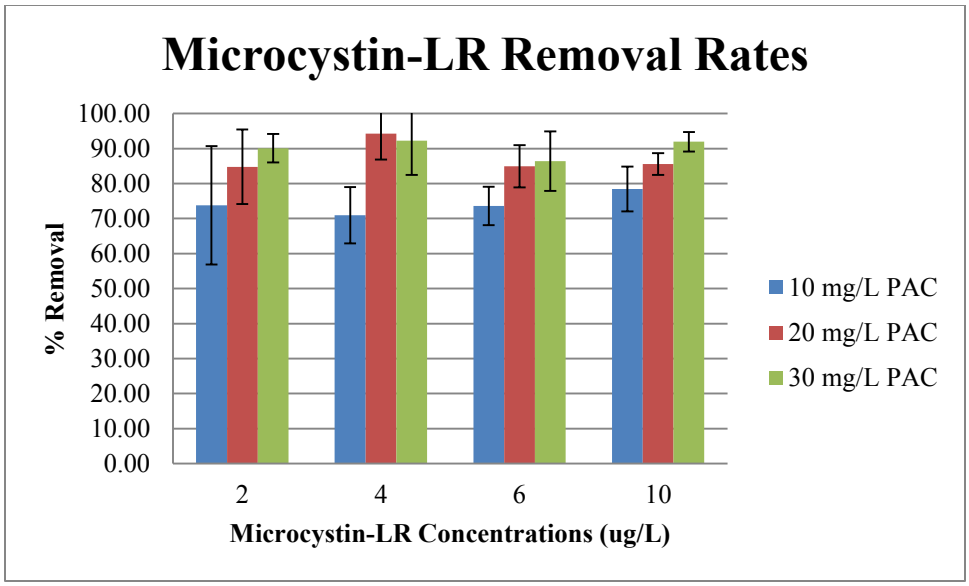

Figure 2-2. Average microcystin-LR removal rates according to PAC doses.
Average error ranged from 3.08 to 16.9%

The removal percentage of microcystin-LR was mostly dependent on the dosage of PAC used, as the kinetics of adsorption was rapid, highlighted by Ho et al. (2011). A competitive adsorptive effect between microcystin-LR and geosmin were observed in some experiments. The removal of lower concentrations of microcystin-LR with the lowest dosage of PAC at 10 mg/L was most affected by the presence of low concentrations of geosmin. Up to a 20% decrease from average removal rate was seen in the combination with concentrations of microcystin-LR at 2 µg/L and PAC at 10 mg/L as seen in Figure 2-3. The presence of low concentrations of geosmin adversely affected the removal of microcystin-LR. Experiments with higher concentrations of microcystin-LR(4 to 10 µg/L) and high PAC dosages were more resistant to fluctuations with presence of geosmin as seen by Figure 2-4.

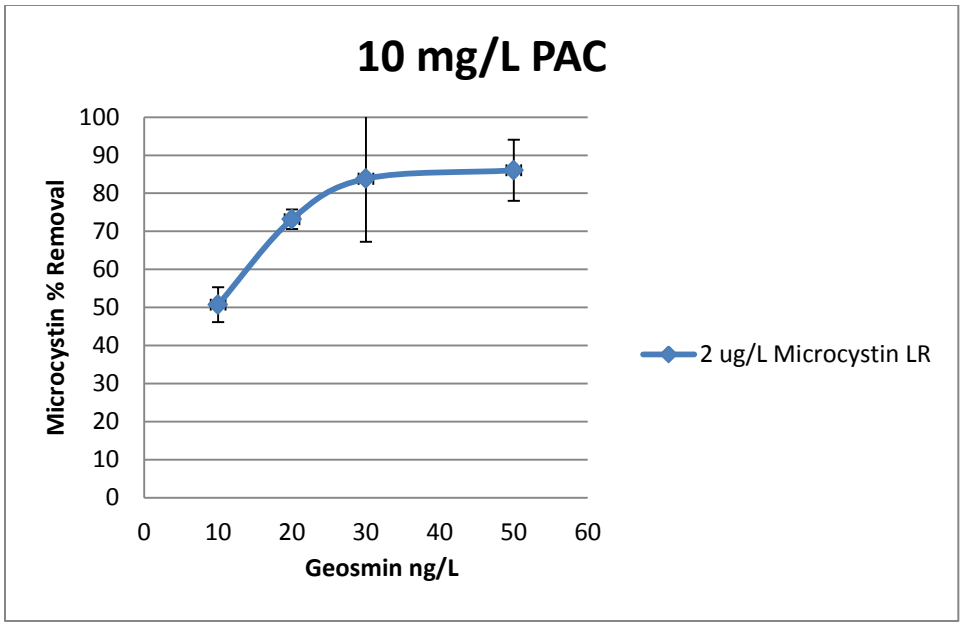

Figure 2-3. The presence of geosmin affecting microcystin-LR removal. N=6

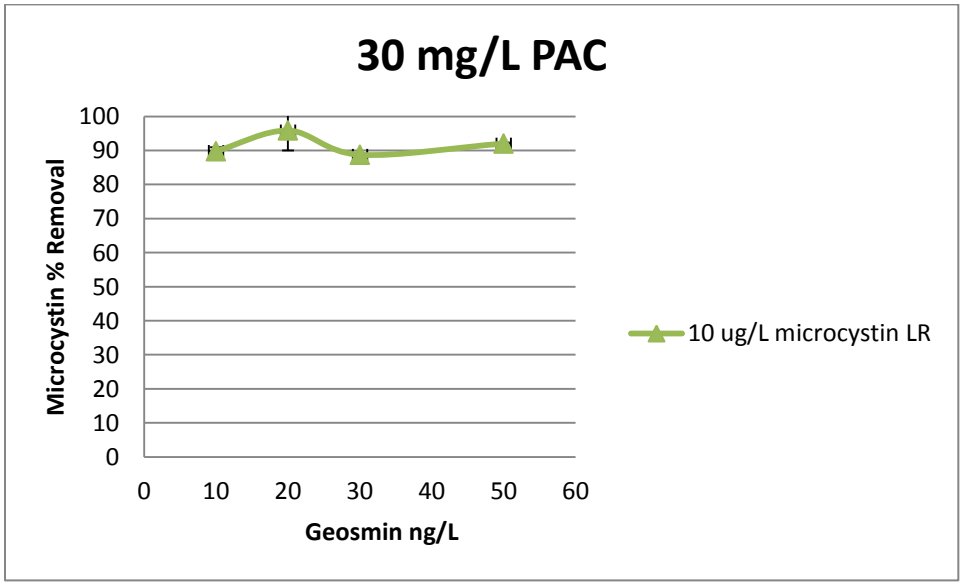

Figure 2-4. The presence of geosmin having little effect on microcystin-LR removal. N=6

2.3.2.2 Geosmin Removal

The removal of geosmin by PAC, with a contact time of 30 minutes and 30 minutes of settling time, resulted in average removal rates ranging from 76% to 95% as seen in Figure 2-5. Geosmin removal followed similar general trend and range as microcystin-LR removal. The

highest removal of geosmin, up to 95%, was achieved by 30 mg/L of PAC. A dosage of 10 mg/L of PAC yielded the lowest average removal of geosmin, at 76% removal, the exception of the experiment with a 20 ng/L concentration of geosmin where removal average reached 84%. Up to 99% removal of was achieved when observing individual experiments contributing to average removal rates. The results are comparable with a study done by Park et al. (2010) and Cook et al. (2001) where PAC doses of 20 mg/L removed between 60-85% of geosmin.

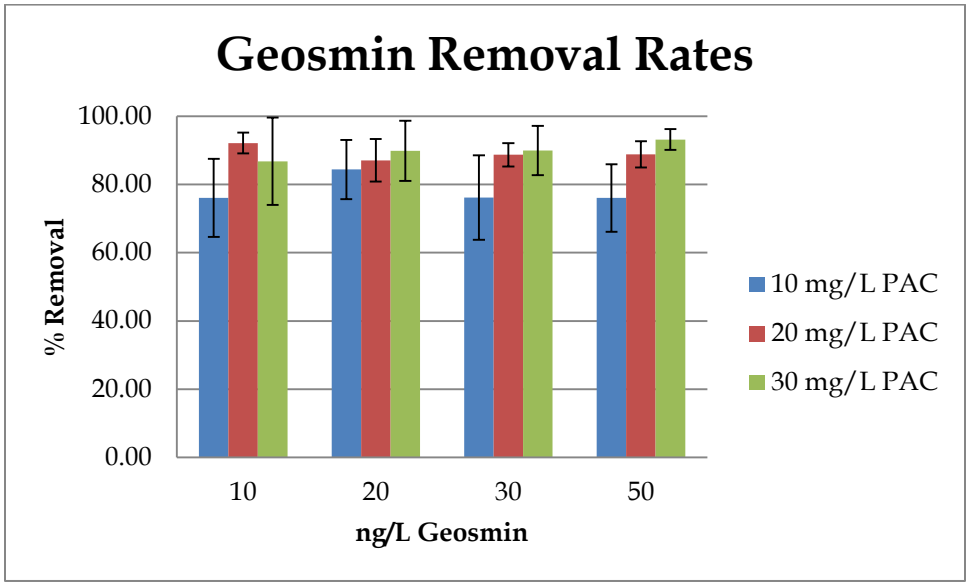

Figure 2-5. Average geosmin removal rates according to PAC dosages
Average error ranged from 3.01 to 12.8%

Average removal rates for geosmin showed minor variation where microcystin-LR concentrations were increased. There was again a competitive effect between both metabolites in some experiments when comparing geosmin removal rates with varying concentrations of microcystin-LR present. It was observed that, at the lowest dosage of PAC (10 mg/L), as the microcystin-LR concentration increased, average geosmin removal decreased. This can trend can be seen in, Figure 2-6. Similar to microcystin-LR removal rates, experiments with higher dosages of PAC were more resistant to fluctuations of the average removal percentage as seen in

Figure 2-7. Again like microcystin-LR, the removal percentage of geosmin was mostly dependent on the dosage of PAC used, as the kinetics of adsorption is fairly rapid with nearly 90% removal of the compound under 30 minutes (Ho et al, 2011).

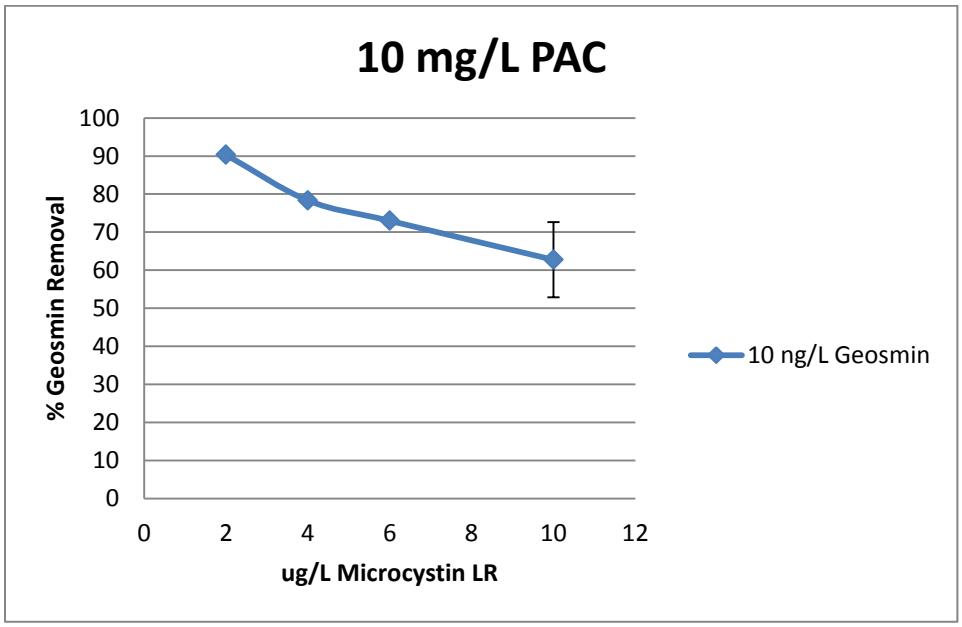

Figure 2-6. The presence of microcystin-LR affecting geosmin removal. N=6

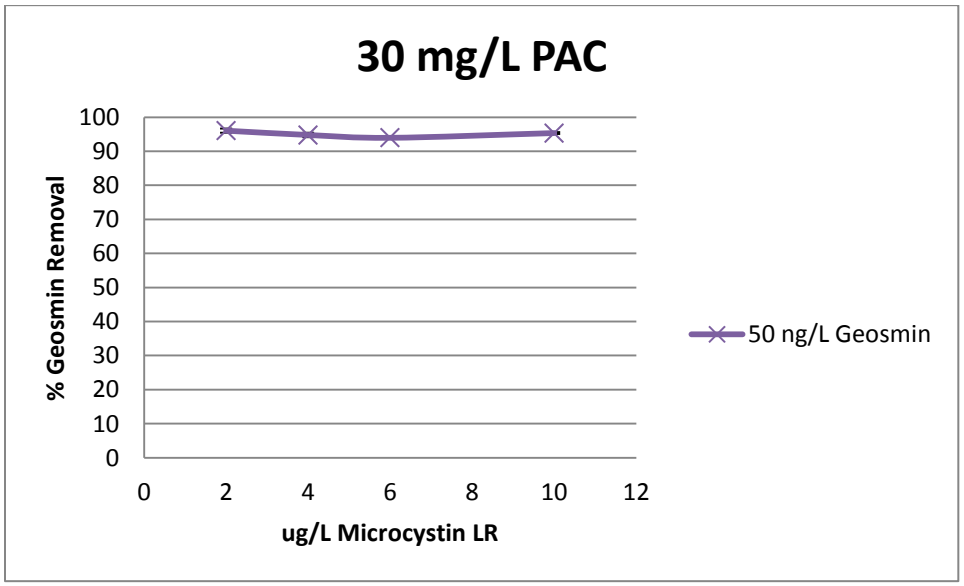

Figure 2-7. The presence of microcystin-LR having little effect on geosmin removal. N=6

Compared to microcystin-LR, geosmin is a flatter and smaller molecule that is fairly water soluble, this allows it to enter smaller pores by diffusion (Zoschke et al., 2011). Microcystin-LR is a larger molecule containing negatively charged groups including carboxyls, D-glutamic acid and d-erythro-β-methyl aspartic acid which is primarily responsible for its high solubility in water (Ho et al. 2011). These factors may allow geosmin to be more readily adsorbed onto a higher variety of pore sizes compared microcystin-LR. Also there was little to no correlation between total organic carbon levels and the removal rates of both of the metabolites. This could be attributed with the relatively small fluctuations of TOC levels throughout the experiments or both metabolites having little to no competing compounds when considering their adsorbance with the PAC. Other water quality parameters such as pH, temperature, DO, alkalinity and hardness also did not have any significant effects on the removal rates of both metabolites.

Diffusion effects, pore size, surface chemistry, surface area volume, pH, temperature and concentration can affect the efficiency of adsorption (Norit Americas 2009; Westrick and Szlag, 2010). However, the adsorption of both microcystin-LR and geosmin is suspected to be mostly driven by entropy. The presence of dissolved natural organic matter (NOM) can reduce the adsorption capacity of activated carbon by restricting access and competing for adsorption surfaces (Zoschke et al., 2011; Donati et al., 1994; Pendleton et al., 2001). However, it was also discussed by Zoschke et al. (2011), that geosmin is relatively resistant to higher natural organic matter (NOM) concentrations indicating that typical conventional treatment only affects larger and less adsorbable NOM components which are irrelevant for competitive adsorption against geosmin. This could explain why a higher degree of variation of removal percentages in microcystin-LR removal was observed when comparing to geosmin removal in this study.

2.4 Conclusions

Through environmental sampling, this study has confirmed that the cyanobacterial metabolites, microcystins and geosmin, co-occur in surface waters of Northern Colorado. Cyanobacterial metabolites were found at higher concentrations in highly eutrophic conditions. Geosmin was found in 44% of the sites sampled and microcystins 29% with 73% of microcystins detected with geosmin. Geosmin occurred more frequently than microcystins. When microcystins were detected, they frequently co-occurred with geosmin. It is suspected that microcystins more frequently co-occur with geosmin than the results show because the maximum detectable limit (MDL) is a few order of magnitudes above the MDL of geosmin. Only one site (Barr Lake, CO) was confirmed to have the most toxic variant of microcystin, microcystin-LR. It may be beneficial to test for microcystins when geosmin is detected, especially if the source water is used for recreational activities and as a drinking water source.

PAC is confirmed to be effective in removing both microcystin-LR and geosmin from drinking water sources below WHO standards of 1 μg/L for microcystin-LR and human detection limits of 4 ng/L for geosmin. Dosages required to remove microcystin-LR and geosmin below the acceptable limits are given in Table 2-2. The removal percentage of the metabolites depends on both PAC dose and the metabolite concentration. As expected, for effective removal of higher concentrations of cyanobacterial metabolites higher dosages of PAC is required.

Table 2-2. Dosages of PAC to remove microcystin-LR and geosmin below acceptable levels in Horsetooth Reservoir waters.

		PAC Dosage		
		10 mg/L	20 mg/L	30 mg/L
Microcystin LR (below 1 ug/L)	2 ug/L	✔	✔	✔
	4 ug/L	⊘	✔	✔
	6 ug/L	⊘	✔	✔
	10 ug/L	⊘	⊘	✔
Geosmin (below4 ng/L)	10 ng/L	✔	✔	✔
	20 ng/L	✔	✔	✔
	30 ng/L	⊘	✔	✔
	50 ng/L	⊘	⊘	✔
Key	⊘	Not below threshold		
	✔	Below threshold		

Prior to this study, there have been no investigations on how microcystin-LR and geosmin concentrations affect each other's removal rates by PAC. Competitive adsorbing between microcystin-LR and geosmin was observed at 10 mg/L of PAC and lower concentrations of metabolites, where up to a 20% removal reduction was observed. Larger concentrations of metabolites and PAC experienced more consistent removal rates. Higher dosages of PAC at 20 mg/L and 30 mg/L are relatively resistant to this effect thus a more consistent high removal of geosmin and microcystin-LR can be achieved. The adsorption of both metabolites onto PAC is primarily driven by entropy where adsorption was somewhat competitive between microcystin-LR and geosmin.

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3.0 In Vitro *Microcystin-LR Toxicity in H4IIE Mouse Liver Cells*

3.1 Introduction

The occurrence of mass blooms of cyanobacteria as of result of eutrophication and climate change has been rapidly increasing throughout the world (Chorus 2001; Svrcek and Smith, 2004). Large cyanobacterial blooms and the release of their metabolites in source waters cause aesthetic issues such as a murky green appearance and unpleasant odors (Chorus and Bartman, 1999; Svrcek and Smith, 2004; Omur-Ozbek and Dietrich, 2005; Miller 2010). However, these blooms may be causing more than environmental and aestetic concerns regarding harmful cyanotoxins that can be released during bloom events (Svrcek and Smith, 2004; Miller, 2010).

It is speculated that cyanotoxins are produced to prevent predation by zooplankton (Oberholster et al., 2006; Rohrlack et al., 2005; Hawkins et al., 2005; Li et al., 2010; Dixon, 2010). Some studies have shown that cyanobacterial toxins inhibit aquatic invertebrate grazers which are key elements to fresh water ecosystems. The cyanotoxins have the ability to disrupt and destroy the digestive systems of daphnia and mosquito larvae. In a recent study by Rohrlack et al. (2005), daphnia that were fed toxic cyanobacteria lost cell to cell epithelial gut cell junctions which ultimately led to their death.

The most common pathway of human exposure to cyanotoxins is from accidental ingestion or contact with water containing cyanobacterial cells through recreational activities. Animals on the other hand, are more commonly affected by the acute cyanotoxin poisonings as humans tend to avoid water bodies with floating mats of cyanobacteria (Svrcek and Smith 2004;

Graham, 2009). Chronic exposure to cyanobacterial toxins may result in carcinogenesis (Hitzfeld et al., 2000; Haddix et al., 2007). China has one of the highest incidents of hepatocellular carcinoma, which may be caused by cyanotoxin exposure. In Quidong county in China, residents who have been drinking surface water with average microcystin-LR concentrations of 0.1 μg/L reported higher frequencies of liver cancer compared to residents drinking from well water (Alvarez et al., 2010). Another study conducted in Florida reported that there was an increased risk for primary hepatocelluar carcinoma for residents living in the service area of water treatment plants drawing from surface waters with a history of blooms compared to groundwater users (Bean et al., 2002).

Due to adverse health effects of cyanotoxin exposure, the World Health Organization has set a drinking water guideline limit of 1 ppb (μg/L) for the cyanotoxin microcystin-LR, which is one of the most common and potent of the cyanotoxins (Chorus and Bartman, 1999). With so many emerging incidents of cyanotoxin exposures, municipalities have begun to be more aware of cyanobacteria blooms and follow in the footsteps of the World Health Organization (WHO) to protect the public from cyanotoxins. Today over eight countries, including Australia, Brazil, Finland and the US, have established water quality and cyanobacteria based programs to address cyanotoxins (Svrcek and Smith, 2004).

Currently, there are over 9 states in the US, which have statewide and local monitoring programs for cyanotoxins in freshwaters. An additional 13 states have event based response procedures or public education materials posted on their websites (Graham et al., 2009). . Cyanotoxins are on the EPA Contaminant Candidate List 3, a list of contaminants that are known to exist in public water systems, in which research is underway about potential national regulation (USEPA, 2011). Currently, there are over 9 states in the US which have statewide and

local monitoring programs for cyanotoxins in freshwaters. An additional 13 states have event based response procedures or public education materials posted on their websites (Graham, 2009).

Microcystins can cause severe liver damage characterized by liver cell cytoskeleton damage, a loss of sinusoidal structure, intrahepatic hemorrhaging, hemodynamic shock, formation of hepatic tumors, apoptosis and progressive liver necrosis. Higher doses can lead to death occurring in as little as a few hours to a few days of ingestion (Chorus and Bartman, 1999; 2003; Ayllon, 2001; Svrcek and Smith, 2004; Agrawal et al., 2006). Of the over 60 variants of microcystins, microcystin-LR is among the most frequently detected and most toxic of the microcystin congeners (Chorus and Bartman, 1999). Microcystin-LR is highly toxic compound with an oral LD_{50} of 5 mg/kg in mice (WHO 2003).

Microcystin-LR is a potent inhibitor of eukaryotic protein serine/threonine phosphates 1 and 2A by covalently binding to these enzymes. Protein phosphatases serve as enzymes that play an important regulatory role in maintaining homeostasis in the cell including tumor suppression (Chorus, 2001). After ingestion of microcystin-LR, the toxin is transported across the ileum into the bloodstream through the liver where it can be concentrated and taken up by hepatocytes (Chorus and Bartman 1999).

The microcystin toxin is organ and cell specific, targeting primary hepatocytes. The cell specificity is not due to metabolic activation in the liver cells but to the specific pathway of uptake. Microcystins have been shown to be taken up through the multi-specific transport system for bile acids, which is also an entrance pathway for several xenobiotic substances. The in-vitro effects of microcystins on hepatocytes can be inhibited by low concentrations of bile acids or

bile acid transport inhibitors. Only primary hepatocytes and intestinal cells are equipped with this bile acid transport system (Chorus and Bartman 1999).

The aim of this study was to observe the toxicity of microcystin-LR on rat hepatocytes, H4IIE cells, *in-vitro*. The H4IIE rat hepatoma cell line is used in many in-vitro tests for cytotoxicity screening and testing. Histological observations and a survey were also performed to investigate signs of cellular damage due to toxin exposure.

3.2 Materials and Methods

3.2.1 Cell Culturing

High purity chemical microcystin-LR (CAS 101043-37-2) was purchased from Cayman Chemicals (Ann Arbor, MI). Optima grade methanol (CAS 67-56-1; Fisher Scientific) was used in preparation of the standard solutions. Standard solutions of microcystin-LR were prepared in methanol at a concentration of 5 mg/L. (TCA, 50mM Tris base (pH 10.5), trypsin, acetic acid, chamber well slides, stains) The above solutions were sterilized by filtering through a 0.2 um filter. MEM/ EBSS 2mM L-Glutamine Hydroclone (Cat#SH30002401) supplemented with 10% fetal bovine serum was used for cell culturing. Sulforhodamine (SRB). All cell culturing procedures followed sterile measures including working under laminar flood hoods, using sterilized pipette tips, syringes, filters and plates.

Standardized growth factors and conditions were maintained due to the H4IIE cell line sensitivity to environmental changes (Whyte and Tillitt, 2004). Cell culturing of H4IIE cells was done following procedures laid out by Ding et al., 2001. H4IIE cells were cultured on pass 25 in plastic T-50 flasks. MEM/EBSS media with 2mm L-Gludamine (Hydroclone ^{TM}) 10% Fetal

bovine serum (FBS) and was utilized through the experiment. The cells were incubated at 37^{o}C with a 5% CO_2 environment concentration until cells reached a density greater than 5 x 10^5 cells/ml. Cell densities was determined by a Bio-Rad TC-10 Automated Cell Counter. Media was changed on a regular basis and cell cultures were split when needed by aspirating the liquid media and adding approximately 1.5 ml of 0.25% trypsin to release the cells. Once the cells were clearly suspended, the trypsin was neutralized by suspending the cells in media with 10% FBS.

A 96 well plate was inoculated with the appropriate amount of cells as indicated in Table 3-1 in a total volume of 200 µl of media. The cells in the plate were allowed to attach over a 24 hour period in the incubator under the conditions mentioned above. After the first incubation period, microcystin-LR exposure tests were begun. The media was aspirated and 200 µl of new media with varying concentrations of microcystin-LR were pipetted into each well as given in Table 1. A stock solution of the media was prepared with a 150 µg/L microcystin-LR in 10% FBS to be added to the wells designated to receive microcystin-LR. All other dilutions of microcystin-LR were made from the 150 µg/L stock solution by adding the respective volume of the microcystin-LR and media to bring up the well to the desired toxin concentration. Selected concentrations of microcystin-LR, 0.78 to 150 µg/L, were tested in triplicates. Two types of controls were used: first with 10% FBS media with a 3% concentration of methanol (MeOH), representing the largest concentration of methanol, used as a vehicle, in the samples. The other control consisted only of 10% FBS media.

Table 3-1. 96 Well array for cytotoxicity observations.

	1	2	3	4	5	6	7	8	9	10	11	12
A	150.00	100.00	75.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	Control MeoH	Control
B	150.00	100.00	75.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	Control MeoH	Control
C	150.00	100.00	75.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	Control MeoH	Control
D	150.00	100.00	75.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	Control MeoH	Control
E	150.00	100.00	75.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	Control MeoH	Control
F	150.00	100.00	75.00	50.00	25.00	12.50	6.25	3.13	1.56	0.78	Control MeoH	Control
G	Control	Control										
H	Control	Control										

(ug/L) 24hr incubation 10^5 cells. Well Vol 200ul

(ug/L) 24hr incubation 5 x 10^4 cells. Well Vol 200ul

3.2.2 Cytotoxicity Screening

An LD_{50} was observed through the sulforhodamine B (SRB) colorimetric assay for cytotoxicity screening. The cells were exposed to the toxin for 24 hours under the same conditions mentioned above. The SRB colorimetric assay was used analyze for cytotoxicity following methods laid out by Fricker, 1994. After the exposure period, the media was aspirated and the cells were fixed with 50 µl of 10% TCA in each well. The plate was then refrigerated for 24 hours. The TCA was then removed and the plate was washed 5 times with tap water to remove the remaining TCA, growth medium and low molecular weight metabolites. The plate was allowed to air dry completely before adding 0.4% SRB dissolved in 1% acetic acid to the TCA fixed wells. The plate was then allowed to sit for 30 minutes for the staining. The SRB was

then removed and each well was washed with 1% acetic acid to remove the dye. This was repeated four times. The plate was then allowed to air dry once more before 50 µl of 50 mM Tris base (pH 10.5) was added to each well to solubilize the cells. The plate was then put on an orbital shaker for 5 minutes. The plate was then read on a GloMax® Multi Microplate Reader from Turner BioSystems, at 560 nm. Two data sets were produced, wells containing 10^5 cells and wells containing 5 x 10^4 cells.

3.2.2.1 Statistical Analysis

The numerical data are presented as the mean +/- standard deviation from at least three independent samples for the experiments performed and analyzed by the one-way analysis of variance (ANOVA) method using Microsoft Excel software.

3.2.4 Histological study

The H4IIE cells were plated and treated under the exact same environmental conditions for the 96 well plates on chamber slides with the same cell densities of 10^5 and 10^6 cells. Each chamber was inoculated with 50 µl of the cell solution with the respected cell densities. The following concentrations of microcystin-LR were plated for the histological study: 0, 25, 50, 100 µg/L. After a 24 hour exposure period, the cells were fixed with LC grade methanol at -20^{o}C for approximately 10 minutes. After fixation, the cells were stained with hematoxylin and eosin and analyzed for signs of damage from the toxin using a Ziess Axiovert 200m microscope equipped with a Hammatsu ORCA-ER cooled charge-coupled device camera at 40x zoom controlled using Slidebook software (v4.1 and 5.0; Intelligent Imaging Innovations, Inc., Denver, CO).

3.3 Results

3.3.1 Cytotoxicity Screening Results

Figure 3-1 shows the number of cells surviving after the 24 hour treatment with the respective dosage of microcystin-LR. Cell viability (confluence) was averaged between the triplicates and normalized against the control wells. Relative standard error for wells containing 10^5 cells ranged between 0.3 to 24.3%. Relative standard error for wells containing 5 x 10^4 cells ranged between 2.1 to 5.4%. Control well containing 10^5 cells exposed only to methanol (0.16%) saw a 22% loss of cell viability. Other control wells had 100% or more cell viability. By observing the wells containing 10^5 cells, wells with microcystin-LR concentrations of 1.56, 3.13, 6.25 and 12.5 µg/L resulted in less than 50% viable cells remaining after exposure. The LD_{50} concentration was observed at 12.5 ug/L with a downward cell viability trend continuing to wells with a 1.56 µg/L microcystin-LR concentration. A similar trend was observed for wells containing 5 x 10^4 cells, where cell viability drops as microcystin-LR concentrations were decreased. It should be noted that an LD_{50} was not observed with wells containing 5 x 10^4 cells. Cell viability only dropped down to 76% by a microcystin-LR dose at 1.56 µg/L.

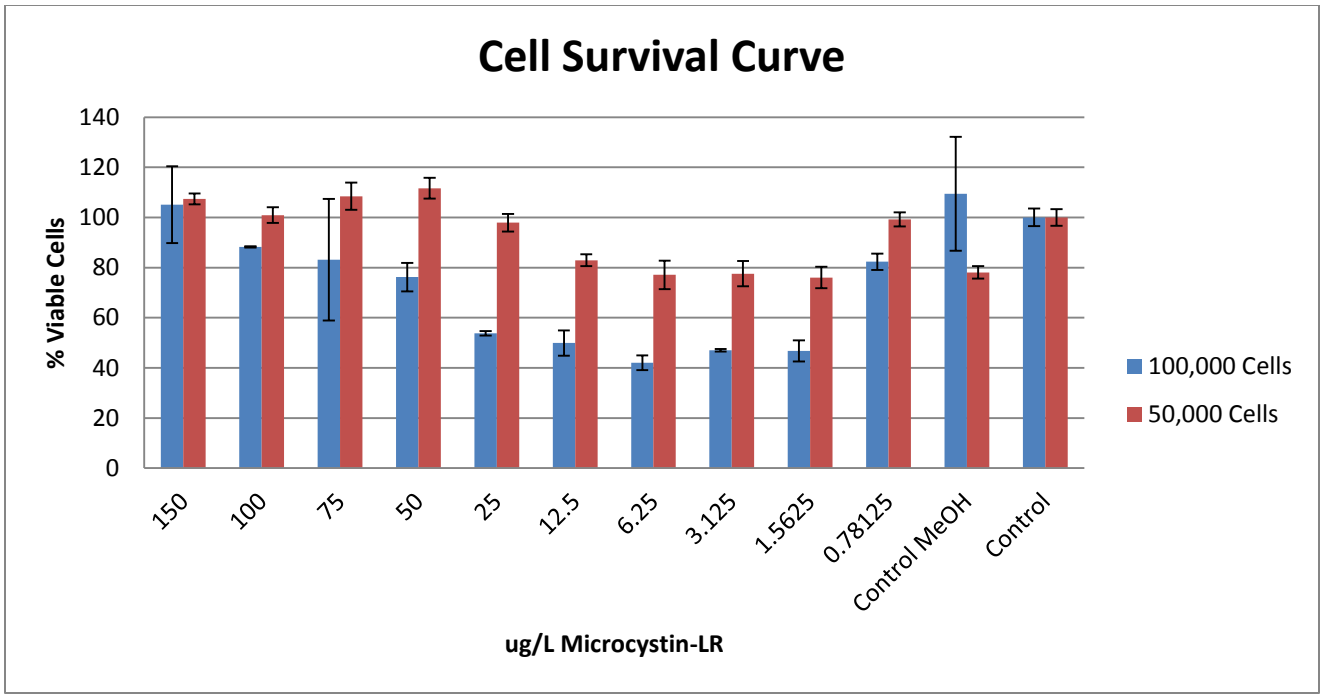

Figure 3-1. Cytotoxicity Results

3.3.2 Histological Observation Results

Cellular stress was observed comparing control cells to cells exposed to 100 µg/L of microcystin-LR. Cellular stress showing signs of apoptosis, as described by Chen et al. 2005, was observed in the form of enlarged nuclei, cell blebbling, vacuolization and detachment of desmosomes in the exposed cells. Compared to the control cells, there was a higher frequency of vacuolization and cell blebbing.

A survey was also conducted with participants comparing cells that were exposed to 100 µg/L of microcystin-LR to cells with no exposure to the cyanotoxin. 76% of participants (n=30) selected the image with cells exposed to 100 µg/L as being damaged or stressed. However, only 17% of participants were able to distinguish between the sets of cells were exposed to a higher dose of toxin versus a lower dose of the toxin.

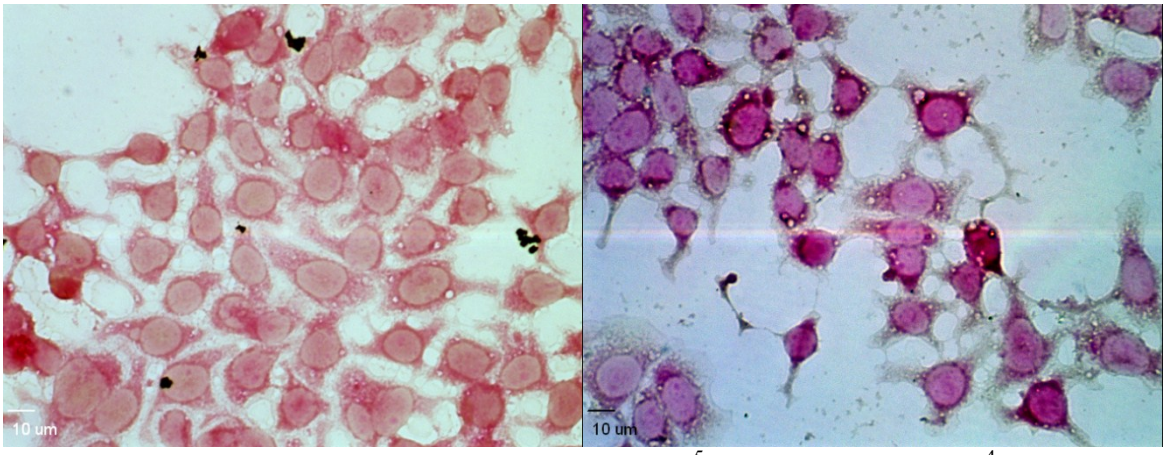

Figure 3-2. Control cells from the wells containing 10^5 cells (left) and 5 x 10^4 cells (right)

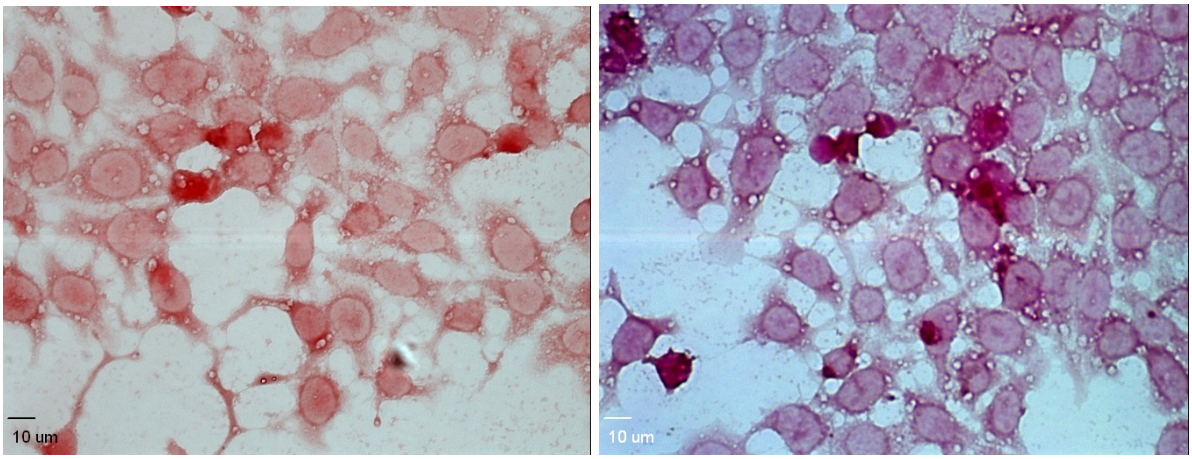

Figure 3-3. Cell exposed to 100 µg/L microcystin-LR from the wells containing 10^5 cells (left) and 5 x 10^4 cells (right)

3.4 Discussion

The only observed loss in cell viability of the control wells occurred in wells containing 10^5 cells and methanol. Other control cells did not experience a drop in cell viability. This may be due to a higher dosage of methanol on a per cell basis. The cytotoxicity analysis showed that cells exposed to a mid-range of toxin between 1.56 to 12.5 µg/L of microcystin-LR exhibited the lowest cell viability while cells exposed to high and lowest dosage of microcystin-LR experienced a high level of cell viability. However, histologically, cells exposed to higher

dosages of microcystin-LR (100 µg/L) showed more prominent signs of apoptosis compared to control cells and cells exposed to lower dosages of the cyanotoxin.

One theory suggests that during the cytotoxicity screening experiment, wells with higher concentrations of microcystin-LR experienced a large wipeout of the cells leaving an abundance of nutrients for surviving cells to utilize. The abundance of nutrients may have come from a lower concentration of cells utilizing available nutrients in their support solution and dead cells which have succumbed to the toxin. Since wells with 5×10^4 cells had more percentage of cells surviving it is suspected that the reduced competition for the nutrients in the support solution was the driving factor to their survival. By histological observations, surviving cells underwent increased stress some toxin levels still remained in solution.

Another theory suggests low dosages and high dosages of microcystin-LR may have different toxicity pathways that are dose dependent. As stated by Chen et al. 2005, at smaller doses of microcystin-LR, apoptosis occurs through the BID-BAX-BCL-2 regulatory protein pathway. Proteins in the BCL-2 family are potent regulators of apoptosis that can influence the permeability of the outer mitochondrial membrane. They contain both pro and anti-apoptotic members that elicit opposing effects on the mitochondrial membrane including the anti-apoptotic protein BCL-2 and pro-apoptotic proteins BAX and BID. Essentially the BID-BAX-BCL-2 pathways are responsible for homeostasis regulation of the cells (Akcali et al., 2004).

The up-regulation of BCL-2 is a host response to toxin exposure, protecting the host cell from destruction by apoptosis. Upon apoptosis, BAX oligomerizes with BAK forming large complexes that create pores in the lipid bilayer and facilitate the release of cytochrome C and other factors. Thus, the up-regulation of BAX at relatively low doses of microcystin-LR could initiate the consequent apoptotic events with BID serving as an initiator of BAX (Chen et al,

2005). Thus an up-regulation of BAX could have induced the apoptosis observed with the experiment.

It is believed that the reactive oxygen species pathway dominates apoptosis during high concentrations of microcystin-LR. As seen in Figure 3-1, there was a higher concentration of viable cells suggesting the reactive oxygen species pathway may take a longer than 24 hours of exposure to take effect to ultimately kill off the cells even though a higher ratio of cells were observed to be experiencing apoptosis. This may explain why cell viability exceeded or was within 50-100% with higher concentrations of microcystin LR. The toxicity pathway may involve a longer path to trigger apoptosis in the cell (Chen et al., 2005). In the reactive oxygen species pathway the protein ferritin serves as the major iron binding protein limiting the catalytic availability of iron for participation in free radical generation. The up regulation of ferritin is suspected to be induced by microcystin-LR. Iron is ubiquitous in cells and present in the structure of many enzymes and proteins that catalyze redox reactions enabling iron to generate radical species from $Fe^{2+} + H_2O_2 \Rightarrow Fe^{3+} + HO^* + OH^-$. Reactive oxygen species cause an increase in cytosolic calcium and has been observed in cells undergoing oxidative stress. This leads to mitochondrial membrane rupture, massive intrahepatic hemorrhaging and damage to the cytoskeleton (Chen et al., 2005).

Though cell densities for the wells containing 100 µg/L of microcystin-LR were greater than the control wells, there were more signs of apoptosis, by histological observations, compared to the control wells and wells with a lower concentration of microcystin-LR. The criteria for histo-pathological alternations include swollen, vacuolar degeneration, plasma membrane blebbling, DNA fragmentation and breakdown of the cell. It was observed that healthy H4IIE cells in the control wells had dendritic characteristics which connect the cells

together. Cells in the exposed wells expressed a retraction of these cellular junctions and some indications of apoptosis, as mentioned before, in the cells incubated with a concentration of microcystin-LR. Cytoskeletal damaged was observed in the form of a higher frequency of misshaped cells which is a major indication of hepatoxicity (Ding et al., 2001). By histological observation, over 76% participants correctly determined which cells were exposed to microcystin-LR and which were not. Participants indicated they observed physical aspects of the cell such as cellular shape, nuclei size and overall uniformity of the cells.

3.5 Conclusion

Exposure to the cyanotoxin microcystin-LR can induce H4IIE cells cultivated *in vitro* to express signs of apoptosis. Initial toxicity may have acted quickly and killed off many cells leaving surviving cells an abundance of nutrients. An LD_{50} was observed at 12.5 µg/L as a result of the 24 hour exposure to microcystin-LR. Cells exposed to lower doses of microcystin LR between 1.56 to 12.5 µg/L experienced a moderate loss of cell viability. Alternatively, since cells exposed to low concentrations of microcystin-LR between 0.78 – 50 µg/L experienced cell viability drop below 50%, apoptosis is suspected to be induced by the up regulation of the BAX regulatory protein pathway during low dose microcystin-LR exposure. At higher concentrations, above 12.5 µg/L, reactive oxygen species pathway is responsible for apoptosis occurring in cells exposed to higher doses of microcystin-LR from to histological observations of cells exposed to high concentrations of microcystin-LR over 100 µg/L.

Through the cytotoxicity test, it was shown that the reactive oxygen species pathway induces a higher frequency of apoptosis to occur, cell growth is either unaffected or increased slightly. However, through histological observations there is a higher ratio between cells showing signs of apoptosis compared to normal cells such as cell blebbing and vacuolization.

Cell viability is lower for cells exposed to low levels of microcystin-LR suggesting a faster apoptic response at low doses. It is concluded that microcystin-LR does have toxic effects on H4IIE cells and that toxicity can be characterized by signs of apoptosis and possible loss in cell viability. Exposure to the toxin may induce severe hepatosis and can be dose dependent. Children are particularly at risk because they can drink a higher volume of water in proportion to their body weight than adults (WHO, 1999). Future studies may compare finer increments of duration of exposure to microcystin-LR to explore and characterized more accurately toxicity pathways and toxicity that is dependent on time. It is expected that the results may deviate from this study.

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Appendix A. Jar Test Results

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**10**	**10**	**11-Jan**

Table A 1. Sample 0,2,10,10, Jan-11 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	603.9	690.4	799.7	1601.6	698	80.12
Area 2	348.7	364.1	418.2	707.4	377	29.80
Concentration (ug/L)	0.91	1.01	1.14	2.07	1.02	0.09
Geosmin						
Area 1	758	755	798	7884	770.33333	19.60
Concentration (ng/L)	1.04	1.01	1.1	10.83	1.05	0.04
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.41	7.41	7.4	7.4	7.41	0.00
Conductivity (µS/cm)	59.5	60.3	62.8	59.2	60.87	1.41
Temperature (oC)	16	16.6	16.5	17.1	16.37	0.26

Table A 2. Microcystin-LR Standard Curve: 0,2,10,10, Jan-11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
2	1476.5	671.6
4	3437.3	1557.2
40	28772.2	13264.6
80	61102.1	26592.5

Table A 3. Geosmin Standard Curve: 0,2,10,10, Jan-11

Standard Conc.	Area
1	315
5	2362
10	5891
25	19031

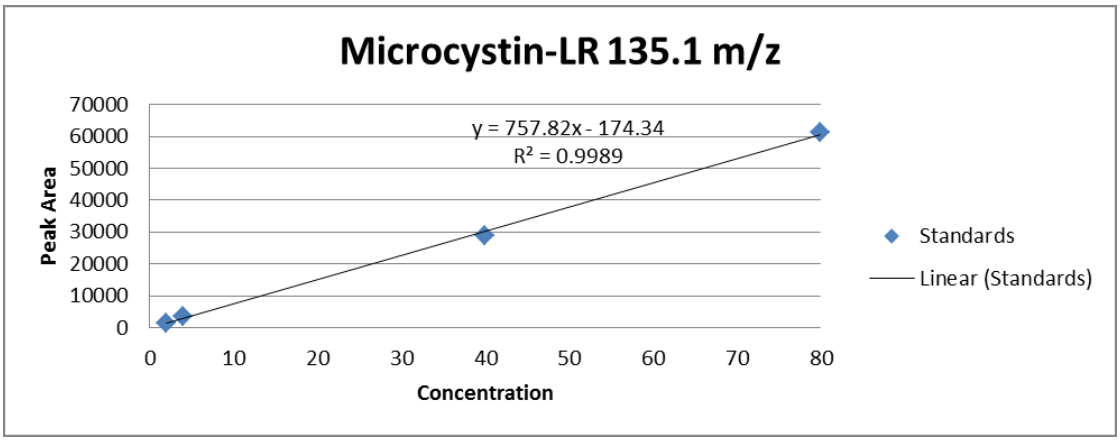


Figure A 1. Microcystin-LR 135.1 ion curve: 0,2,10,10, Jan-11

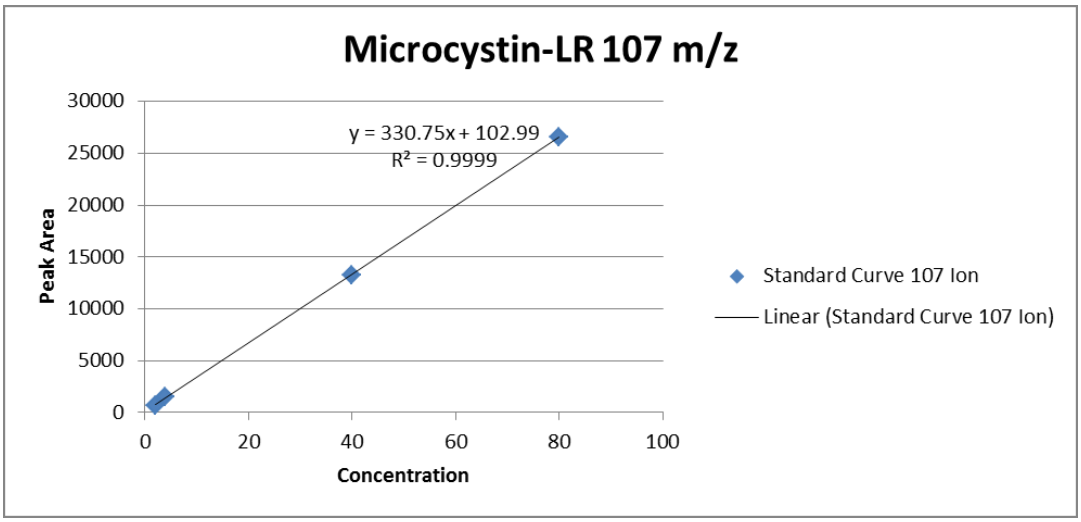


Figure A 2. Microcystin-LR 107 ion curve: 0,2,10,10, Jan-11

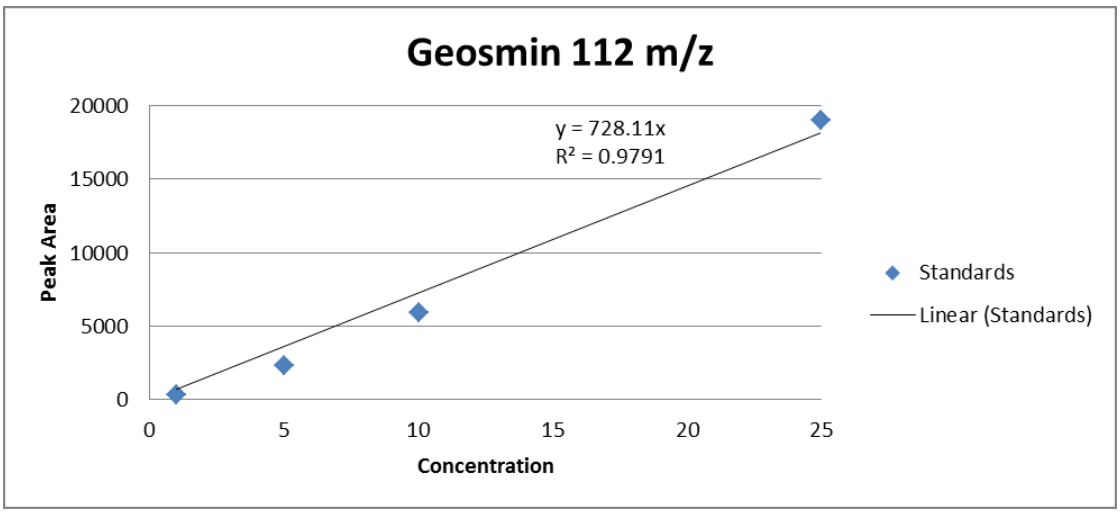


Figure A 3. Geosmin 112 ion curve: 0,2,10,10, Jan-11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**20**	**10**	**17-Sep**

Table A 4. Sample 0,2,20,10, Sept 17 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	20	20	17	1711.6	442.15	1.41
Area 2	5	10	10	776.6	200.40	2.36
Concentration (ug/L)	0.71	0.71	0.6	2.2	1.06	0.05
Geosmin						
Area 1	3880	4097	3864	14753	3947	106.267
Concentration (ng/L)	3.23	3.22	3.41	20.26	7.53	0.09
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	30	40.00	0.00
pH	6.95	6.9	6.9	7.4	6.92	0.02
Conductivity (µS/cm)	58.7	58.9	58.6	59.5	58.73	0.12
Temperature (oC)	17.2	17.8	17.5	16.3	17.50	0.24

Table A 5. Microcystin-LR Standard Curve: 0,2,20,10, Sept 17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	15	8
1	34	11
10	255	111
20	555	247

Table A 6. Geosmin Standard Curve: 0,2,20,10, Sept 17

Standard Conc.	Area
1	315
5	2362
10	5891
25	19031

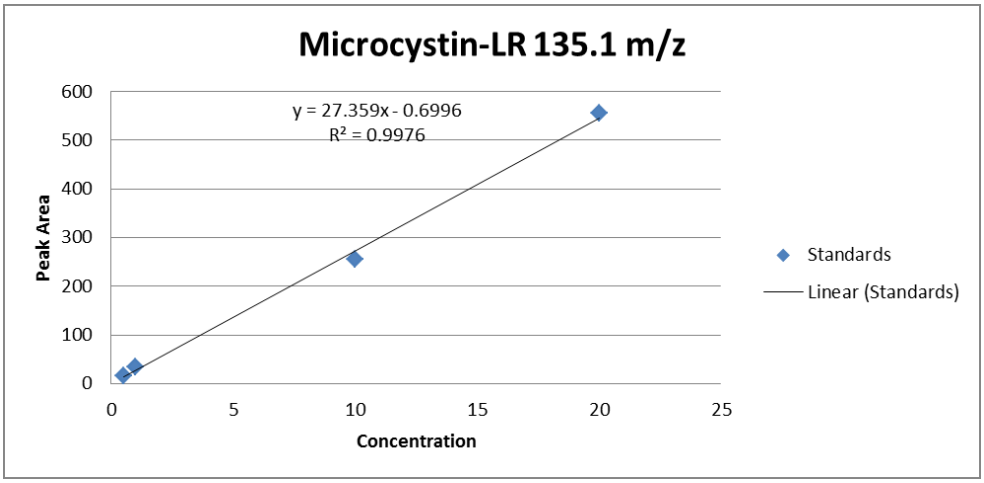


Figure A 4. Microcystin-LR 135.1 ion curve: 0,2,20,10, Sept 17

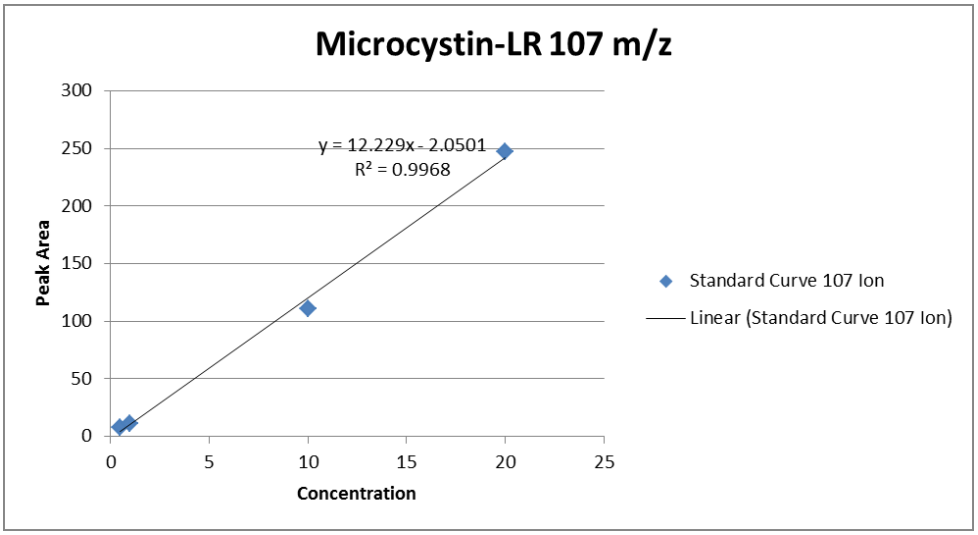


Figure A 5. Microcystin-LR 107 ion curve: 0,2,20,10, Sept 17

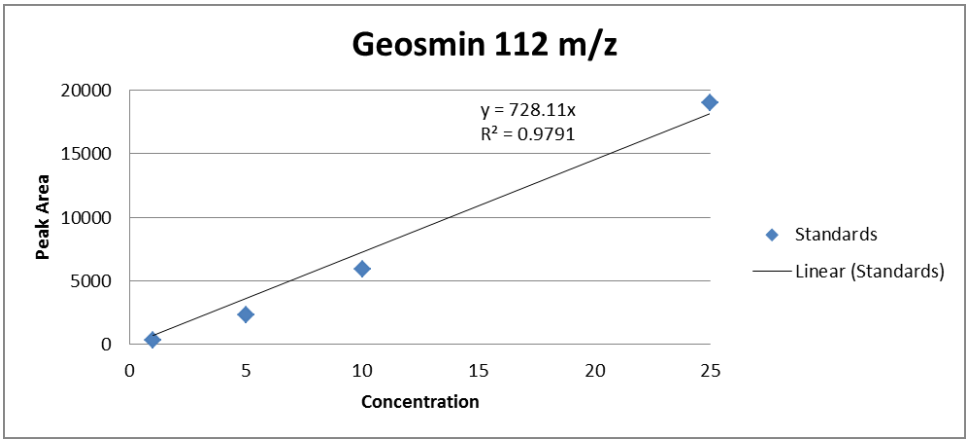


Figure A 6. Geosmin 112 ion curve: 0,2,20,10, Sept 17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**30**	**10**	**11-Jan**

Table A 7. Sample 0,2,30,10, Jan-11 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	10	0	541	1711.6	183.67	252.71
Area 2	0	0	246	776.6	82.00	115.97
Concentration (ug/L)	0	0	0.78	2.2	0.26	0.37
Geosmin						
Area 1	8029	8254	8154	15730	8145.6667	92.04468
Concentration (ng/L)	10.24	10.52	10.41	20.26	10.39	0.12
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.2	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30	0.00
pH	7.6	7.7	7.75	7.4	7.6833333	0.06
Conductivity (µS/cm)	60.5	60.5	60.3	59.5	60.433333	0.09
Temperature (oC)	19.5	19.4	19.7	16.3	19.533333	0.12

Table A 8. Microcystin-LR Standard Curve: 0,2,30,10, Jan-11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	0	0
1	20	0
5	97	34
10	207	83
20	332	131
40	605	228

Table A 9. Geosmin Standard Curve: 0,2,30,10, Jan-11

Standard Conc.	Area
1	576
5	4872
10	6936
25	20017
50	39099

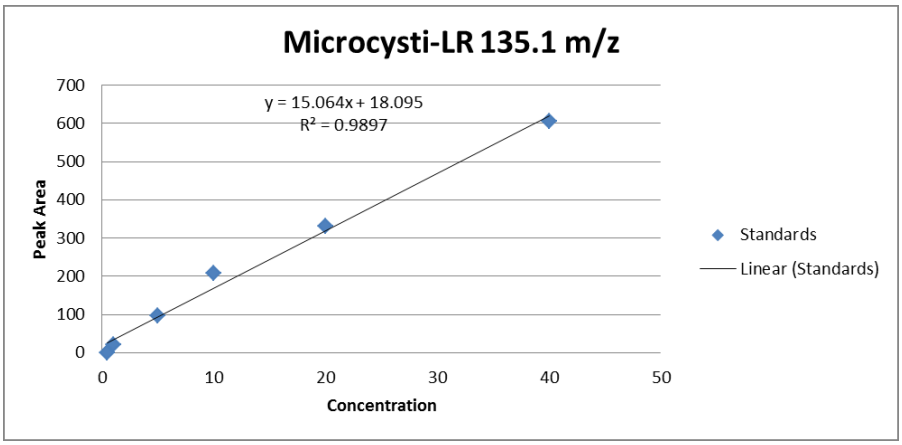


Figure A 7. Microcystin-LR 135.1 ion curve: 0,2,30,10, Jan-11

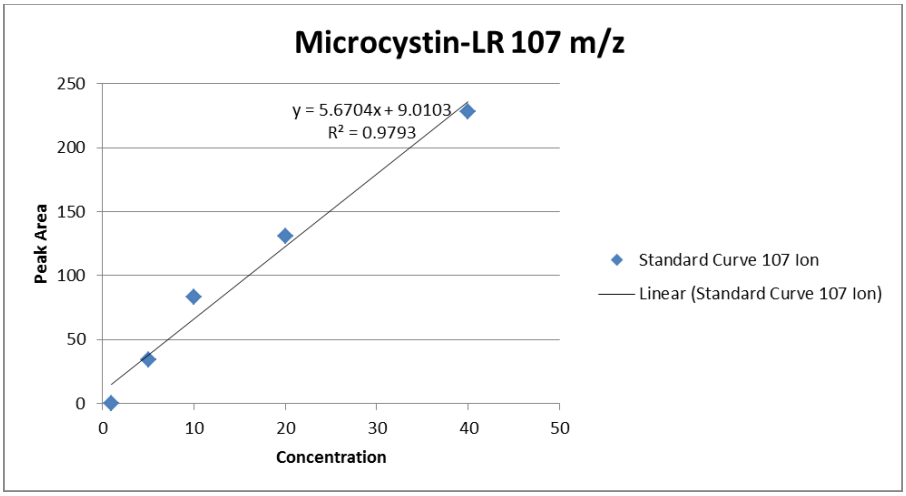


Figure A 8. Microcystin-LR 107 ion curve: 0,2,30,10, Jan-11

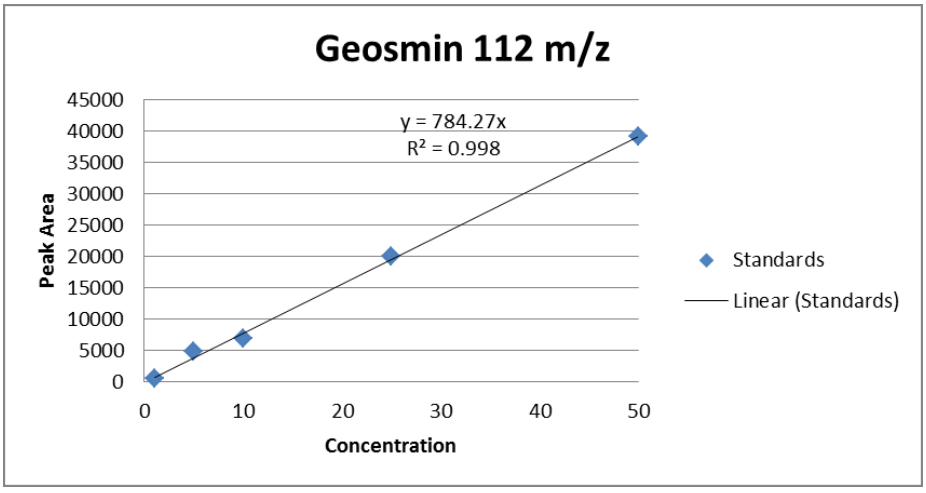


Figure A 9. Geosmin 112 ion curve: 0,2,30,10, Jan-11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**50**	**10**	**16-Nov**

Table A 10. Sample 0,2,50,10, Nov 16 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	371.3	352.3	249	895	324.20	53.74
Area 2	168.6	162	124	470	151.53	19.65
Concentration (ug/L)	0.31	0.27	0.04	1.48	0.21	0.12
Geosmin						
Area 1	6421	6596	6598	44244.52	6538.3333	82.97
Concentration (ng/L)	7.32	7.52	7.52	50.43	7.4533333	0.09
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.25	7.3	7.31	7.2	7.29	0.03
Conductivity (µS/cm)	61.3	61.5	61.6	60.5	61.47	0.12
Temperature (oC)	19.6	19.5	19.4	19.2	19.50	0.08

Table A 11. Microcystin-LR Standard Curve: 0,2,50,10, Nov 16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	231.5	137.8
1	544.5	265.5
10	5406	2350
20	8847	4088

Table A 12. Geosmin Standard Curve: 0,2,50,10, Nov 16

Standard Conc.	Area
1	469
5	3664
10	6254
25	22220
50	44308

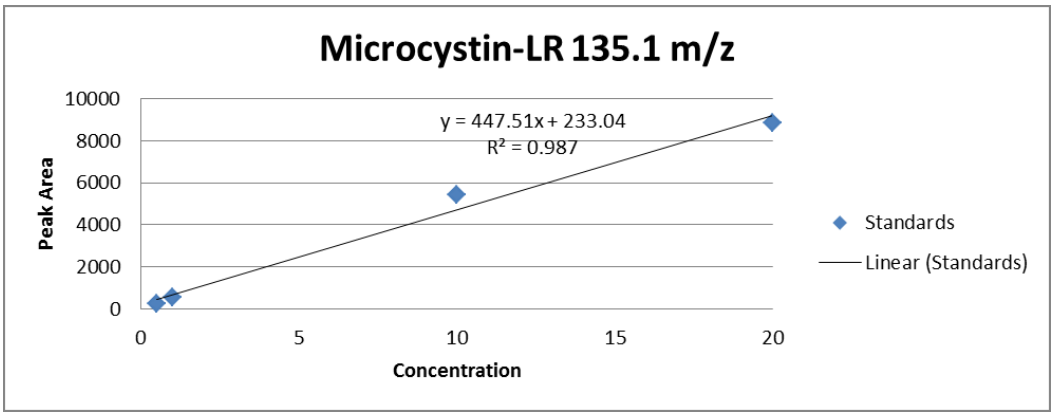


Figure A 10. Microcystin-LR 135.1 ion curve: 0,2,50,10, Nov 16

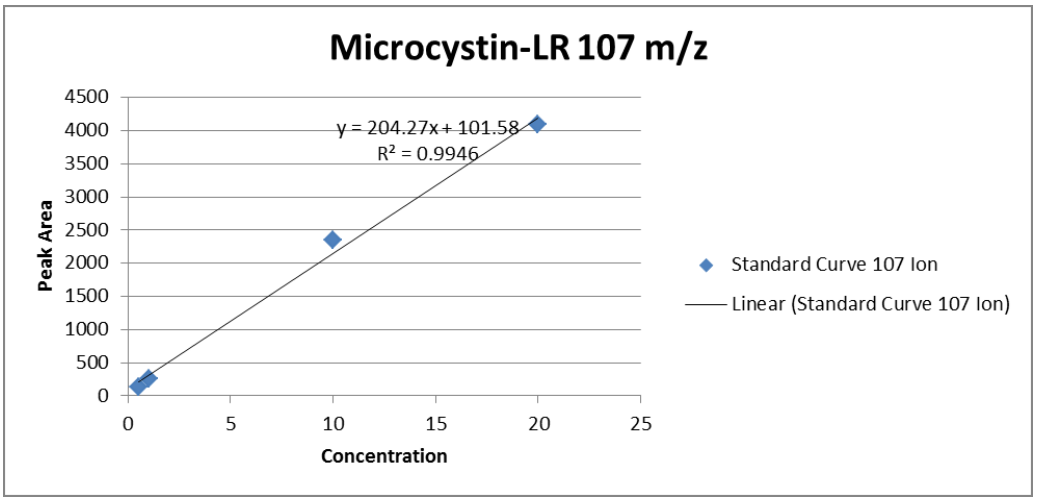


Figure A 11. Microcystin-LR 107 ion curve: 0,2,50,10, Nov 16

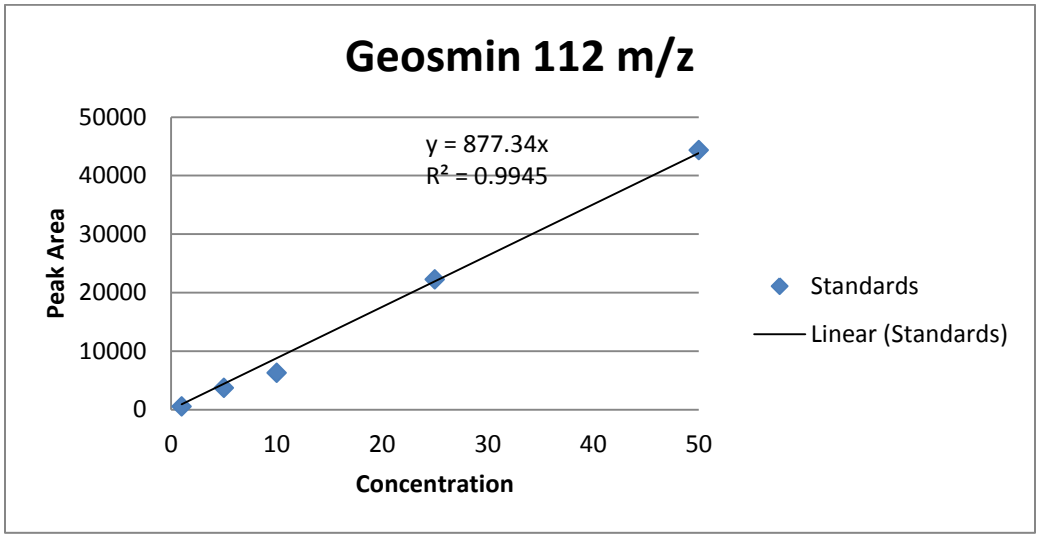


Figure A 12. Geosmin 112 ion curve: 0,2,50,10, Nov 16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**10**	**10**	**25-Jan**

Table A 13. Sample 0,4,10,10, Jan-25 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	0	786.3	1127.2	2486.5	637.83	472.00
Area 2	0	348.6	505.3	1093.3	284.63	211.19
Concentration (ug/L)	0	1.2	1.77	4.04	0.99	0.74
Geosmin						
Area 1	1939	1839	1882	8708	1886.67	40.96
Concentration (ng/L)	2.23	2.12	2.17	10.03	2.17	0.04
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.55	7.52	7.58	7.6	7.55	0.02
Turbidity (NTU)	1.89	1.78	1.41	3.76	1.69	0.21
Conductivity (µS/cm)	62.9	62.9	62.9	61.4	62.90	0.00
Temperature (oC)	22.4	22.4	22.6	22.4	22.47	0.09

Table A 14. Microcystin-LR Standard Curve: 0,4,10,10, Jan-25

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.01	70.6	37.2
0.02	86.7	45.1
1	625.9	256.8
10	6111	2669
20	12008	5561

Table A 15. Geosmin Standard Curve: 0,4,10,10, Jan-25

Standard Conc.	Area
1	530
5	3768
10	9639
25	21446

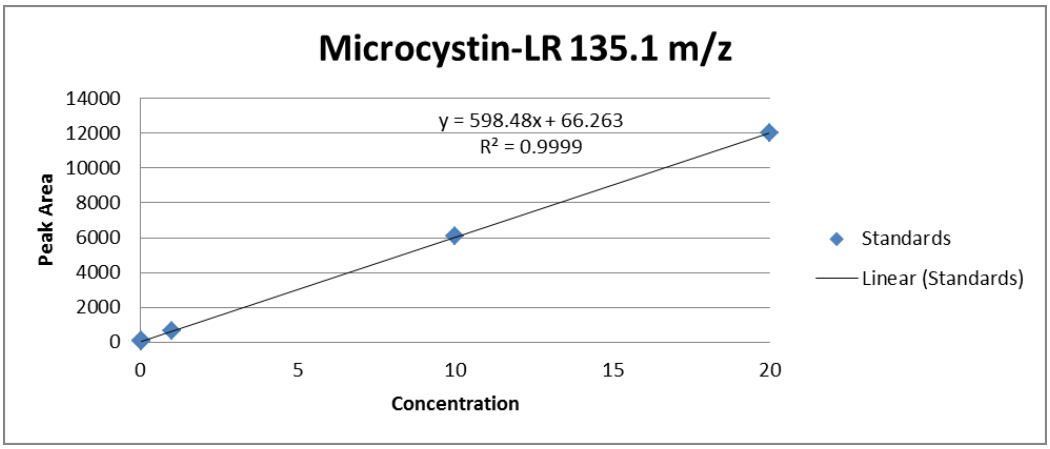


Figure A 13. Microcystin-LR 135.1 ion curve: 0,4,10,10, Jan-25

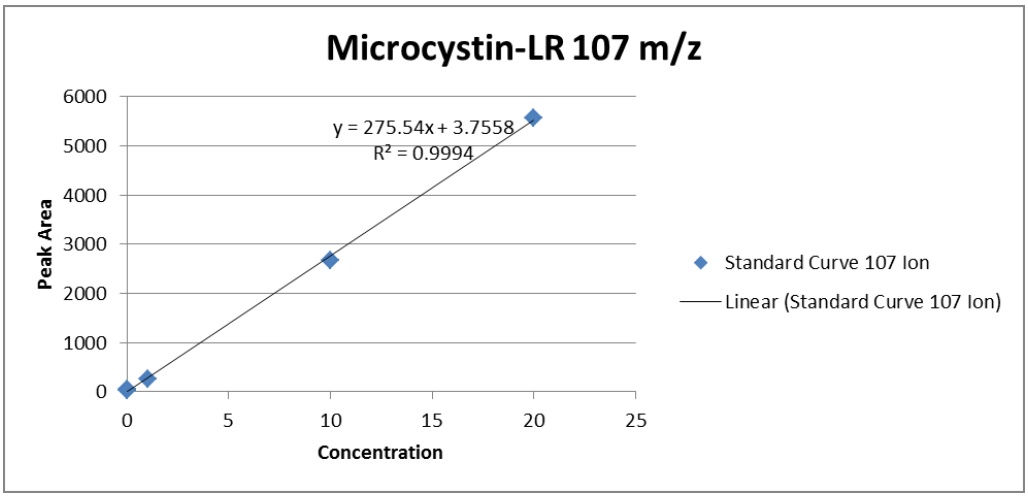


Figure A 14. Microcystin-LR 107 ion curve: 0,4,10,10, Jan-25

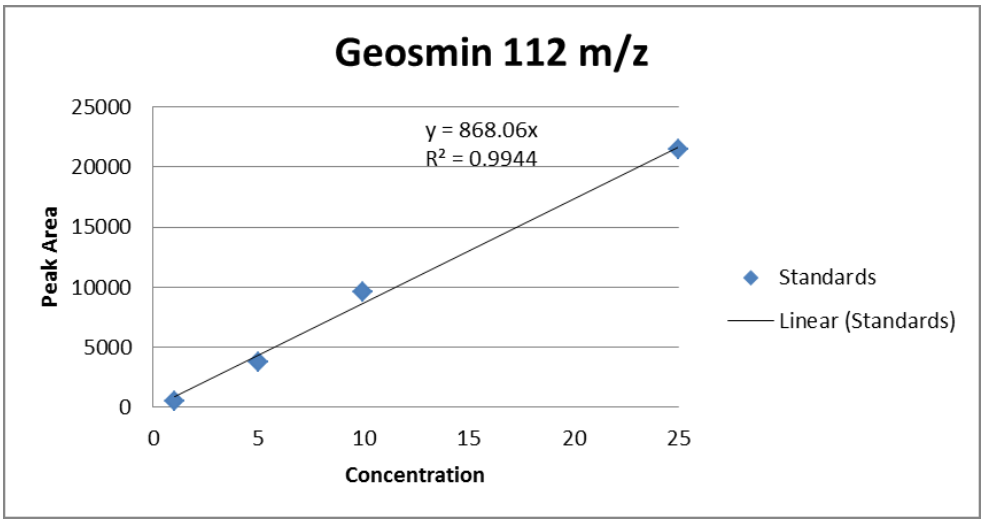


Figure A 15. Geosmin 112 ion curve: 0,4,10,10, Jan-25

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**20**	**10**	**9-Aug**

Table A 16. Sample 0,4,20,10, Aug 9 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	64	60	62	2486.5	62	1.63
Area 2	28	30	26	1093.3	28	1.63
Concentration (ug/L)	1.38	1.42	1.34	4.04	1.38	0.03
Geosmin						
Area 1	2801	1703	2209	9067	2237.67	448.71
Concentration (ng/L)	6.66	4.05	5.25	21.55	5.32	1.07
Water Quality						
Hardness (mg/L CaCO3)	34.2	51.3	34.2	34.2	39.90	8.06
Alkalinity (mg/L CaCO3)	40	35	35	30	36.67	2.36
pH	7.23	6.56	6.5	7.6	6.76	0.33
Conductivity (µS/cm)	62.3	62.4	65.3	61.4	63.33	1.39
Temperature (oC)	20.4	20.6	20.1	22.4	20.37	0.21

Table A 17. Microcystin-LR Standard Curve: 0,4,20,10, Aug 9

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	24	11
1	54	53
10	498	209
20	1095	467

Table A 18. Geosmin Standard Curve: 0,4,20,10, Aug 9

Standard Curve	Area
5	1801
10	3976
25	10671

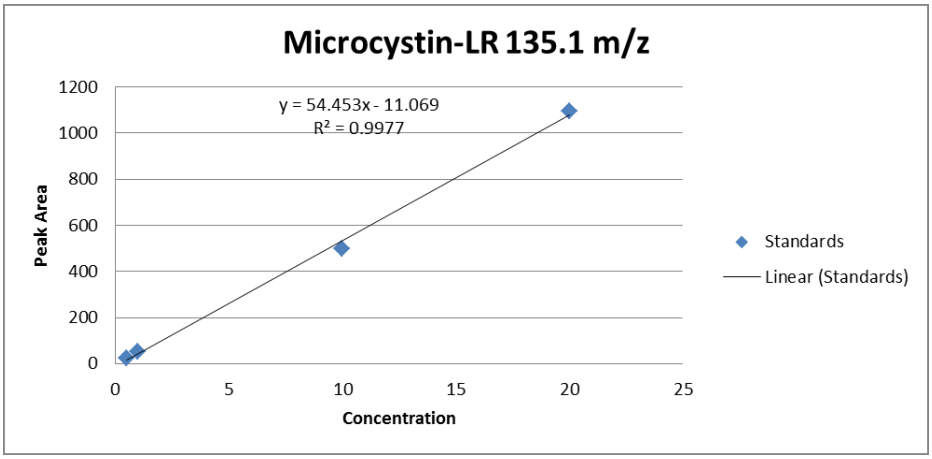


Figure A 16. Microcystin-LR 135.1 ion curve: 0,4,20,10, Aug 9

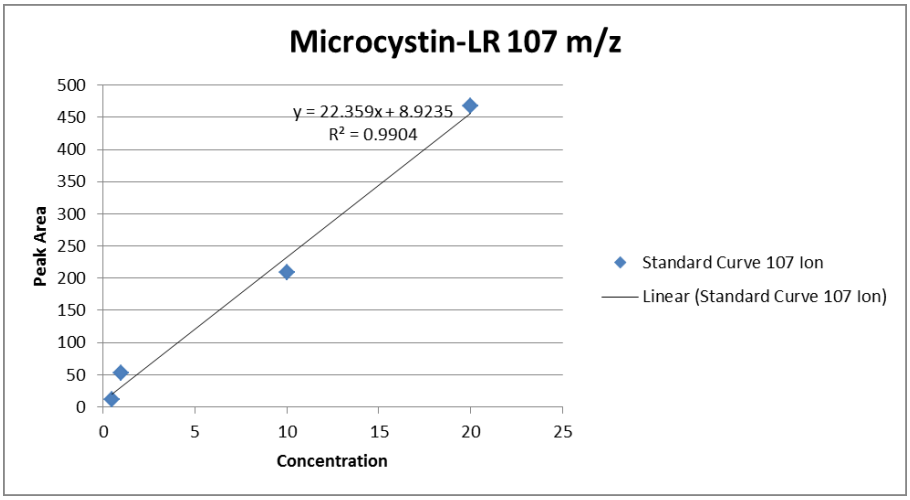


Figure A 17. Microcystin-LR 107 ion curve: 0,4,20,10, Aug 9

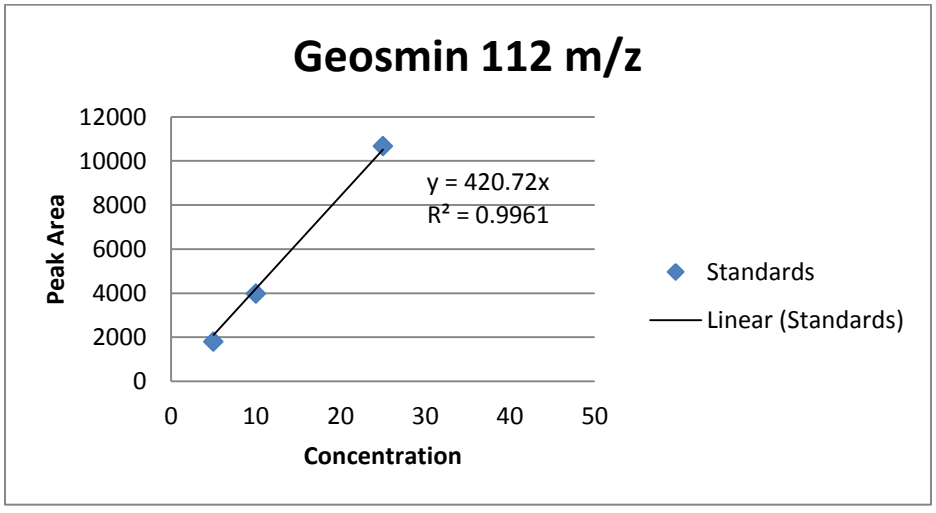


Figure A 18. Geosmin 112 ion curve: 0,4,20,10, Aug 9

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**30**	**10**	**19-Jul**

Table A 19. Sample 0,4,30,10, Jul 19 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	26	19	25	2486.5	23.33	3.09
Area 2	0	13	15	1093.3	9.33	6.65
Concentration (ug/L)	0	0.98	1.17	4.04	0.72	0.51
Geosmin						
Area 1	2399	2130	2946	12219	2491.67	339.51
Concentration (ng/L)	6.28	5.58	7.72	32.01	6.53	0.89
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	30	40.00	0.00
pH	7.1	7.03	7.06	7.6	7.06	0.03
Conductivity (µS/cm)	62.8	62.8	61.8	61.4	62.47	0.47
Temperature (oC)	19.8	19.8	19.9	22.4	19.83	0.05

Table A 20. Microcystin-LR Standard Curve: 0,4,30,10, Jul 19

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.8	24	0
1	30	15
5	146	59
10	261	106
20	669	265
40	1271	511

Table A 21. Geosmin Standard Curve: 0,4,30,10, Jul 19

Standard Conc.	Area
1	362
5	2335
10	4034
25	10217
50	18665

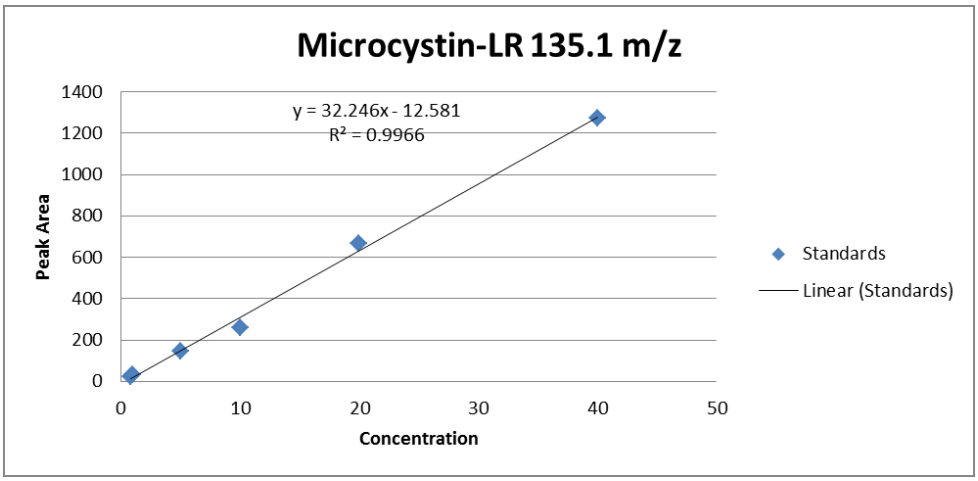


Figure A 19. Microcystin-LR 135.1 ion curve: 0,4,30,10, Jul 19

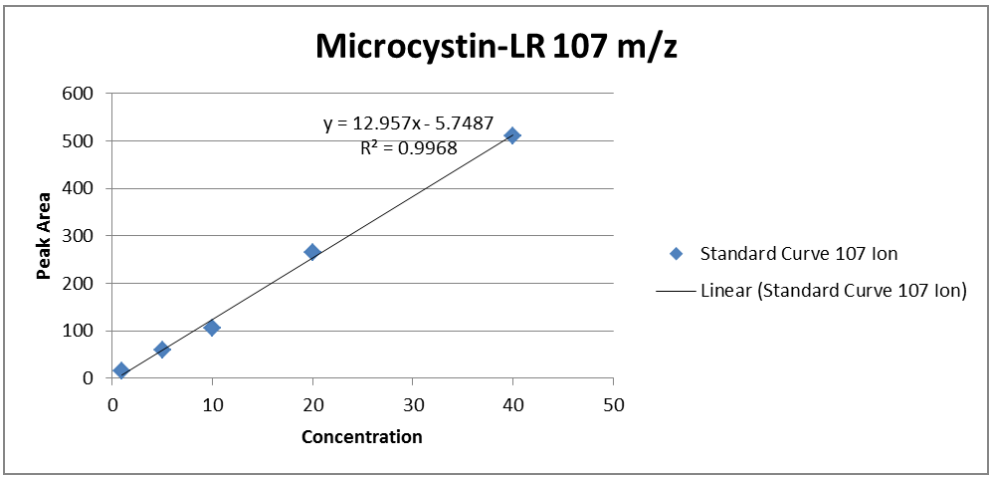


Figure A 20. Microcystin-LR 107 ion curve: 0,4,30,10, Jul 19

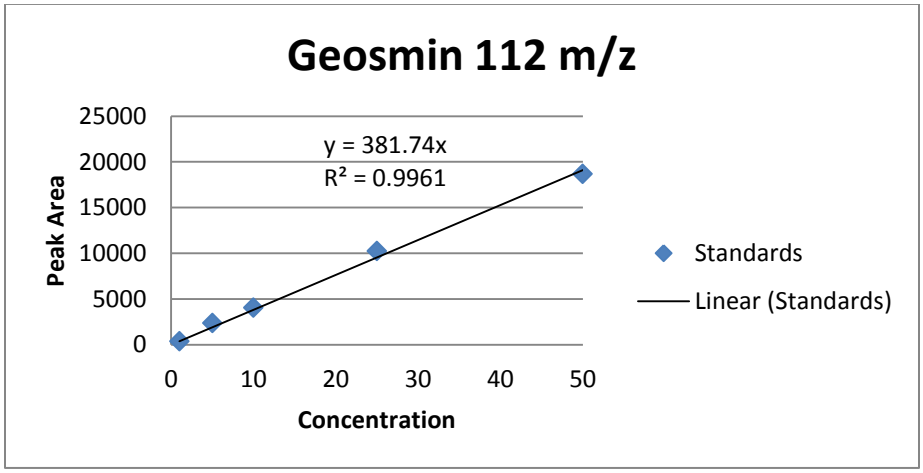


Figure A 21. Geosmin 112 ion curve: 0,4,30,10, Jul 19

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**10**	**10**	**17-Oct**

Table A 22. Sample 0,6,10,10, Oct-17 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	54	46	43	152	47.67	4.64
Area 2	26	20	22	74	22.67	2.49
Concentration (ug/L)	1.99	1.68	1.56	5.79	1.74	0.18
Geosmin						
Area 1	2366	2384	2431	6379	2393.6667	27.40
Concentration (ng/L)	2.17	2.19	2.23	5.86	2.1966667	0.02
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	6.9	6.8	6.8	6.9	6.83	0.05
Conductivity (µS/cm)	70.2	70.5	70.8	69	70.50	0.24
Temperature (oC)	19	19.2	18.9	19.7	19.03	0.12

Table A 23. Microcystin-LR Standard Curve: 0,6,10,10, Oct-17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	12	8
20	525	232
40	1029	479

Table A 24. Geosmin Standard Curve: 0,6,10,10, Oct-17

Standard Conc.	Area
1	779
5	6320
10	11982
25	26628

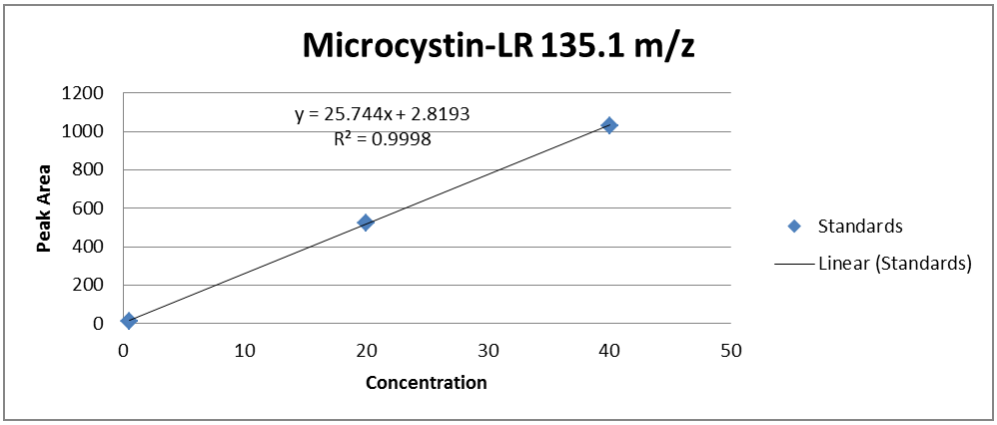


Figure A 22. Microcystin-LR 135.1 ion curve: 0,6,10,10, Oct-17

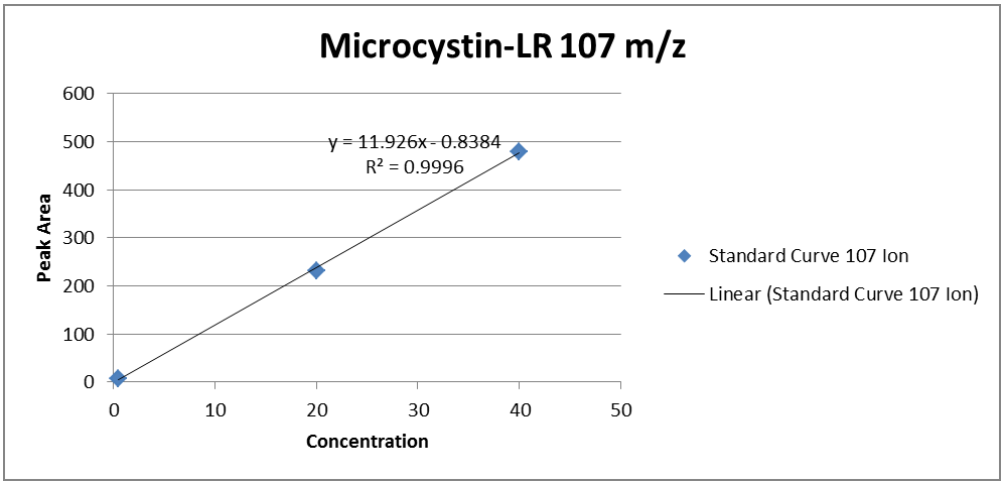


Figure A 23. Microcystin-LR 107 ion curve: 0,6,10,10, Oct-17

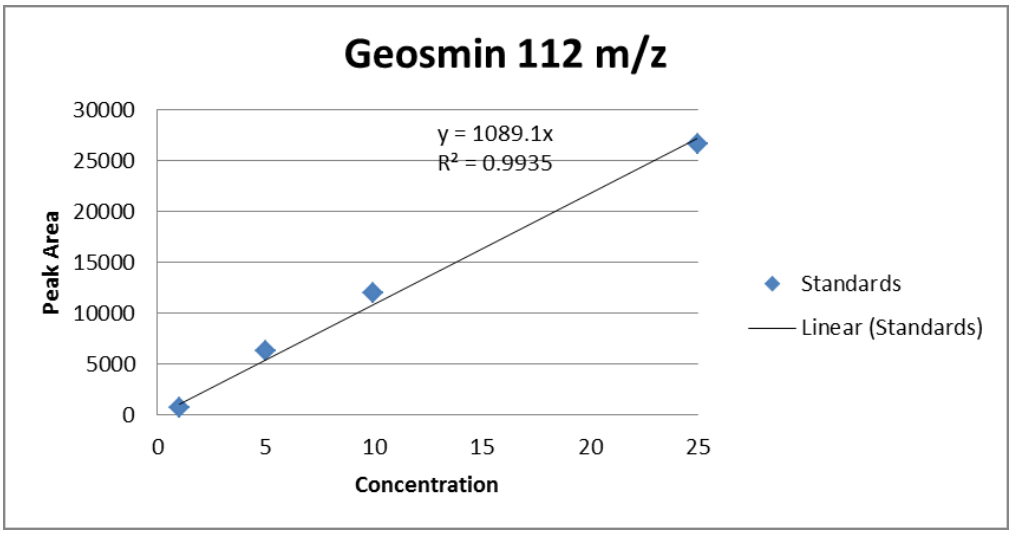


Figure A 24. Geosmin 112 ion curve: 0,6,10,10, Oct-17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**20**	**10**	**16-Nov**

Table A 25. Sample 0,6,20,10, Nov-16 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	315.7	302.7	271.4	895.4	296.60	18.59
Area 2	156.7	138.2	119.4	428	138.10	15.23
Concentration (ug/L)	2.43	2.21	1.91	7.84	2.18	0.21
Geosmin						
Area 1	1558	1512	1361	26480	1477	84.15
Concentration (ng/L)	1.78	1.72	1.55	30.18	1.6833333	0.10
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	35	30.00	0.00
pH	6.9	7	7.1	6.9	7.00	0.08
Conductivity (µS/cm)	51.7	50.9	50.1	45	50.90	0.65
Temperature (oC)	20.1	20.1	21.1	17.9	20.43	0.47

Table A 26. Microcystin-LR Standard Curve: 0,6,20,10, Nov-16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	66.2	32.3
1	141.4	74.3
20	2355	1110
40	4194	1987

Table A 27. Geosmin Standard Curve: 0,6,20,10, Nov-16

Standard Conc.	Area
1	469
5	3664
10	6254
25	22220
50	44308

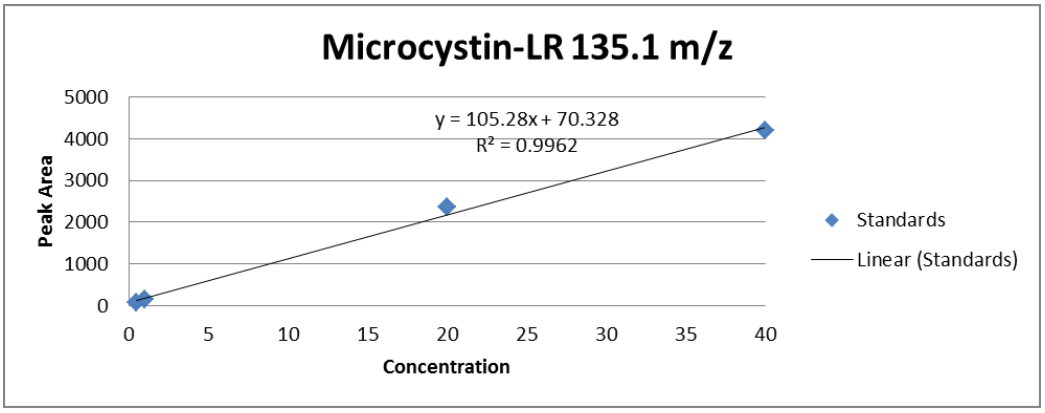


Figure A 25. Microcystin-LR 135.1 ion curve: 0,6,20,10, Nov-16

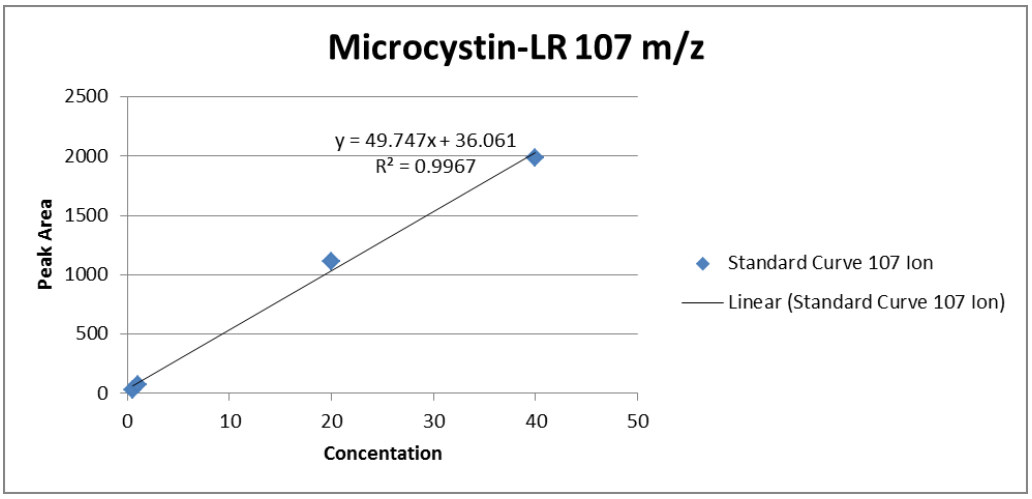


Figure A 26. Microcystin-LR 107 ion curve: 0,6,20,10, Nov-16

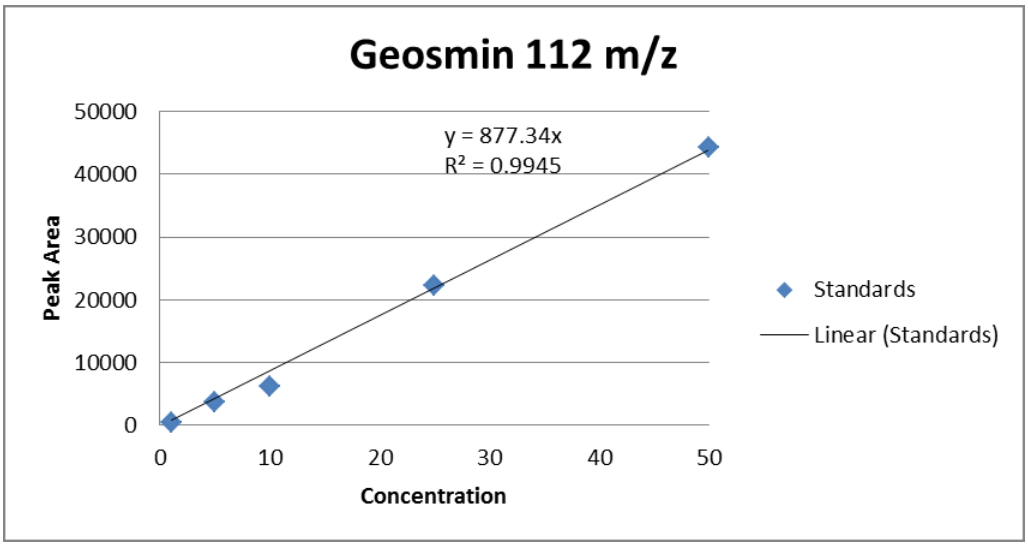


Figure A 27. Geosmin 112 ion curve: 0,6,20,10, Nov-16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**30**	**10**	**16-Nov**

Table A 28. Sample 0,6,10,10, Nov-16 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	202.3	230.3	260.5	895.4	231.03	23.77
Area 2	106.2	93.9	112.3	428	104.13	7.65
Concentration (ug/L)	1.25	1.52	1.53	7.84	1.43	0.13
Geosmin						
Area 1	1879	1877	1903	26480	1886.3333	11.81
Concentration (ng/L)	2.14	2.14	2.17	30.18	2.15	0.01
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	30	35	33.33	2.36
pH	6.9	6.9	7.1	6.9	6.97	0.09
Conductivity (µS/cm)	45.5	45.6	52.9	45	48.00	3.47
Temperature (oC)	18.3	19.4	19.8	17.9	19.17	0.63

Table A 29. Microcystin-LR Standard Curve: 0,6,10,10, Nov-16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	66.2	32.3
1	141.4	74.3
20	2355	1110
40	4194	1987

Table A 30. Geosmin Standard Curve: 0,6,10,10, Nov-16

Standard Conc.	Area
1	469
5	3664
10	6254
25	22220
50	44308

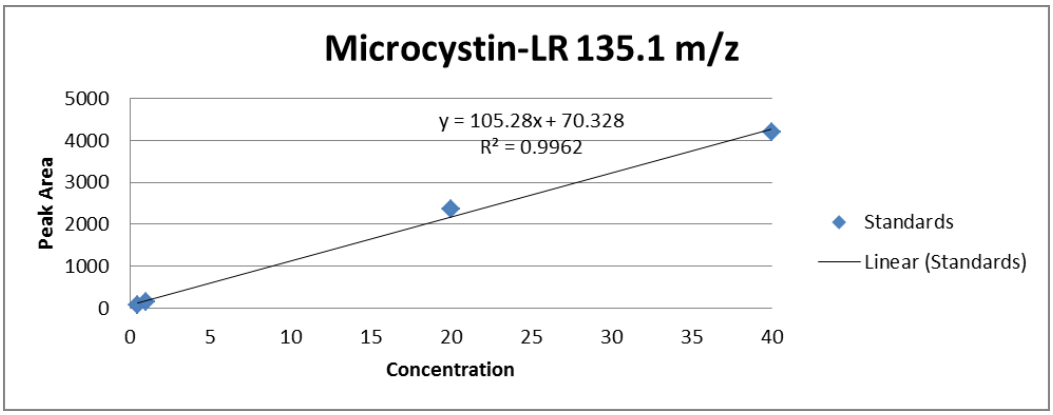


Figure A 28. Microcystin-LR 135.1 ion curve: 0,6,10,10, Nov-16

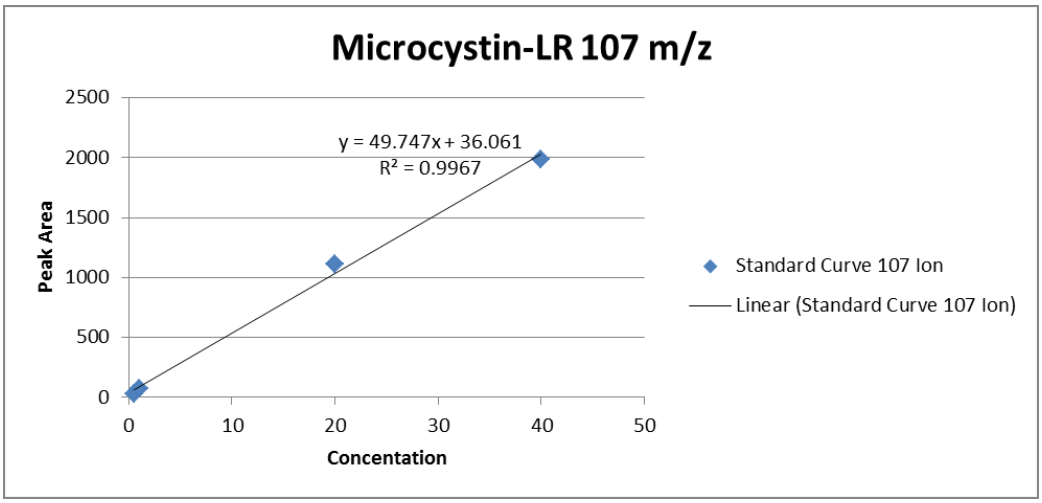


Figure A 29. Microcystin-LR 107 ion curve: 0,6,10,10, Nov-16

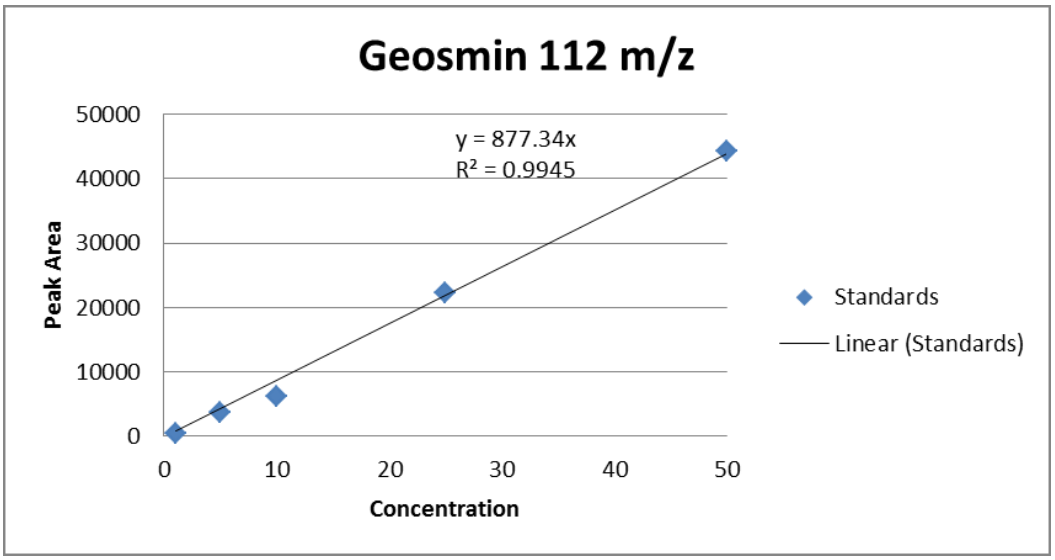


Figure A 30. Geosmin 112 ion curve: 0,6,10,10, Nov-16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**50**	**10**	**17-Oct**

Table A 31. Sample 0,2,10,10, Jan-11 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	53	47	19	112	39.67	14.82
Area 2	22	7	8	51	12.33	6.85
Concentration (ug/L)	2.39	2.06	0.51	5.65	1.65	0.82
Geosmin						
Area 1	38367	37522	39283	71744	38390.667	719.12
Concentration (ng/L)	3.23	3.35	3.18	50.48	3.2533333	0.07
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7	7.1	7.2	6.9	7.10	0.08
Conductivity (µS/cm)	72.9	72.9	72.7	72.2	72.83	0.09
Temperature (oC)	19	18.8	18.6	19.1	18.80	0.16

Table A 32. Microcystin-LR Standard Curve: 0,2,10,10, Jan-11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	1	1
1	19	9
10	242	104
20	347	159

Table A 33. Geosmin Standard Curve: 0,2,10,10, Jan-11

Standard Conc.	Area
1	959
5	5374
10	12644
25	32248
50	73188

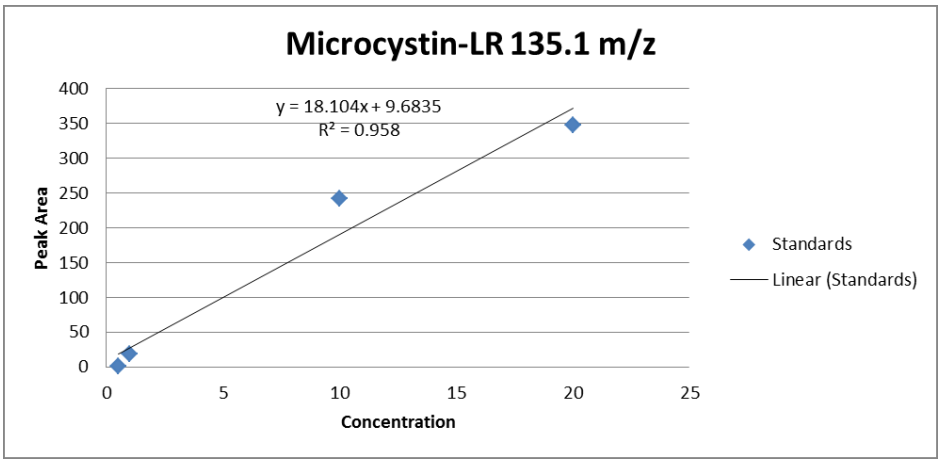


Figure A 31. Microcystin-LR 135.1 ion curve: 0,2,10,10, Jan-11

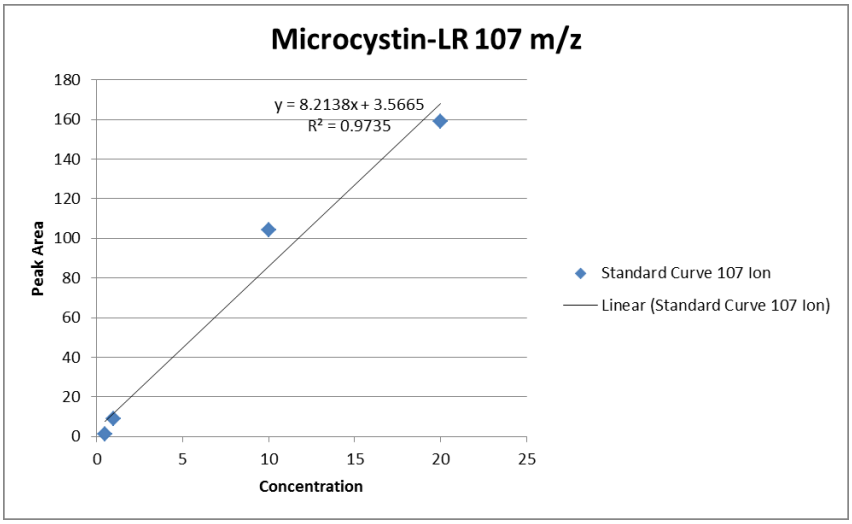


Figure A 32. Microcystin-LR 107 ion curve: 0,2,10,10, Jan-11

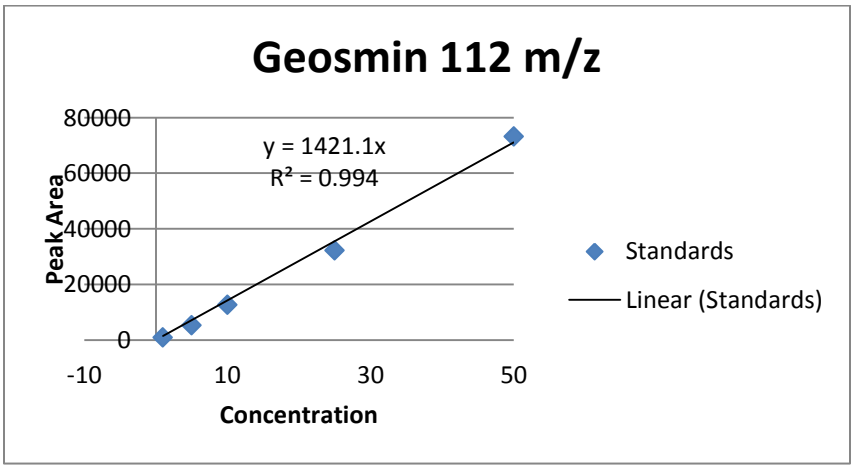


Figure A 33. Geosmin 112 ion curve: 0,2,10,10, Jan-11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**10**	**10**	**16-Nov**

Table A 34. Sample 0,2,10,10, Jan-11 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	36.7	37.4	14.8	130	29.63	10.49
Area 2	17.4	20.7	7.7	53.8	15.27	5.52
Concentration (ug/L)	2.67	2.73	0.67	11.2	2.02	0.96
Geosmin						
Area 1	6960	3579	6604	15323	5714.3333	1516.89
Concentration (ng/L)	4.68	2.41	4.44	10.31	3.84	1.02
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	6.9	6.9	7	6.9	6.93	0.05
Conductivity (µS/cm)	45.21	45.2	45.3	45.4	45.24	0.04
Temperature (oC)	21.2	21.4	21.3	21	21.30	0.08

Table A 35. Microcystin-LR Standard Curve: 0,2,10,10, Jan-11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11.9	4.9
1	19.5	5.9
20	226.4	106.3

Table A 36. Geosmin Standard Curve: 0,2,10,10, Jan-11

Standard Conc.	Area
1	1063
5	4184
10	15624
25	33298
50	76419

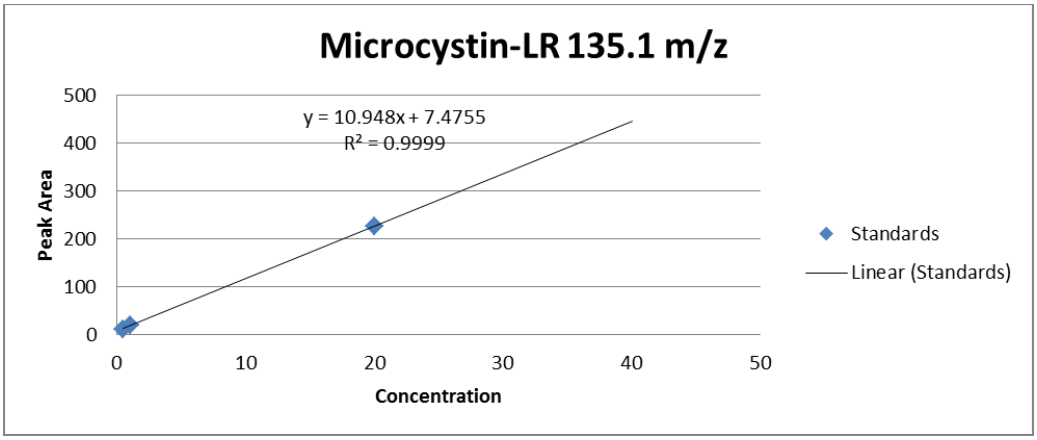


Figure A 34. Microcystin-LR 135.1 ion curve: 0,2,10,10, Jan-11

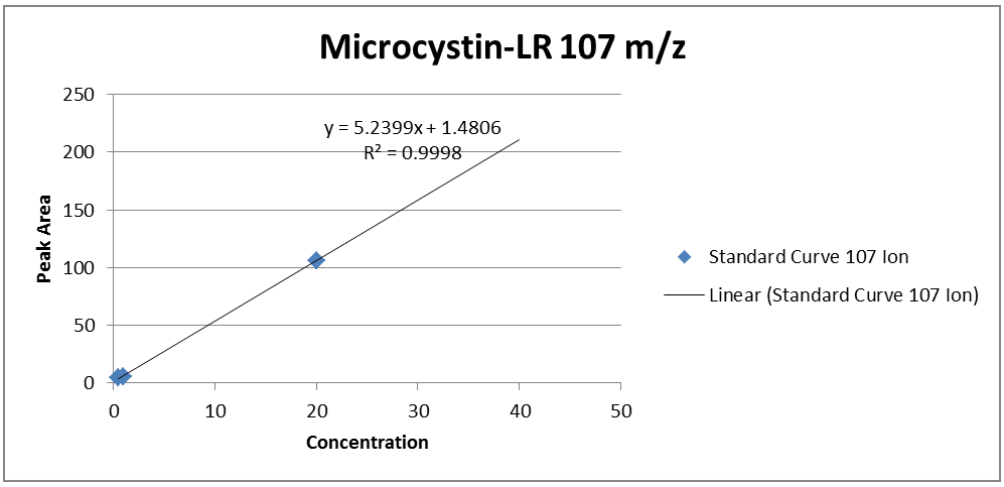


Figure A 35. Microcystin-LR 107 ion curve: 0,2,10,10, Jan-11

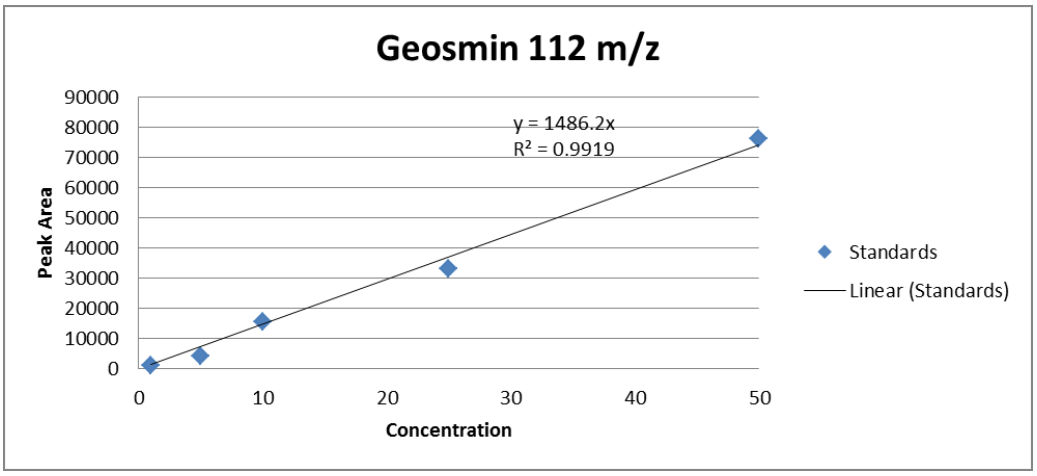


Figure A 36. Geosmin 112 ion curve: 0,2,10,10, Jan-11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**20**	**10**	**3-Oct**

Table A 37. Sample 0,10,20,10, Oct-3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	92	68	70	236	76.67	10.87
Area 2	38	28	32	109	32.67	4.11
Concentration (ug/L)	4.55	3.22	3.33	12.5	3.89	0.67
Geosmin						
Area 1	4637	4632	4320	32952	4529.6667	148.27
Concentration (ng/L)	3.26	3.26	3.04	23.19	3.19	0.10
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	35	35	35	33.33	2.36
pH	7.04	7.13	7.11	7.13	7.09	0.04
Conductivity (µS/cm)	73.2	73.6	73.4	71.9	73.40	0.16
Temperature (oC)	19	19.1	19.1	20.4	19.07	0.05

Table A 38. Microcystin-LR Standard Curve: 0,10,20,10, Oct-3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	1	1
1	19	9
10	242	104
20	347	159

Table A 39. Geosmin Standard Curve: 0,10,20,10, Oct-3

Standard Conc.	Area
1	959
5	5374
10	12644
25	32248
50	73188

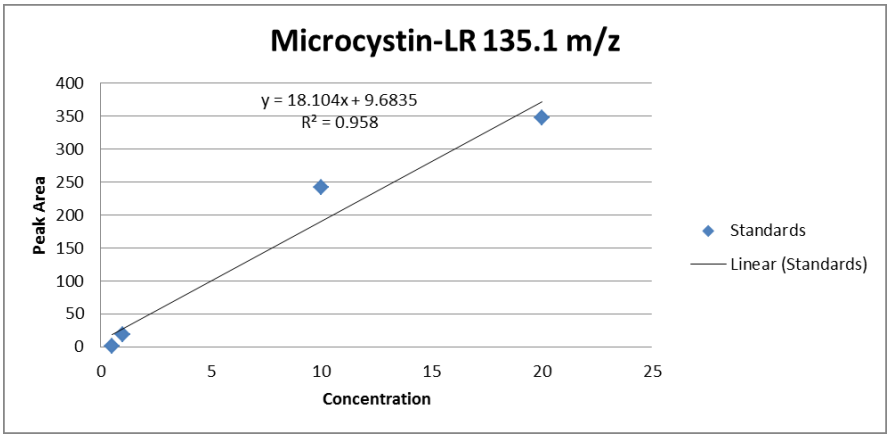


Figure A 37. Microcystin-LR 135.1 ion curve: 0,10,20,10, Oct-3

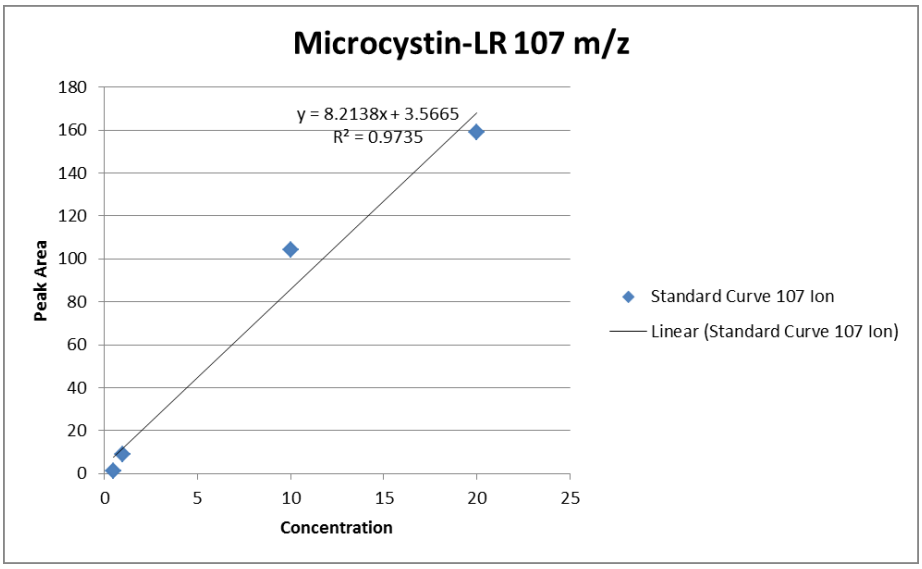


Figure A 38. Microcystin-LR 107 ion curve: 0,10,20,10, Oct-3

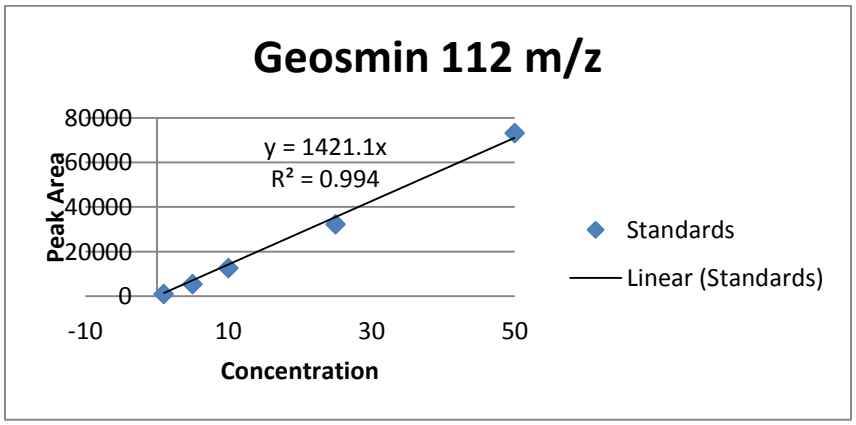


Figure A 39. Geosmin 112 ion curve: 0,10,20,10, Oct-3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**30**	**10**	**11-Jan**

Table A 40. Sample 0,10,30,10, Jan-11 results

	Replicate1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	2485	1096	198	7330	1259.67	940.81
Area 2	1236	509	61	3227	602.00	484.18
Concentration (ug/L)	2.96	1.4	0.4	8.38	1.59	1.05
Geosmin						
Area 1	10107	10147	10371	25789	10208.333	116.18
Concentration (ng/L)	11.96	12.01	12.27	30.51	12.08	0.14
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.8	7.8	7.9	7.8	7.83	0.05
Conductivity (µS/cm)	60.4	60.3	60.3	60.6	60.33	0.05
Temperature (oC)	19.4	19.9	19.8	19.9	19.70	0.22

Table A 41. Microcystin-LR Standard Curve: 0,10,30,10, Jan-11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	425.1	208.4
1	685.9	287
10	8614	3896
20	17796	7545

Table A 42. Geosmin Standard Curve 0,10,30,10, Jan-11

Standard Conc.	Area
1	374
5	4701
10	6433
25	21275
50	42553

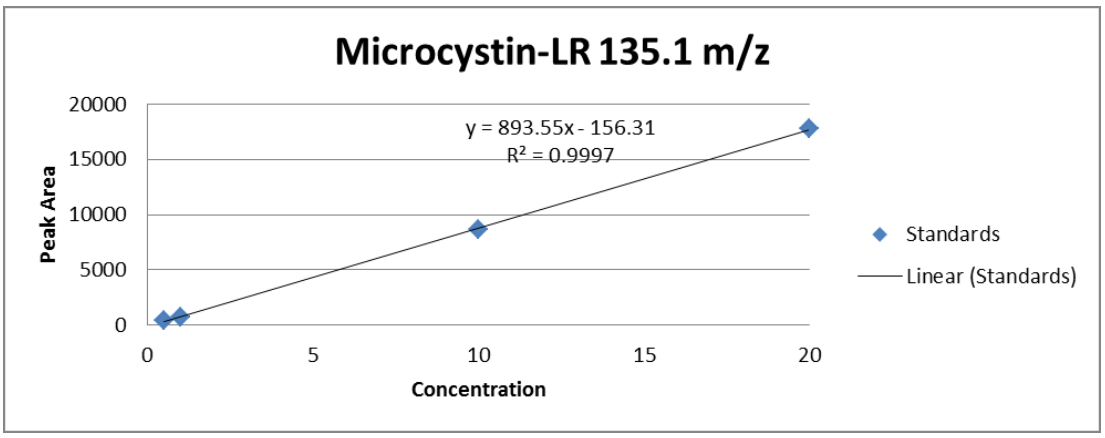


Figure A 40. Microcystin-LR 135.1 ion curve: 0,10,30,10, Jan-11

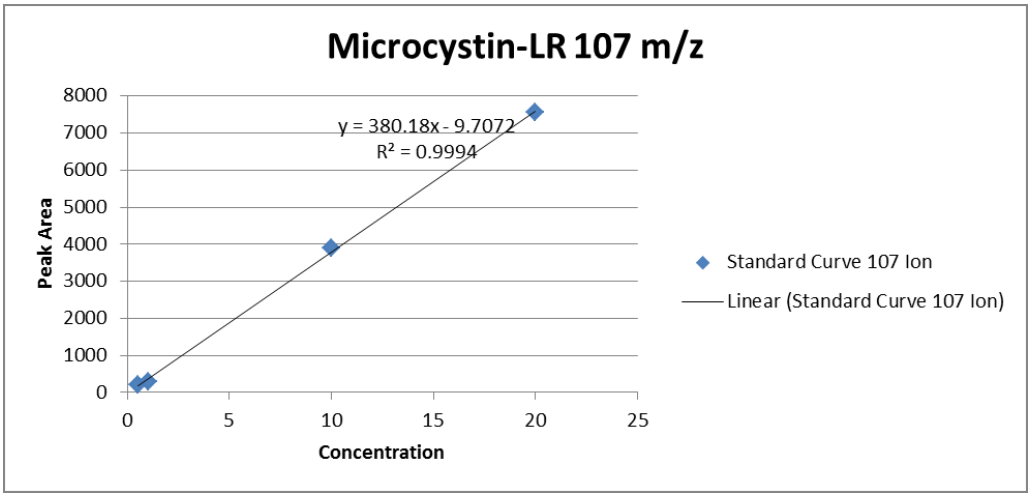


Figure A 41. Microcystin-LR 107 ion curve: 0,10,30,10, Jan-11

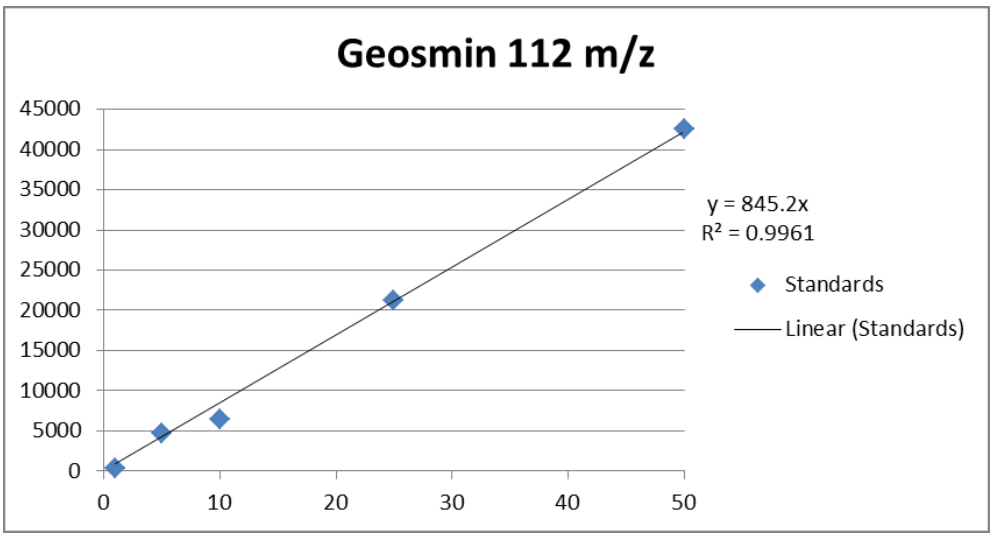


Figure A 42. Geosmin 112 ion curve: 0,10,30,10, Jan-11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**50**	**10**	**3-Oct**

Table A 43. Sample 0,10,50,10, Oct 3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	38	25	43	223	35.33	7.59
Area 2	16	11	18	97	15.00	2.94
Concentration (ug/L)	2.05	1.45	2.29	10.69	1.93	0.35
Geosmin						
Area 1	12308	12176	11908	64556	12130.667	166.42
Concentration (ng/L)	9.72	9.62	9.41	51	9.58	0.13
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	35	40.00	0.00
pH	7.09	7.2	7.2	6.82	7.16	0.05
Conductivity (µS/cm)	73.9	74.8	74.6	72.8	74.43	0.39
Temperature (oC)	20.9	21.1	21.1	20	21.03	0.09

Table A 44. Microcystin-LR Standard Curve: 0,10,50,10, Oct 3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11	5
1	26	13
10	176	82
20	438	204

Table A 45. Geosmin Standard Curve: 0,10,50,10, Oct 3

Standard Conc.	Area
1	1075
5	3245
10	11843
25	29177
50	65000

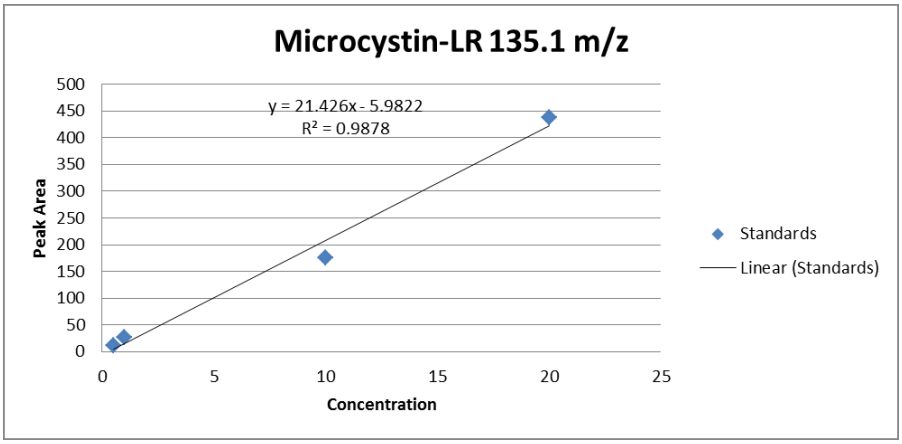


Figure A 43. Microcystin-LR 135.1 ion curve: 0,10,50,10, Oct 3

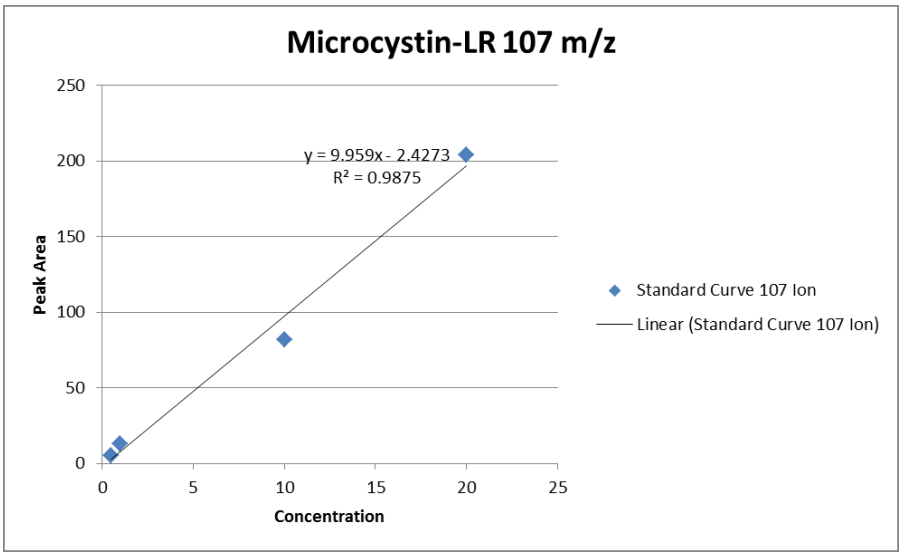


Figure A 44. Microcystin-LR 107 ion curve: 0,10,50,10, Oct 3

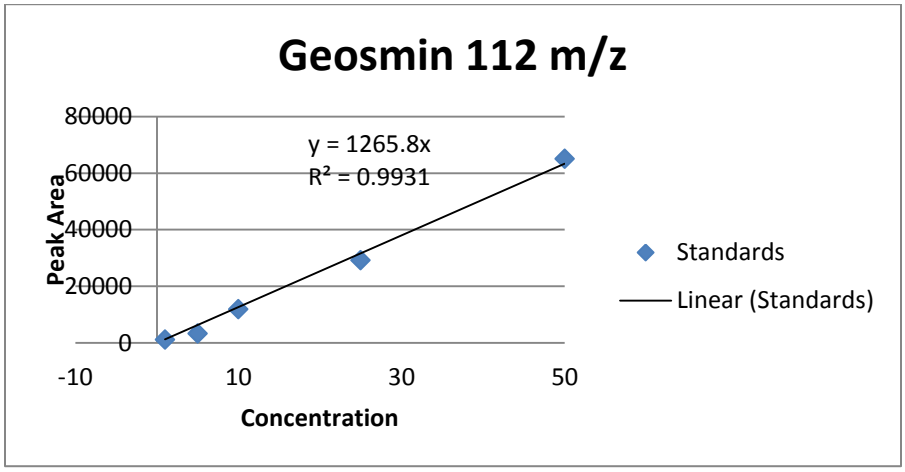


Figure A 45. Geosmin 112 ion curve: 0,10,50,10, Oct 3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**10**	**20**	**22-Feb**

Table A 46. Sample 0,2,10,20, Feb 22 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	6	40	31	158.39	25.67	14.38
Area 2	6	23	17	96.4	15.33	7.04
Concentration (ug/L)	0.01	0.48	0.35	2.01	0.28	0.20
Geosmin						
Area 1	1398	1462	1398	9011	1419.33333	30.17
Concentration (ng/L)	1.54	1.61	1.54	9.94	1.56	0.03
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7	7.2	7.3	7.1	7.17	0.12
Conductivity (µS/cm)	63.3	62.7	62.5	62.8	62.83	0.34
Temperature (oC)	17	17.2	17.1	17.5	17.10	0.08

Table A 47. Microcystin-LR Standard Curve: 0,2,10,20, Feb 22

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	40	33
1	105	45
10	687	360
20	1487	698

Table A 48. Geosmin Standard Curve: 0,2,10,20, Feb 22

Standard Conc.	Area
1	475
5	2966
10	7200
25	23734

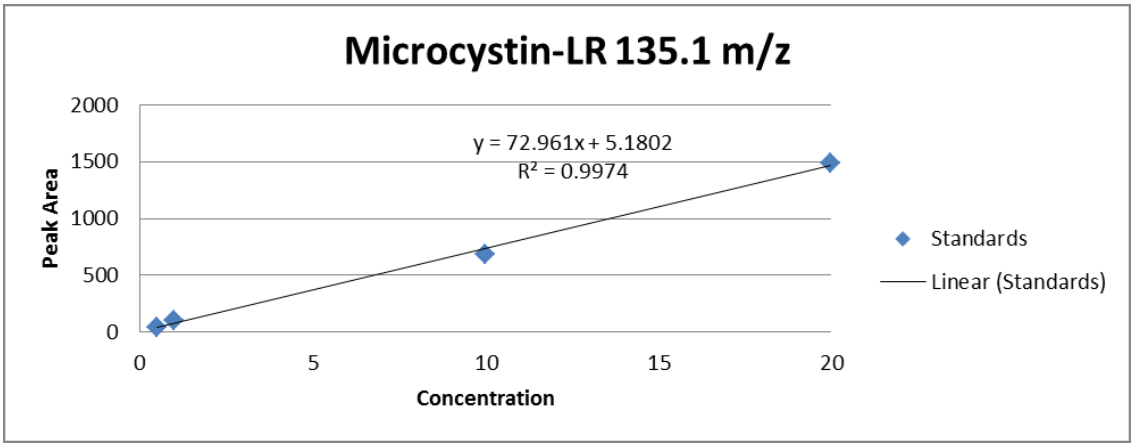


Figure A 46. Microcystin-LR 135.1 ion curve: 0,2,10,20, Feb 22

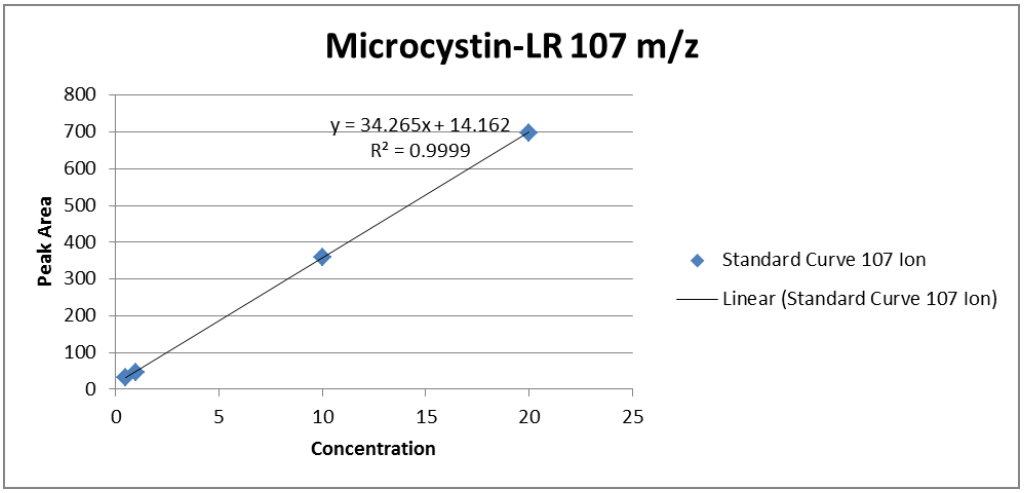


Figure A 47. Microcystin-LR 107 ion curve: 0,2,10,20, Feb 22

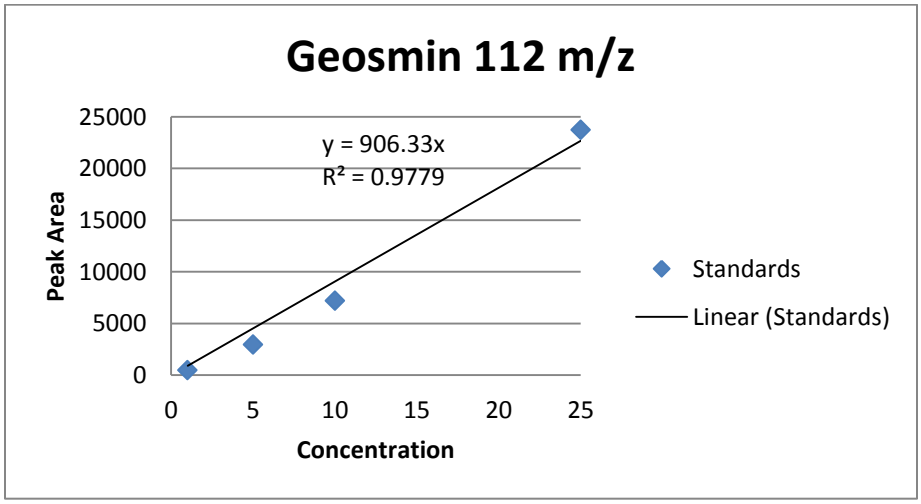


Figure A 48. Geosmin 112 ion curve: 0,2,10,20, Feb 22

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**20**	**20**	**17-Sep**

Table A 49. Sample 0,2,20,20, Sept 17 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	7	6	10	57.85	7.67	1.70
Area 2	0	4	5	24.12	3.00	2.16
Concentration (ug/L)	0	0.19	0.34	2.14	0.18	0.14
Geosmin						
Area 1	960	879	965	17359	934.67	39.42
Concentration (ng/L)	1.1	1.01	1.11	19.95	1.07	0.04
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	40	40.00	0.00
pH	7.29	7.36	7.36	7.4	7.34	0.03
Conductivity (µS/cm)	61.9	61.9	70	62.5	64.60	3.82
Temperature (oC)	18.5	18.4	18.4	19.1	18.43	0.05

Table A 50. Microcystin-LR Standard Curve: 0,2,20,20, Sept 17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	15	8
1	34	11
10	255	111
20	555	247

Table A 51. Geosmin Standard Curve: 0,2,20,20, Sept 17

Standard Conc.	Area
1	878
5	4790
10	8989
25	21550

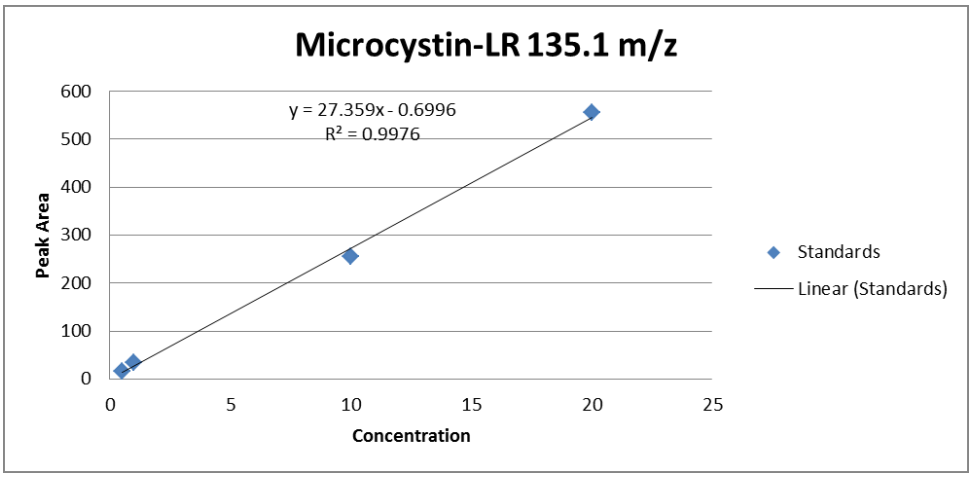


Figure A 49. Microcystin-LR 135.1 ion curve: 0,2,20,20, Sept 17

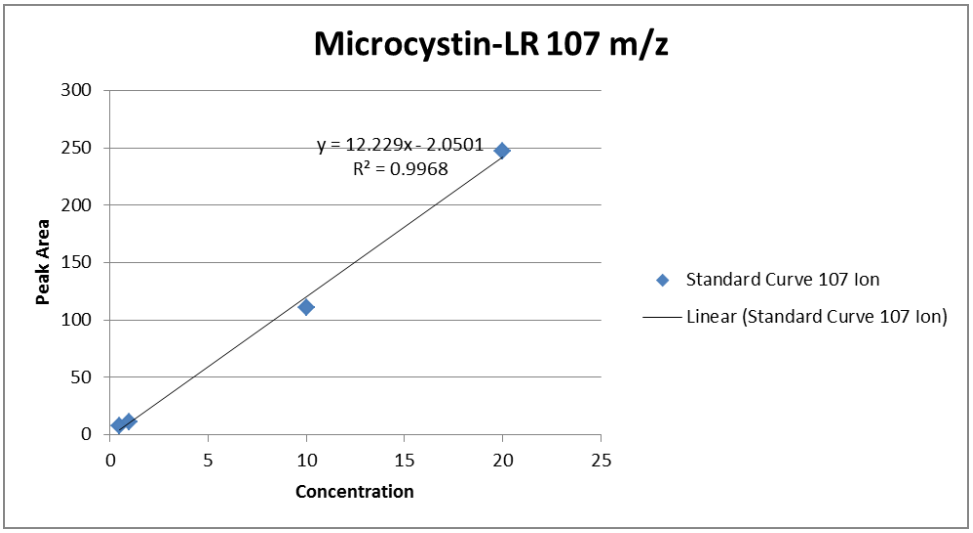


Figure A 50. Microcystin-LR 107 ion curve: 0,2,20,20, Sept 17

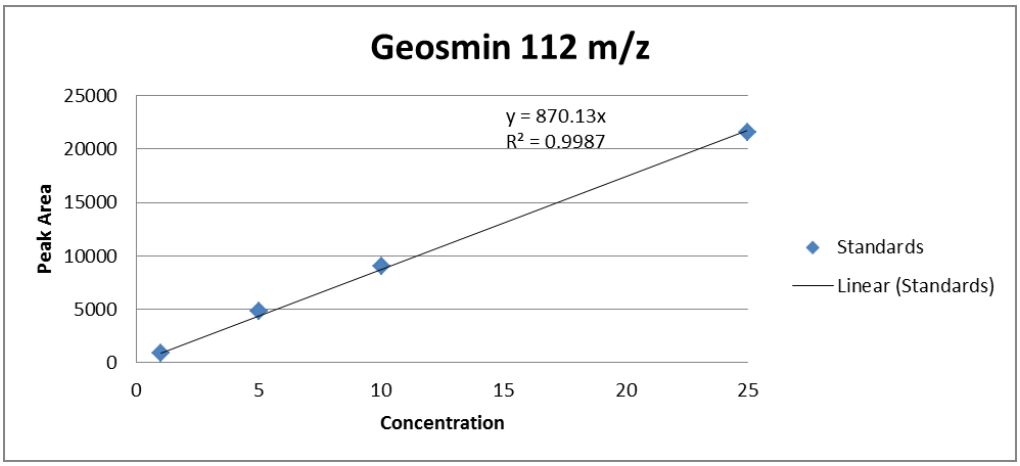


Figure A 51. Geosmin 112 ion curve: 0,2,20,20, Sept 17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**30**	**20**	**25-Jan**

Table A 52. Sample 0,2,30,200, Jan-25 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	0	64.5	206.6	1368.6	90.37	86.30
Area 2	8	120.6	64.4	600	64.33	45.97
Concentration (ug/L)	0	0.34	0.21	2.49	0.18	0.14
Geosmin						
Area 1	2293	2023	2171	19256	2162.33	110.40
Concentration (ng/L)	3.09	2.68	2.9	29.57	2.89	0.17
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.9	7.1	7.2	7.1	7.40	0.36
Conductivity (µS/cm)	60.4	62.5	62.4	60.4	61.77	0.97
Temperature (oC)	19.7	16.9	16.9	20.1	17.83	1.32

Table A 53. Microcystin-LR Standard Curve: 0,2,30,200, Jan-25

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0	0	0
0.5	257.2	124.7
1	506.3	280.4
10	5328	2595
20	10829	4952

Table A 54. Geosmin Standard Curve: 0,2,30,200, Jan-25

Standard Conc.	Area
1	383
5	2743
10	5567
25	15507
50	33193

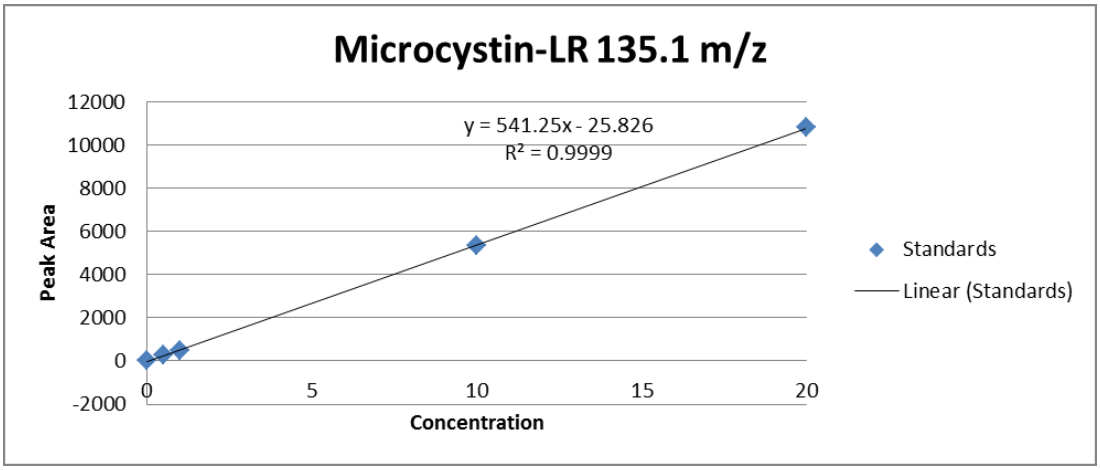


Figure A 52. Microcystin-LR 135.1 ion curve: 0,2,30,200, Jan-25

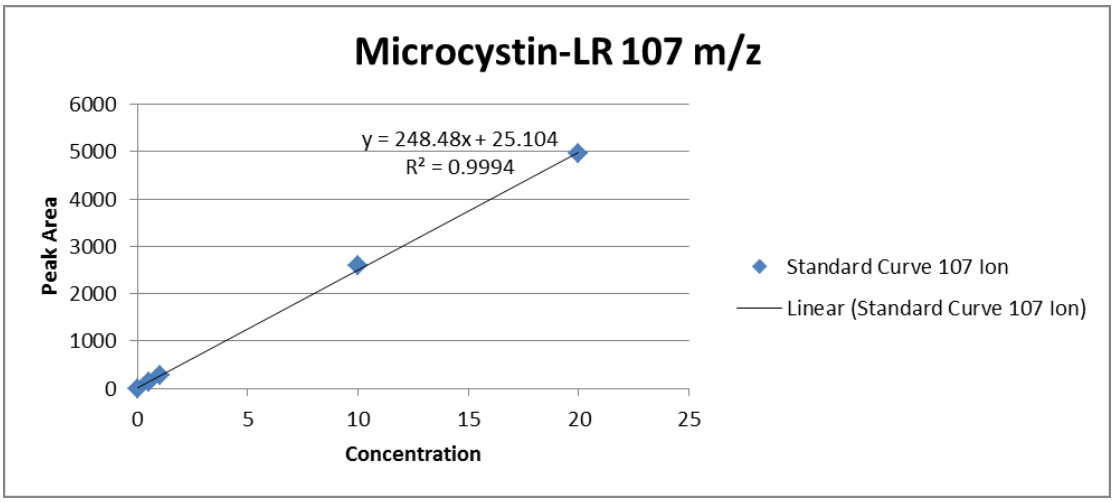


Figure A 53. Microcystin-LR 107 ion curve: 0,2,30,200, Jan-25

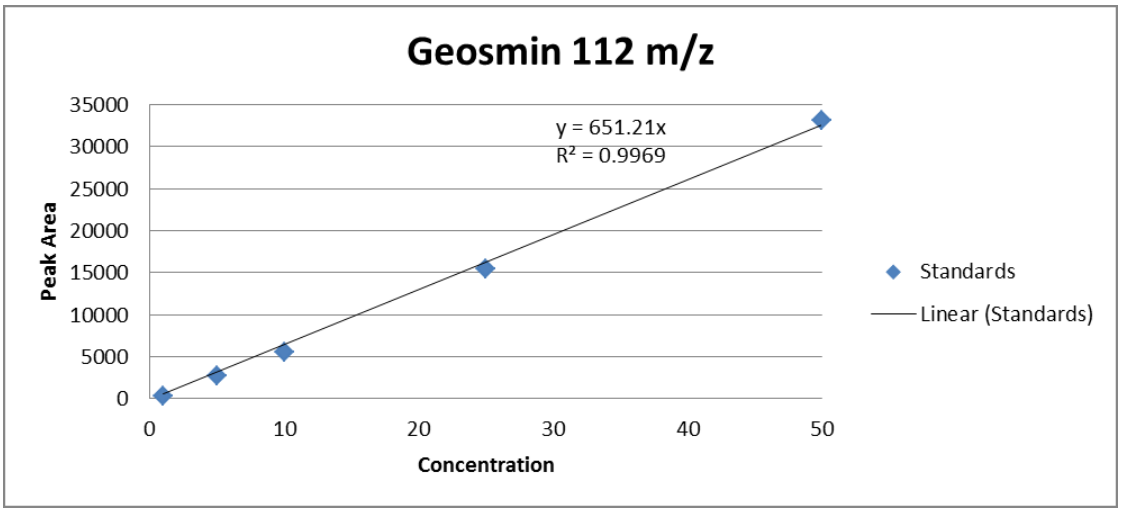


Figure A 54. Geosmin 112 ion curve: 0,2,30,200, Jan-25

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**50**	**20**	**16-Nov**

Table A 55. Sample 0,2,50,20, Nov 16 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	230.3	37.2	374.4	895	213.97	138.14
Area 2	87.5	34.4	194.4	470	105.43	66.54
Concentration (ug/L)	0.47	0.25	0.64	1.48	0.45	0.16
Geosmin						
Area 1	4202	4398	4229	37000	4276.33	86.73
Concentration (ng/L)	5.77	6.04	5.81	50.81	5.87	0.12
Water Quality						
Hardness (mg/L CaCO3)	51.3	34.2	34.2	34.2	39.90	8.06
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.3	7.3	7.3	7.2	7.30	0.00
Conductivity (µS/cm)	59.4	59.8	59.3	60.5	59.50	0.22
Temperature (oC)	19.7	19.9	19.3	19.2	19.63	0.25

Table A 56. Microcystin-LR Standard Curve: 0,2,50,20, Nov 16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
2	1477	671.6
4	3437	1557
40	28772	13265
80	61102	26593

Table A 57. Geosmin Standard Curve: 0,2,50,20, Nov 16

Standard Conc.	Area
1	315
5	2362
10	5891
25	19031

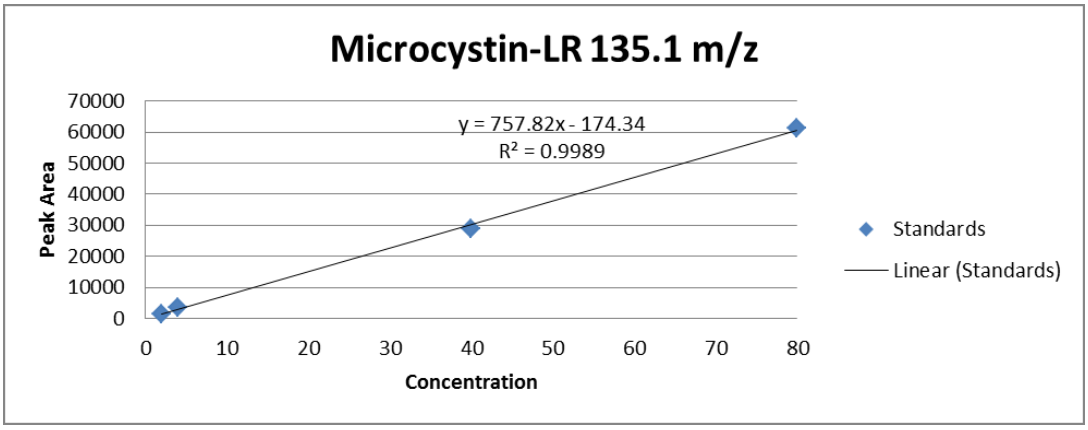


Figure A 55. Microcystin-LR 135.1 ion curve: 0,2,50,20, Nov 16

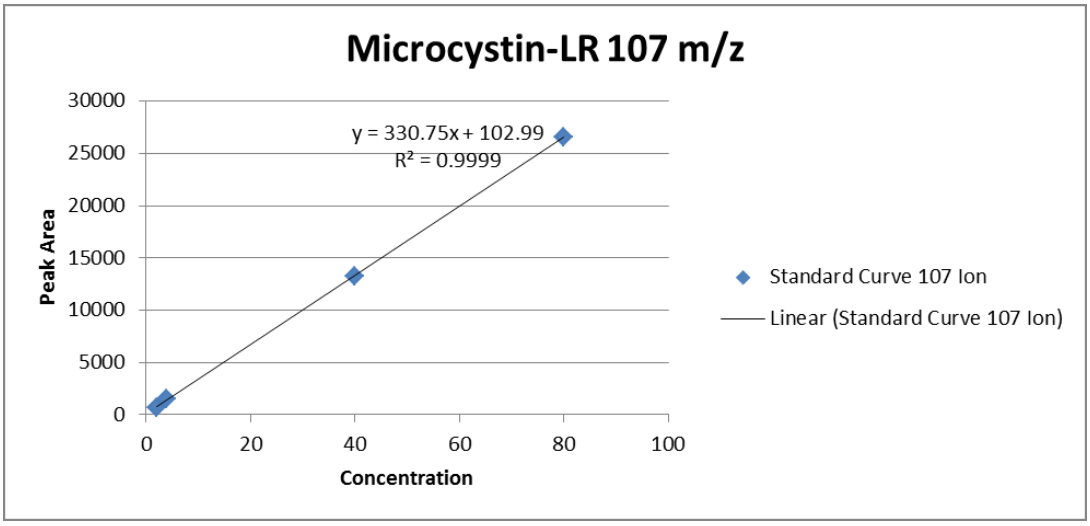


Figure A 56. Microcystin-LR 107 ion curve: 0,2,50,20, Nov 16

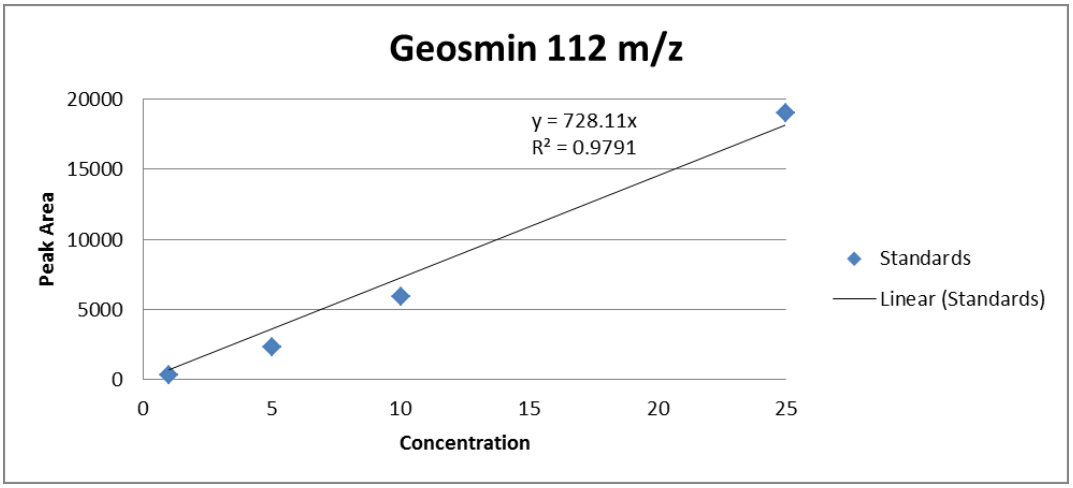


Figure A 57. Geosmin 112 ion curve: 0,2,50,20, Nov 16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**10**	**20**	**25-Jan**

Table A 58. Sample 0,2,10,20, Jan-15 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	418.2	288.6	394.2	1372.9	367.00	56.30
Area 2	237.1	159.3	169.3	954.6	188.57	34.56
Concentration (ug/L)	0.73	0.49	0.69	4.03	0.64	0.10
Geosmin						
Area 1	587	539	542	6340	556	21.95
Concentration (ng/L)	0.47	0.4	0.4	9.74	0.42	0.03
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.5	7.5	7.6	7.5	7.53	0.05
Conductivity (µS/cm)	61	61	61.1	61.1	61.03	0.05
Temperature (oC)	20.7	20.4	21	21	20.70	0.24

Table A 59. Microcystin-LR Standard Curve: 0,2,10,20, Jan-15

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0	0	0
0.5	257.2	124.7
1	506.3	280.4
10	5328	2595
20	10829	4952

Table A 60. Geosmin Standard Curve: 0,2,10,20, Jan-15

Standard Conc.	Area
1	383
5	2743
10	5567
25	15507
50	33193

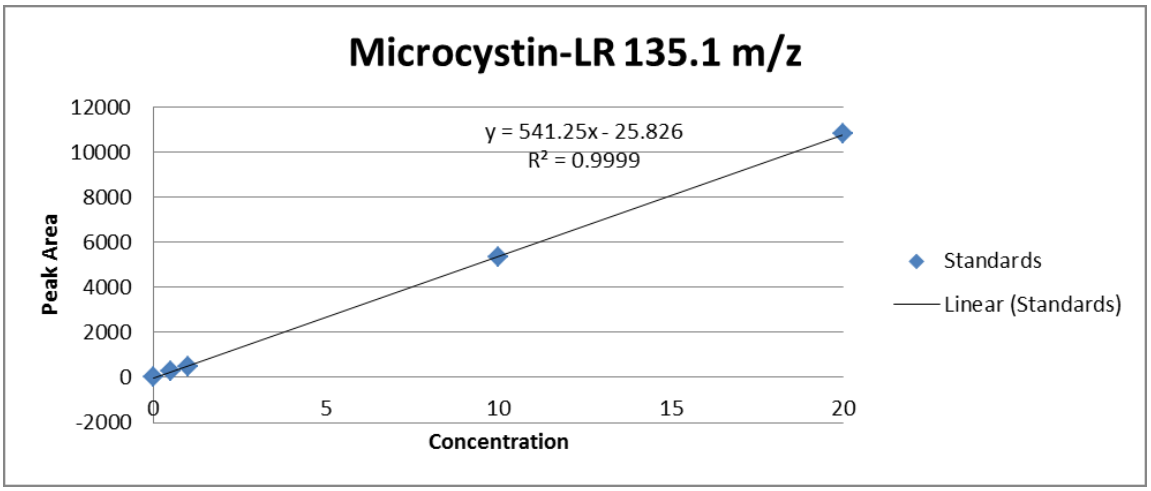


Figure A 58. Microcystin-LR 135.1 ion curve: 0,2,10,20, Jan-15

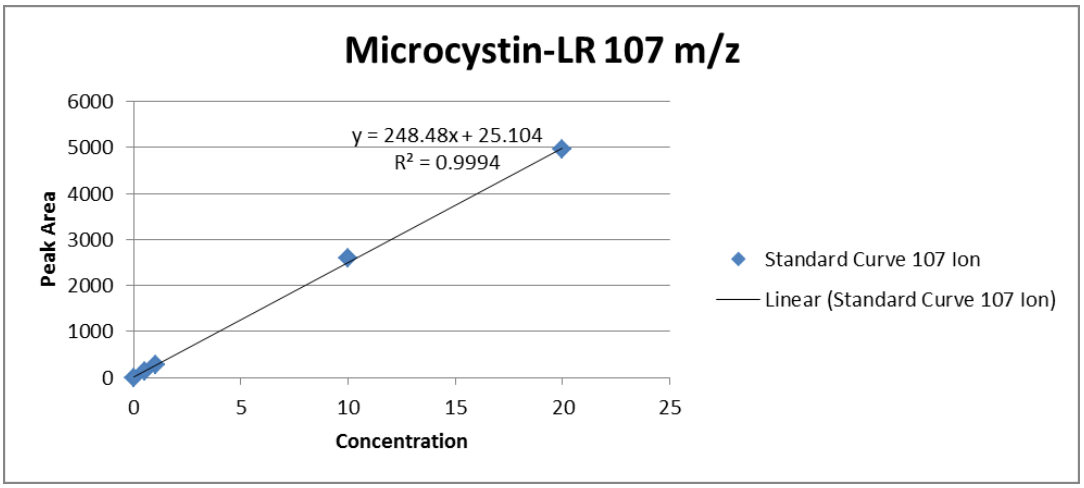


Figure A 59. Microcystin-LR 107 ion curve: 0,2,10,20, Jan-15

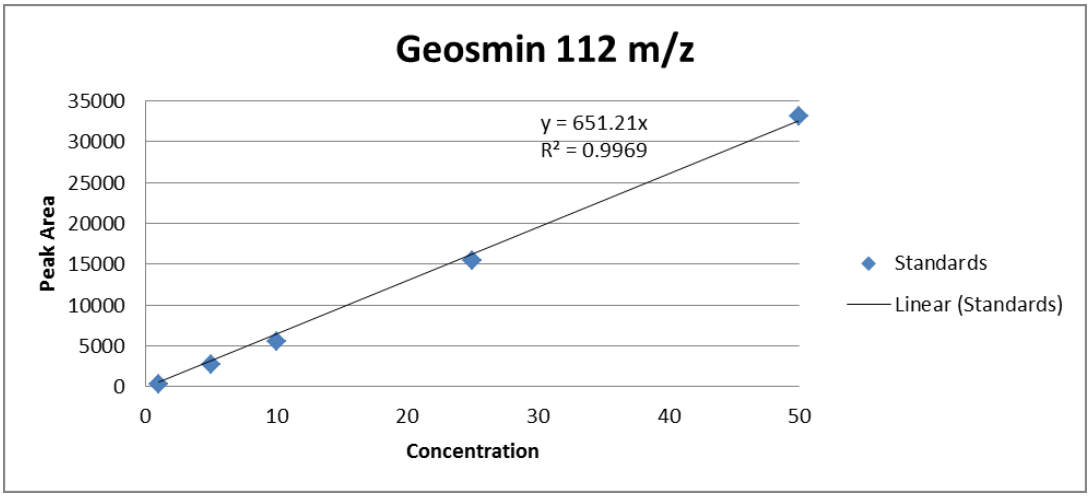


Figure A 60. Geosmin 112 ion curve: 0,2,10,20, Jan-15

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**20**	**20**	**25-Jan**

Table A 61. Sample 0,4,20,20, Jan-15 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	500	124.9	79	2486.5	234.63	188.58
Area 2	238	46.7	39.8	1093.3	108.17	91.85
Concentration (ug/L)	0.72	0.1	0.02	4.04	0.28	0.31
Geosmin						
Area 1	1474	1491	1528	10710	1497.66667	22.54
Concentration (ng/L)	2.73	2.76	2.83	19.85	2.77	0.04
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.6	7.5	7.6	7.6	7.57	0.05
Conductivity (µS/cm)	63.3	63.6	63.7	61.4	63.53	0.17
Temperature (oC)	20.4	20.2	20.8	22.4	20.47	0.25

Table A 62. Microcystin-LR Standard Curve: 0,4,20,20, Jan-15

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.01	70.6	37.2
0.02	86.7	45.1
1	625.9	256.8
10	6111	2669
20	12008	5561

Table A 63. Geosmin Standard Curve: 0,4,20,20, Jan-15

Standard Conc.	Area
1	374
5	1678
10	5053
25	13836

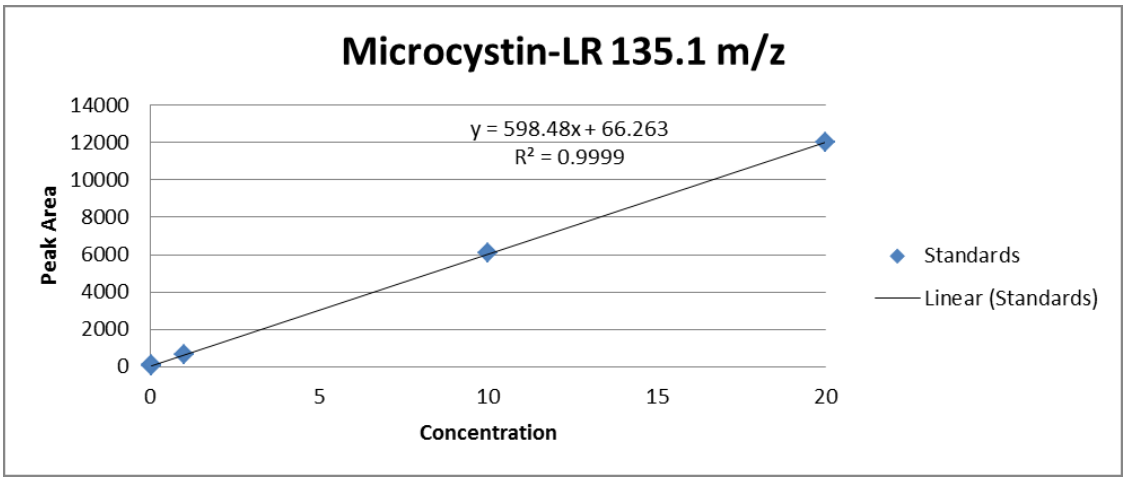


Figure A 61. Microcystin-LR 135.1 ion curve: 0,4,20,20, Jan-15

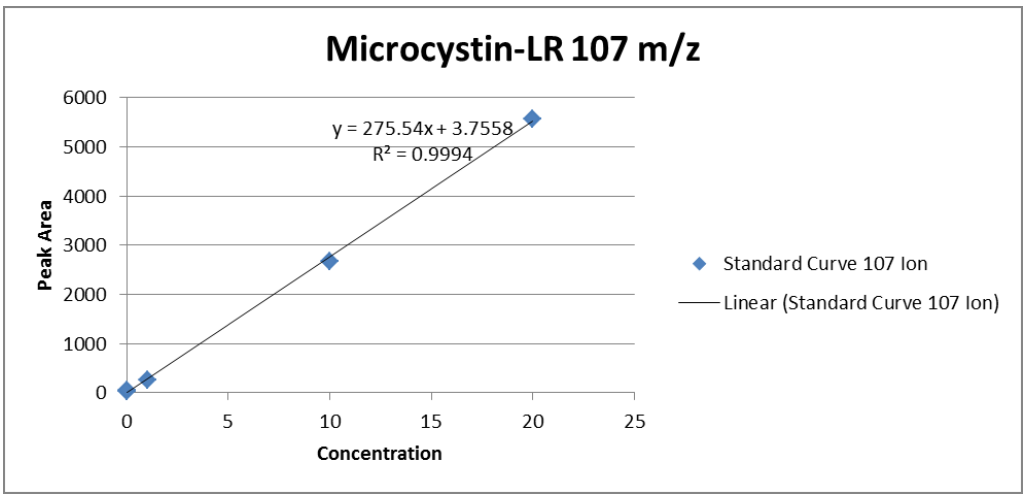


Figure A 62. Microcystin-LR 107 ion curve: 0,4,20,20, Jan-15

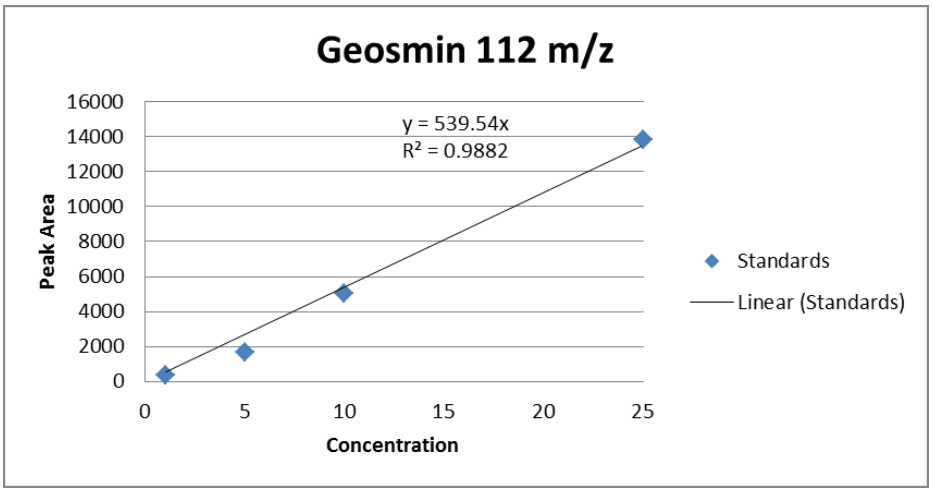


Figure A 63. Geosmin 112 ion curve: 0,4,20,20, Jan-15

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**30**	**20**	**25-Jan**

Table A 64. Sample 0,2,30,20, Jan-15 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	12.2	67.6	60	2486.5	46.60	24.52
Area 2	0	22	28	1093.3	16.67	12.04
Concentration (ug/L)	0	0	0	4.04	0.00	0.00
Geosmin						
Area 1	1047	1079	1198	10710	1108	64.97
Concentration (ng/L)	1.21	1.24	1.38	19.85	1.277	0.07
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.6	7.6	7.5	7.6	7.57	0.05
Conductivity (µS/cm)	63.9	63.8	63.8	61.4	63.83	0.05
Temperature (oC)	21.4	20.4	21.2	22.4	21.00	0.43

Table A 65. Microcystin-LR Standard Curve: 0,2,30,20, Jan-15

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.01	70.6	37.2
0.02	86.7	45.1
1	625.9	256.8
10	6111	2669
20	12008	5561

Table A 66. Geosmin Standard Curve: 0,2,30,20, Jan-15

Standard Conc.	Area
1	530
5	3768
10	9639
25	21446

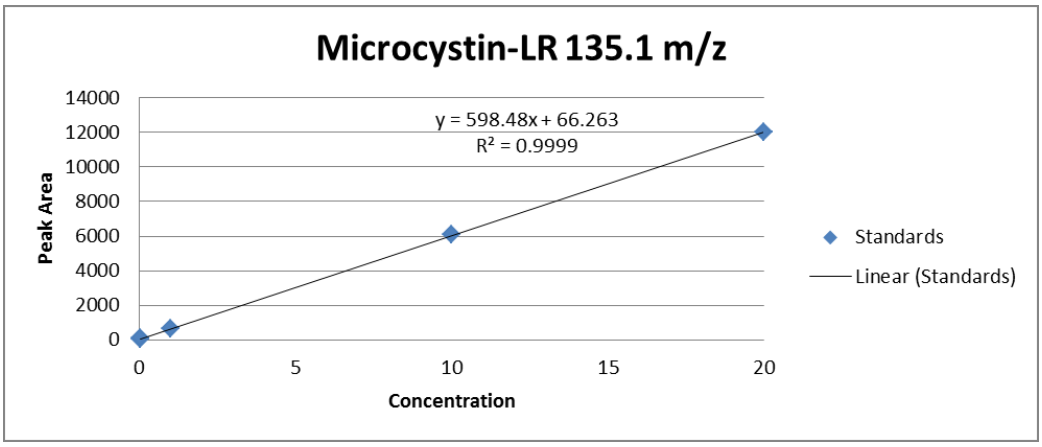


Figure A 64. Microcystin-LR 135.1 ion curve: 0,2,30,20, Jan-15

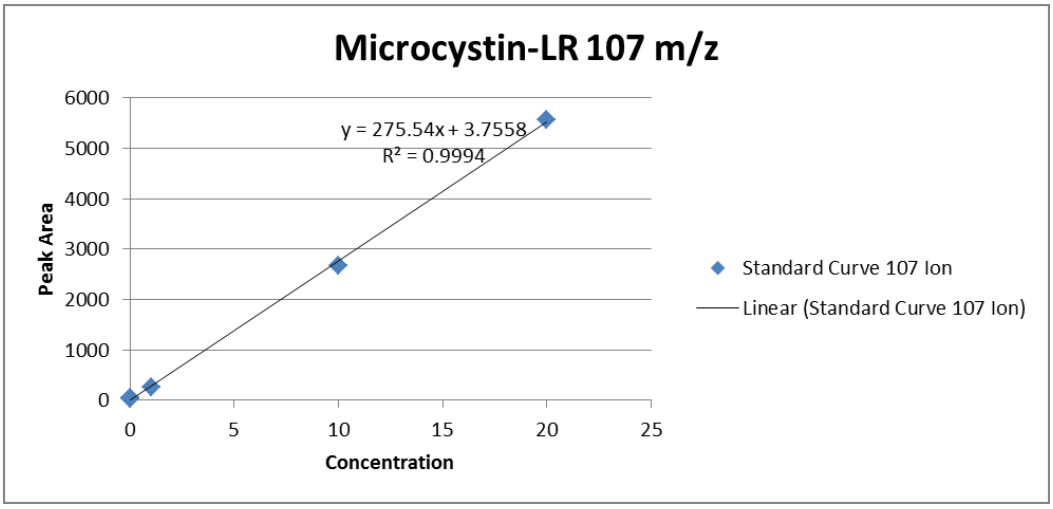


Figure A 65. Microcystin-LR 107 ion curve: 0,2,30,20, Jan-15

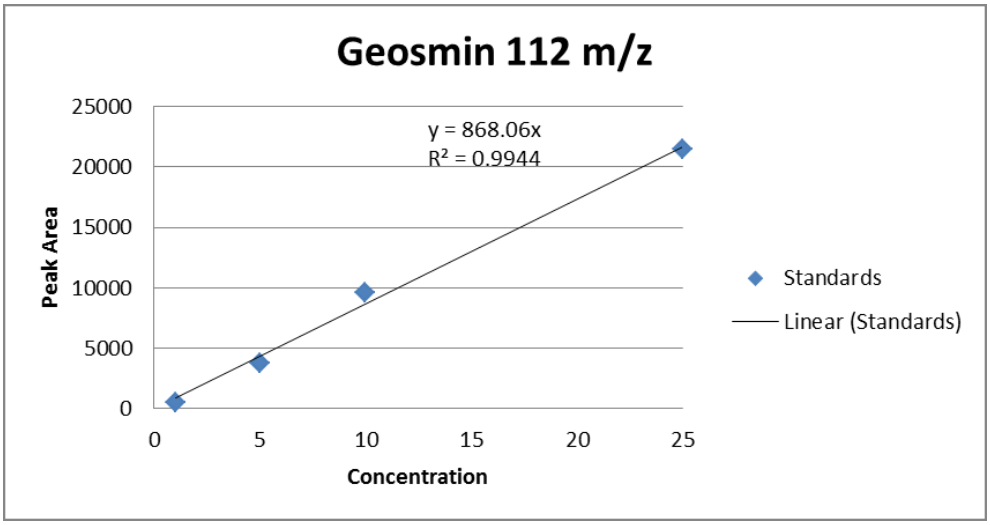


Figure A 66. Geosmin 112 ion curve: 0,2,30,20, Jan-15

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**50**	**20**	**16-Nov**

Table A 67. Sample 0,4,50,20, Nov 16 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	7.9	0	3.6	86	3.83	3.23
Area 2	4.2	0	1.6	37	1.93	1.73
Concentration (ug/L)	0	0	0	3.6	0.00	0.00
Geosmin						
Area 1	9107	9187	9043	84874	9112.33333	58.91
Concentration (ng/L)	5.35	5.4	5.31	49.85	5.35	0.04
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	6.9	6.9	7	6.9	6.93	0.05
Conductivity (µS/cm)	45.4	45.6	45.5	65.3	45.50	0.08
Temperature (oC)	19.5	19.6	18.9	21.2	19.33	0.31

Table A 68. Microcystin-LR Standard Curve: 0,4,50,20, Nov 16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11.9	4.9
1	19.5	5.9
20	226.4	106.3

Table A 69. Geosmin Standard Curve: 0,4,50,20, Nov 16

Standard Conc.	Area
1	752
5	8734
10	13202
25	37445
50	88447

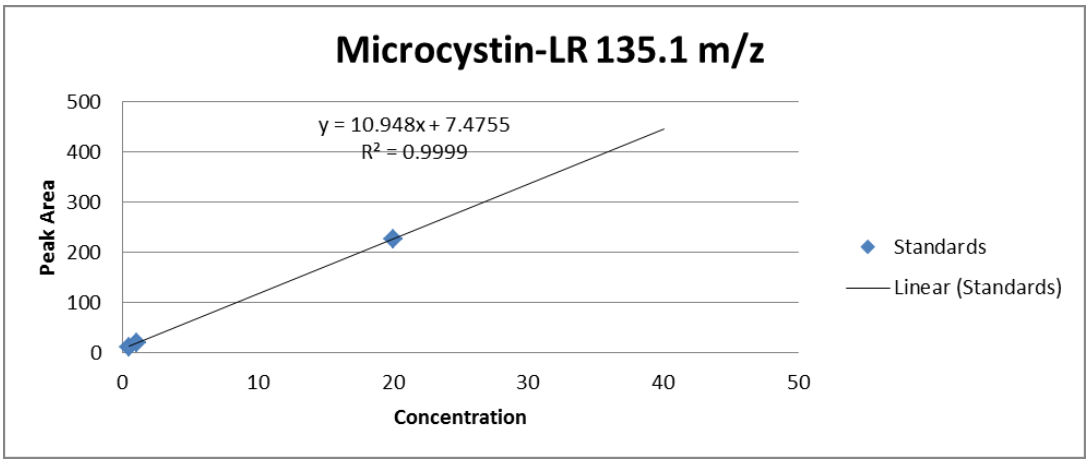


Figure A 67. Microcystin-LR 135.1 ion curve: 0,4,50,20, Nov 16

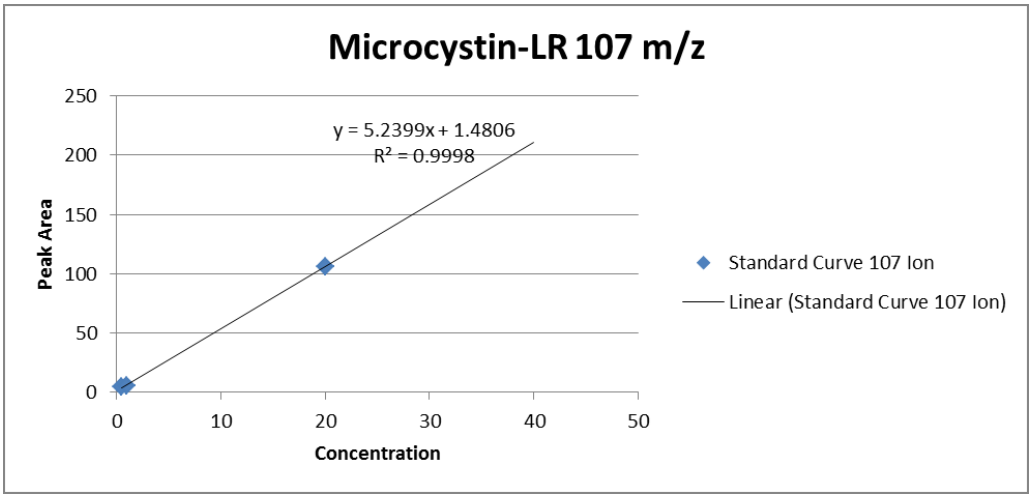


Figure A 68. Microcystin-LR 107 ion curve: 0,4,50,20, Nov 16

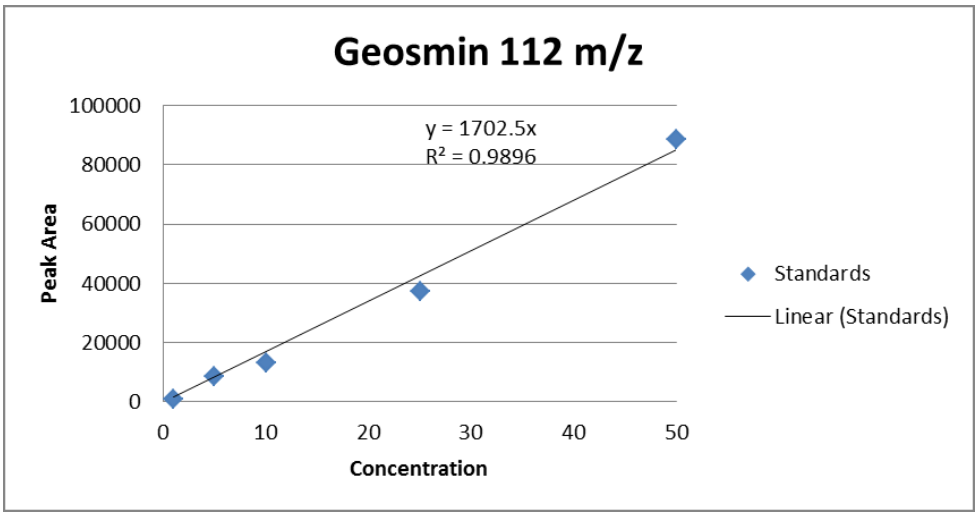


Figure A 69. Geosmin 112 ion curve: 0,4,50,20, Nov 16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**10**	**20**	**16-Nov**

Table A 70. Sample 0,6,10,20, Nov 16 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	297.2	383.5	509.7	609	396.80	87.26
Area 2	141	164.2	237.2	298.3	180.80	40.99
Concentration (ug/L)	0.14	0.34	0.62	5.12	0.37	0.20
Geosmin						
Area 1	913	922	890	9129	908.333333	13.47
Concentration (ng/L)	1.04	1.05	1.01	10.41	1.03	0.02
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.1	7.1	7.1	7.1	7.10	0.00
Conductivity (µS/cm)	59.2	59.5	59.7	50.1	59.47	0.21
Temperature (oC)	18.2	18.2	18.2	20.1	18.20	0.00

Table A 71. Microcystin-LR Standard Curve: 0,6,10,20, Nov 16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	231.5	137.8
1	544.5	265.5
10	5406	2350
20	8847	4088

Table A 72. Geosmin Standard Curve: 0,6,10,20, Nov 16

Standard Conc.	Area
1	469
5	3664
10	6254
25	22220
50	44308

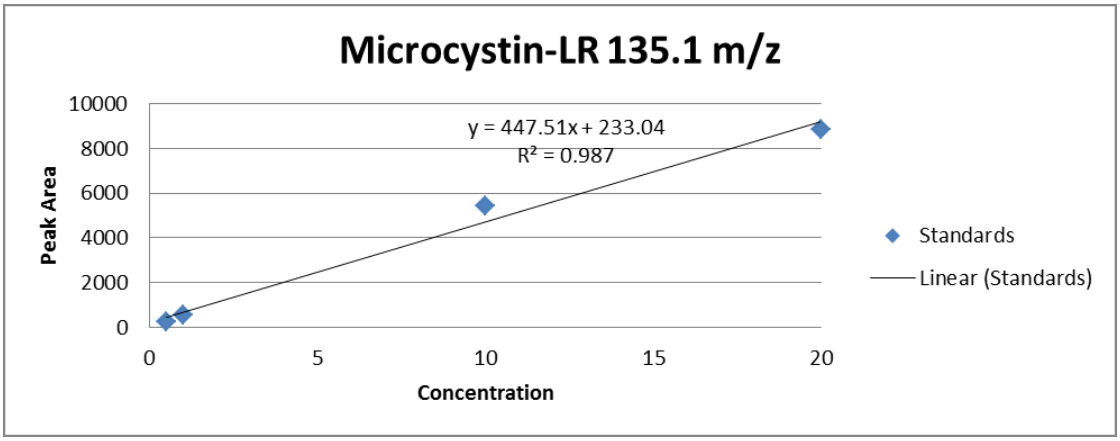


Figure A 70. Microcystin-LR 135.1 ion curve: 0,6,10,20, Nov 16

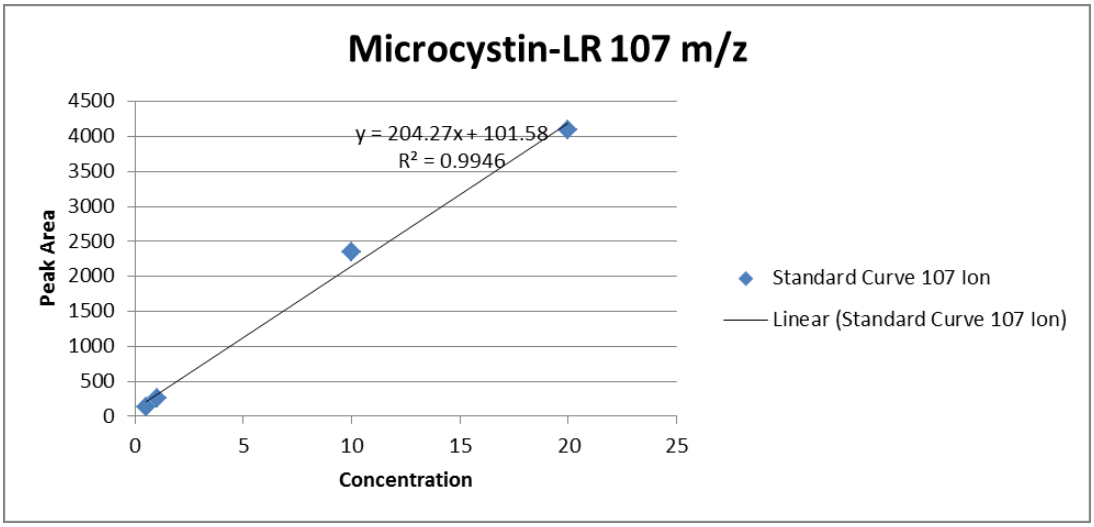


Figure A 71. Microcystin-LR 107 ion curve: 0,6,10,20, Nov 16

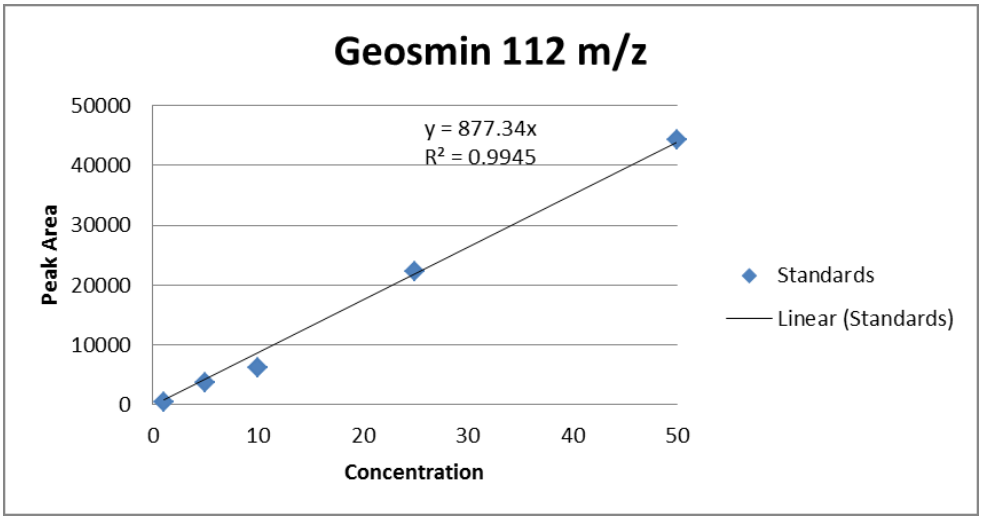


Figure A 72. Geosmin 112 ion curve: 0,6,10,20, Nov 16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**20**	**20**	**17-Sep**

Table A 73. Sample 0,6,20,20, Sept 17 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	27	28	25	400	26.67	1.25
Area 2	11	22	9	189	14.00	5.72
Concentration (ug/L)	1.27	1.32	1.17	19.86	1.25	0.06
Geosmin						
Area 1	3218	3027	2899	14811	3048.00	131.08
Concentration (ng/L)	4.33	4.08	3.9	19.95	4.10	0.18
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	40	40.00	0.00
pH	7.52	7.48	7.48	7.58	7.49	0.02
Conductivity (µS/cm)	58.8	58.6	58.3	58.5	58.57	0.21
Temperature (oC)	19.1	18.9	18.6	18.7	18.87	0.21

Table A 74. Microcystin-LR Standard Curve: 0,6,20,20, Sept 17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	10	5
1	21	10
5	106	44
10	201	90

Table A 75. Geosmin Standard Curve: 0,6,20,20, Sept 17

Standard Conc.	Area
1	731
5	3414
10	7111
25	18746

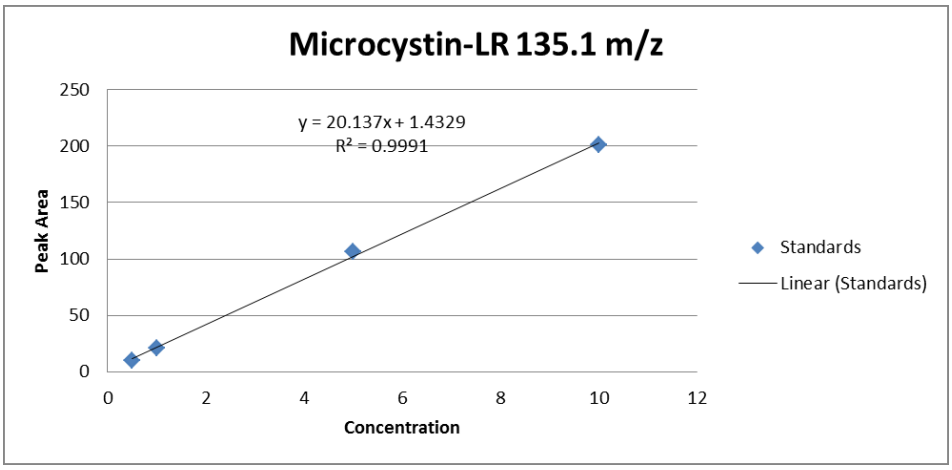


Figure A 73. Microcystin-LR 135.1 ion curve: 0,6,20,20, Sept 17

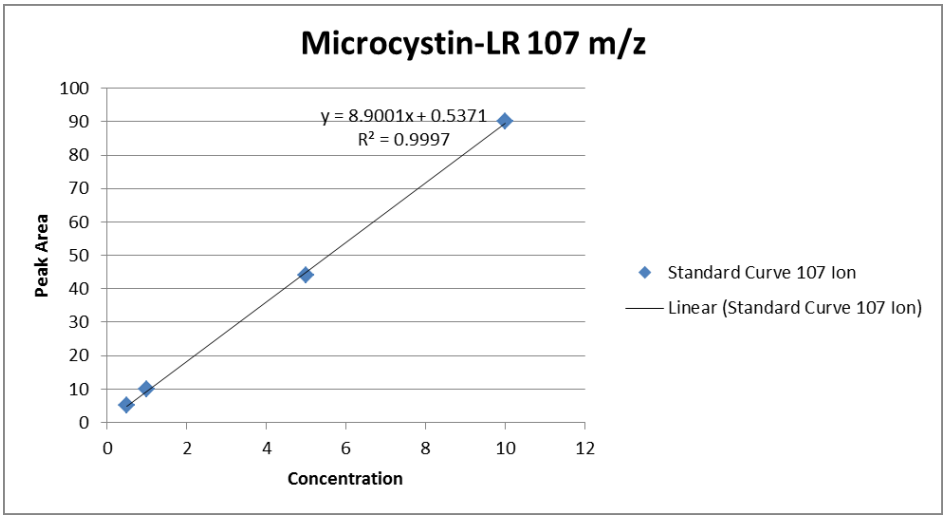


Figure A 74. Microcystin-LR 107 ion curve: 0,6,20,20, Sept 17

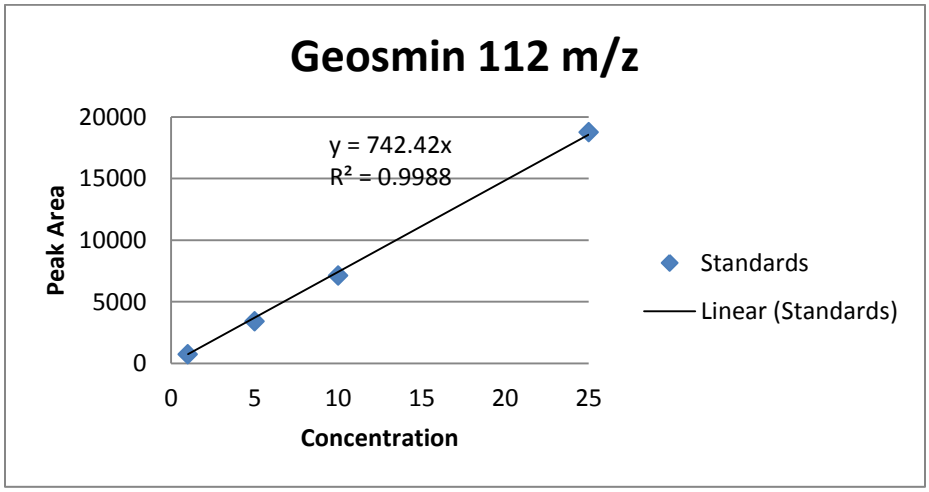


Figure A 75. Geosmin 112 ion curve: 0,6,20,20, Sept 17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**30**	**20**	**17-Sep**

Table A 76. Sample 0,6,30,20, Sept 17 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	20	20	15	400	18.33	2.36
Area 2	6	7	7	189	6.67	0.47
Concentration (ug/L)	0.92	0.92	0.67	19.86	0.84	0.12
Geosmin						
Area 1	2773	2546	2629	22450	2649.33333	93.78
Concentration (ng/L)	3.74	3.43	3.54	30.24	3.57	0.13
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	40	40.00	0.00
pH	6.93	6.3	6.4	6.5	6.54	0.28
Conductivity (µS/cm)	58.4	58.5	58.3	58.5	58.40	0.08
Temperature (oC)	18.9	18.6	18.7	18.8	18.73	0.12

Table A 77. Microcystin-LR Standard Curve: 0,6,30,20, Sept 17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	10	5
1	21	10
5	106	44
10	201	90

Table A 78. Geosmin Standard Curve: 0,6,30,20, Sept 17

Standard Conc.	Area
1	731
5	3414
10	7111
25	18746

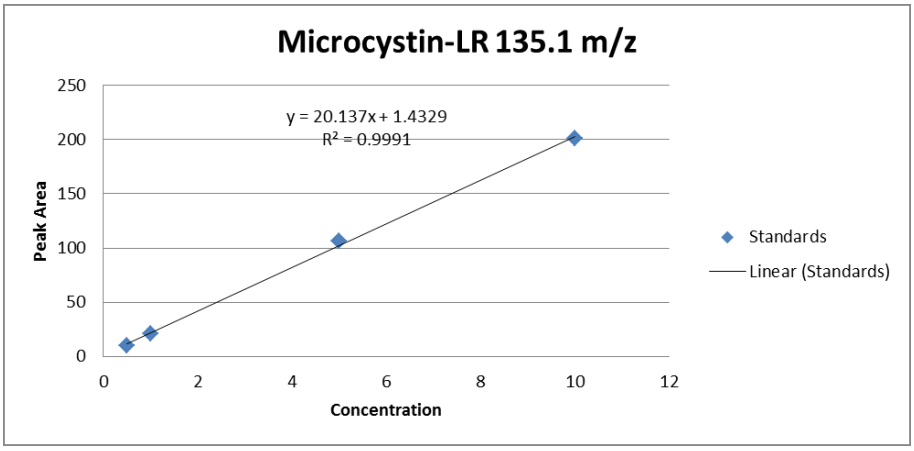


Figure A 76. Microcystin-LR 135.1 ion curve: 0,6,30,20, Sept 17

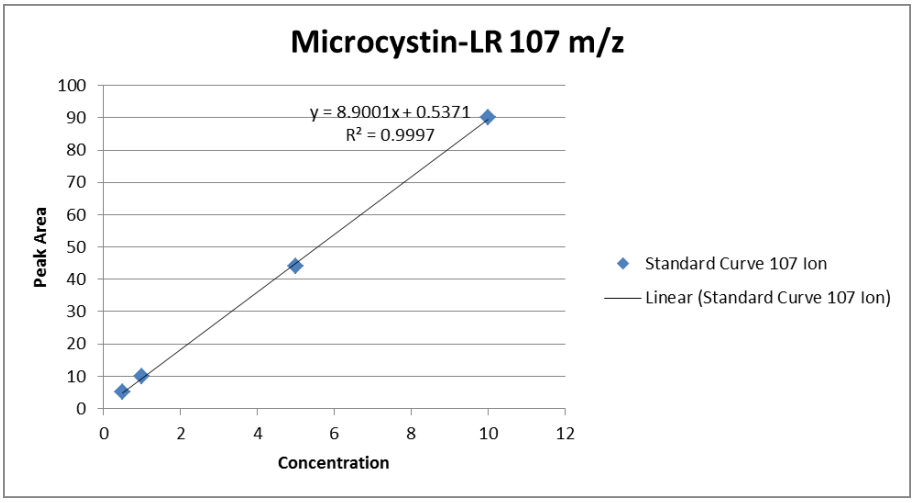


Figure A 77. Microcystin-LR 107 ion curve: 0,6,30,20, Sept 17

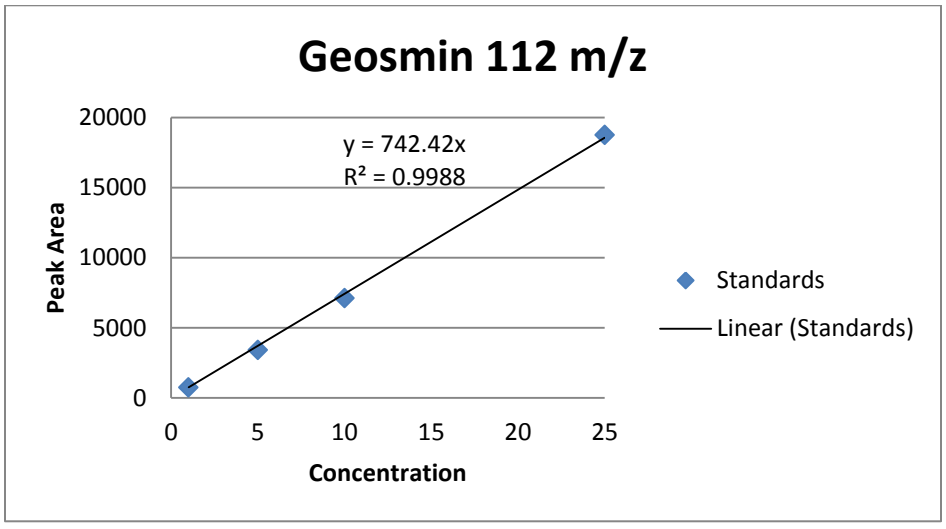


Figure A 78. Geosmin 112 ion curve: 0,6,30,20, Sept 17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**50**	**20**	**17-Oct**

Table A 79. Sample 0,6,50,20, Oct 17 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	29	24	23	112	25.33	2.62
Area 2	8	10	11	51	9.67	1.25
Concentration (ug/L)	1.07	0.79	1.25	5.65	1.04	0.19
Geosmin						
Area 1	4596	4765	4302	71744	4554.33	191.30
Concentration (ng/L)	3.23	3.35	3.18	50.48	3.25	0.07
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7.2	7.2	7	7.2	7.13	0.09
Conductivity (µS/cm)	73.6	73.6	71.2	72.2	72.80	1.13
Temperature (oC)	18.9	18.9	19.4	19.1	19.07	0.24

Table A 80. Microcystin-LR Standard Curve: 0,6,50,20, Oct 17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	1	1
1	19	9
10	242	104
20	347	159

Table A 81. Geosmin Standard Curve: 0,6,50,20, Oct 17

Standard Conc.	Area
1	959
5	5374
10	12644
25	32248
50	73188

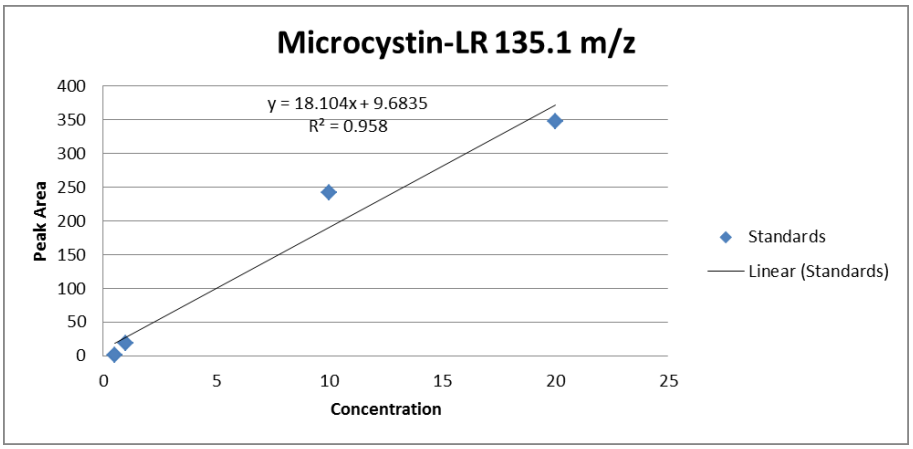


Figure A 79. Microcystin-LR 135.1 ion curve: 0,6,50,20, Oct 17

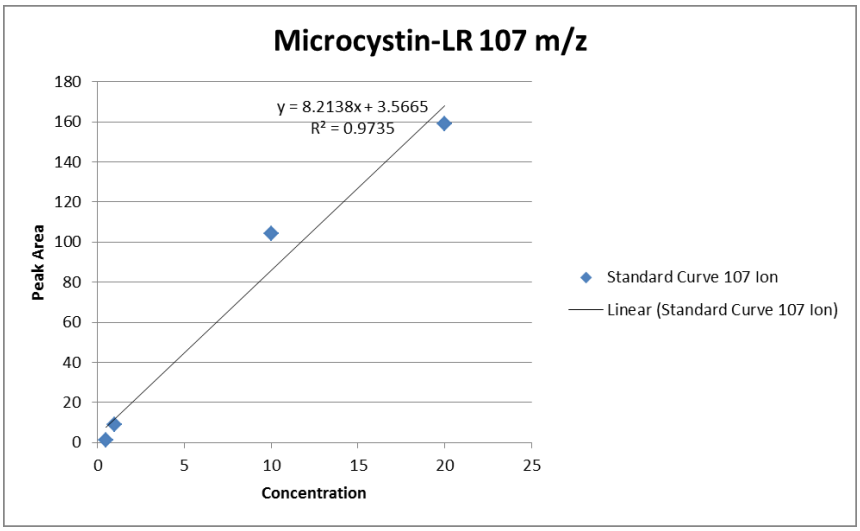


Figure A 80. Microcystin-LR 107 ion curve: 0,6,50,20, Oct 17

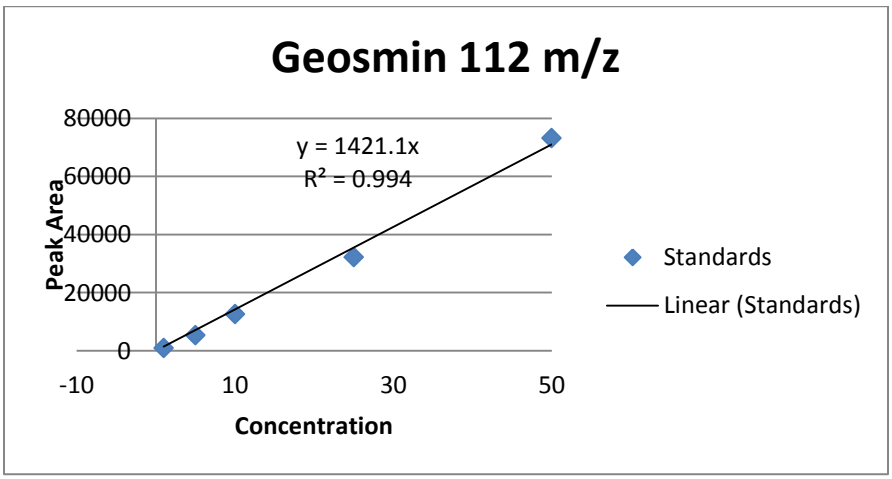


Figure A 81. Geosmin 112 ion curve: 0,6,50,20, Oct 17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**10**	**20**	**16-Nov**

Table A 82. Sample 0,10,10,20, Nov 16 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	138.1	291.5	208	130	212.53	62.71
Area 2	59.2	135.2	106	53.8	100.13	31.30
Concentration (ug/L)	0.64	2.1	1.31	11.2	1.35	0.60
Geosmin						
Area 1	834	825	835	15323	831.33	4.50
Concentration (ng/L)	0.95	0.94	0.95	10.31	0.95	0.00
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7.4	7.4	6.9	6.9	7.23	0.24
Conductivity (µS/cm)	45	45	44.7	45.4	44.90	0.14
Temperature (oC)	18.1	18.1	17.4	21	17.87	0.33

Table A 83. Microcystin-LR Standard Curve: 0,10,10,20, Nov 16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	66.2	32.3
1	141.4	74.3
20	2355	1110
40	4194	1987

Table A 84. Geosmin Standard Curve: 0,10,10,20, Nov 16

Standard Conc.	Area
1	469
5	3664
10	6254
25	22220
50	44308

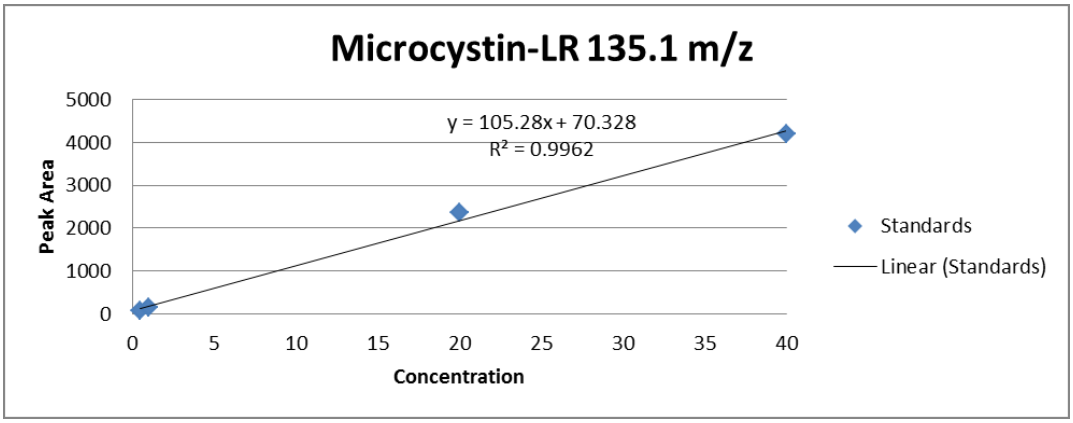


Figure A 82. Microcystin-LR 135.1 ion curve: 0,10,10,20, Nov 16

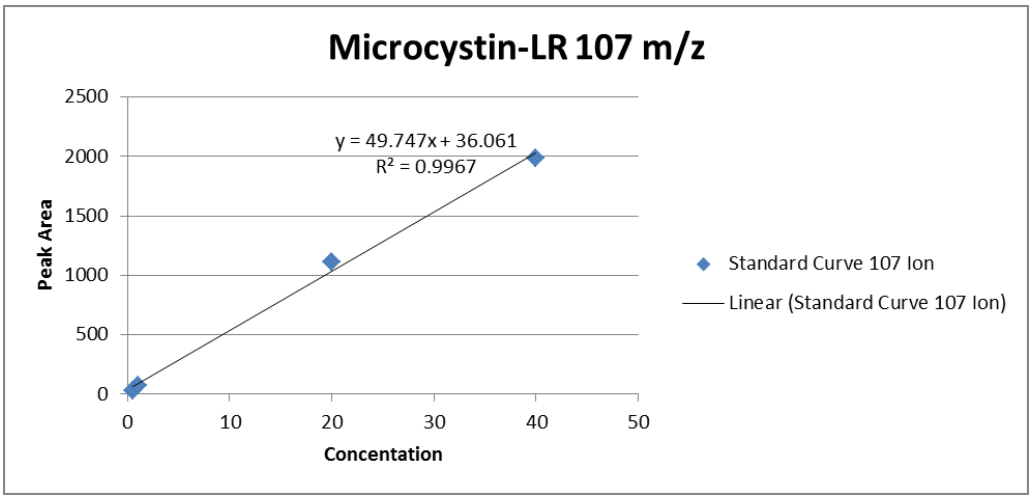


Figure A 83. Microcystin-LR 107 ion curve: 0,10,10,20, Nov 16

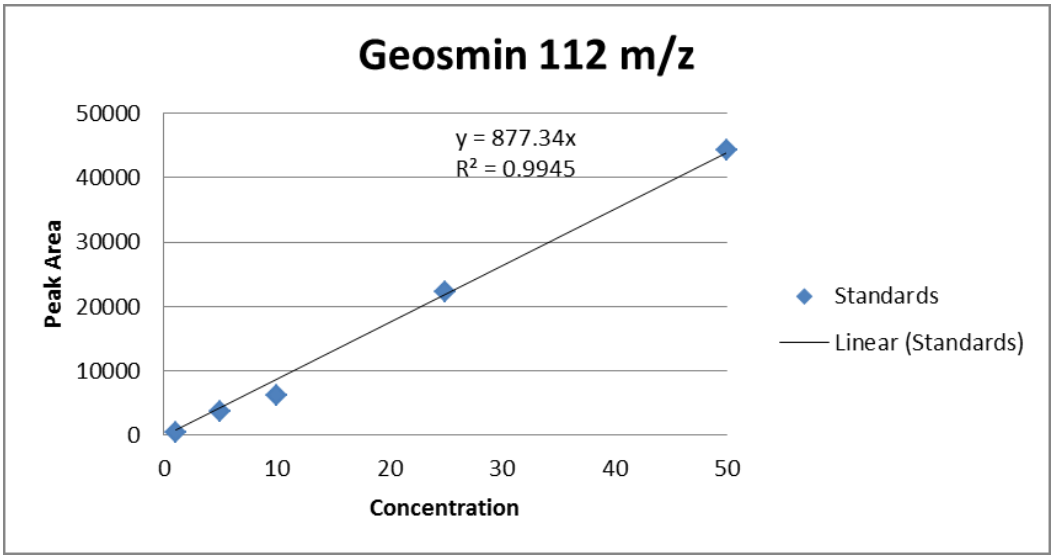


Figure A 84. Geosmin 112 ion curve: 0,10,10,20, Nov 16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**20**	**20**	**3-Oct**

Table A 85. Sample 0,10,20,20, Oct 3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	49	33	29	236	37.00	8.64
Area 2	59.2	135.2	106	109	100.13	31.30
Concentration (ug/L)	0.64	2.1	1.31	12.5	1.35	0.60
Geosmin						
Area 1	4002	3461	3621	32952	3694.67	226.92
Concentration (ng/L)	2.82	2.44	2.55	23.19	2.60	0.16
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	6.97	7.06	7.05	6.9	7.13	0.04
Conductivity (µS/cm)	74.3	74.1	74.1	71.9	74.17	0.09
Temperature (oC)	19	18.9	19	20.4	18.97	0.05

Table A 86. Microcystin-LR Standard Curve: 0,10,20,20, Oct 3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	1	1
1	19	9
10	242	104
20	347	159

Table A 87. Geosmin Standard Curve: 0,10,20,20, Oct 3

Standard Conc.	Area
1	959
5	5374
10	12644
25	32248
50	73188

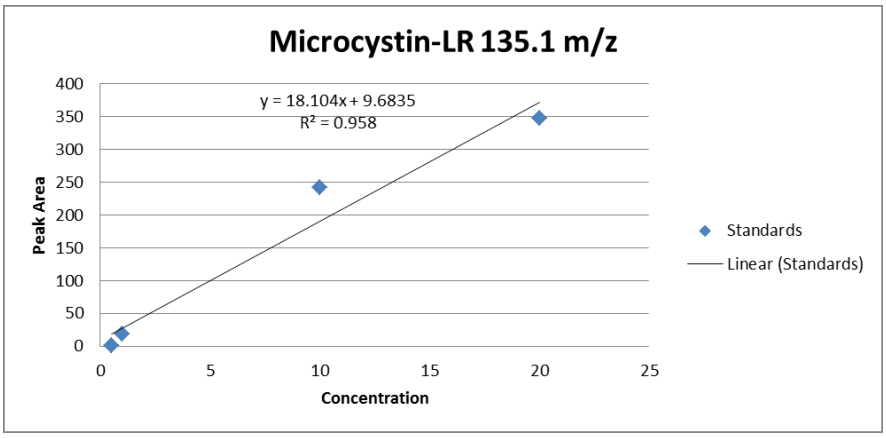


Figure A 85. Microcystin-LR 135.1 ion curve: 0,10,20,20, Oct 3

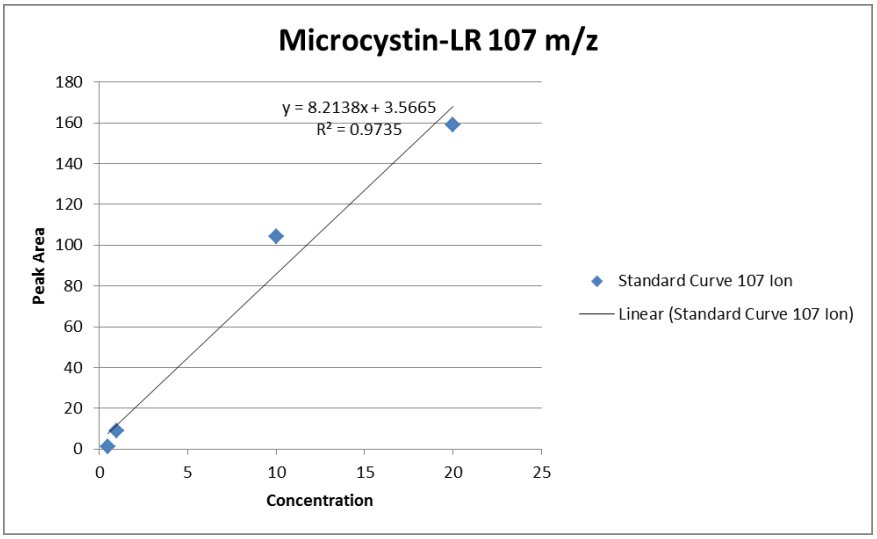


Figure A 86. Microcystin-LR 107 ion curve: 0,10,20,20, Oct 3

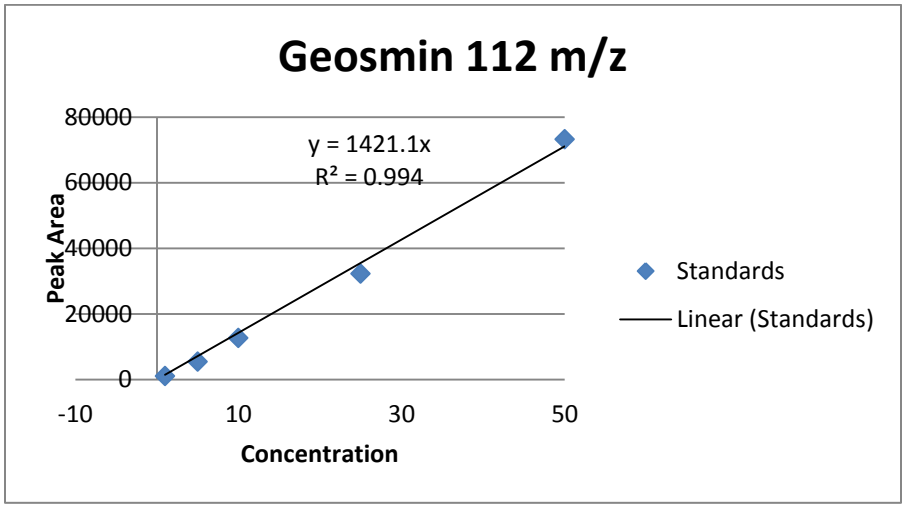


Figure A 87. Geosmin 112 ion curve: 0,10,20,20, Oct 3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**30**	**20**	**3-Oct**

Table A 88. Sample 0,10,30,20, Oct 3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	22	26	46	239	31.33	10.50
Area 2	9	12	23	108	14.67	6.02
Concentration (ug/L)	1.31	1.49	2.43	11.43	1.74	0.49
Geosmin						
Area 1	6299	6519	6202	51324	6340.00	132.62
Concentration (ng/L)	4.1	4.24	4.03	33.39	4.12	0.09
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	35	40.00	0.00
pH	6.94	7	6.9	7.18	7.13	0.04
Conductivity (µS/cm)	76.6	69.6	69.8	72.4	72.00	3.25
Temperature (oC)	21.5	15.6	15.2	20.9	17.43	2.88

Table A 89. Microcystin-LR Standard Curve: 0,10,30,20, Oct 3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11	5
1	26	13
10	176	82
20	438	204

Table A 90. Geosmin Standard Curve: 0,10,30,20, Oct 3

Standard Conc.	Area
1	1619
5	8325
10	17947
25	37272

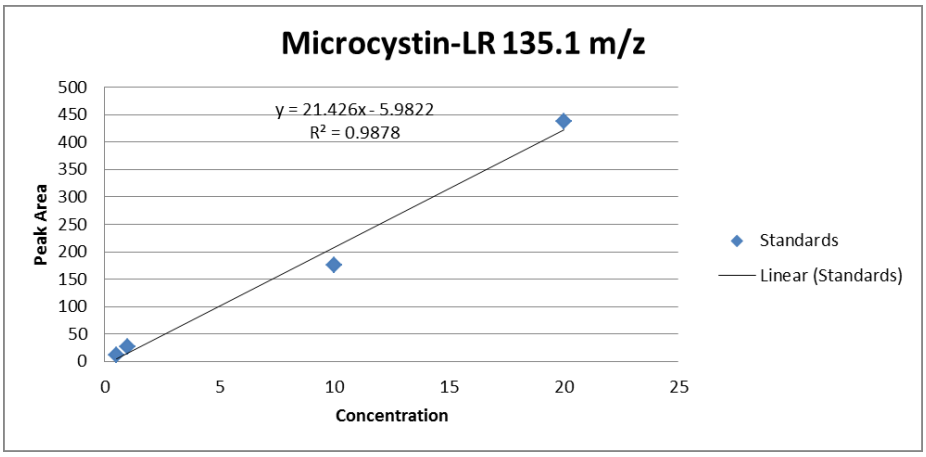


Figure A 88. Microcystin-LR 135.1 ion curve: 0,10,30,20, Oct 3

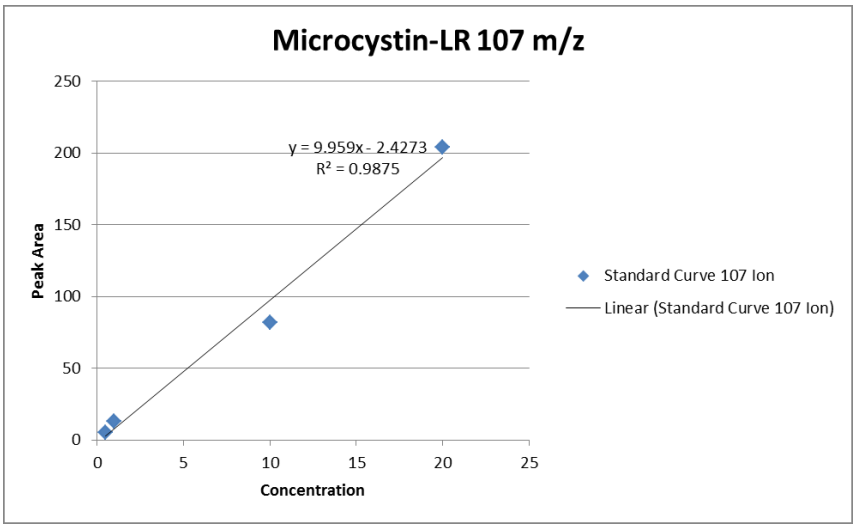


Figure A 89. Microcystin-LR 107 ion curve: 0,10,30,20, Oct 3

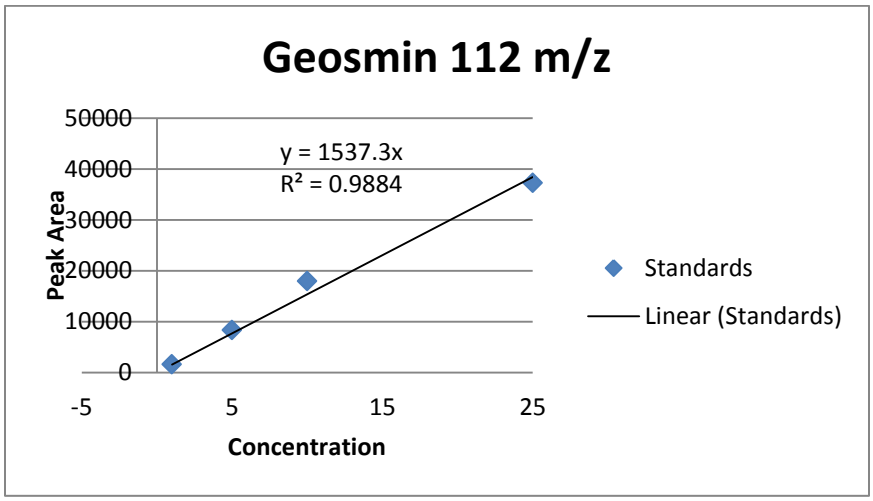


Figure A 90. Geosmin 112 ion curve: 0,10,30,20, Oct 3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**50**	**20**	**3-Oct**

Table A 91. Sample 0,10,50,20, Oct-3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	9	24	31	223	21.33	9.18
Area 2	5	11	15	97	10.33	4.11
Concentration (ug/L)	0.7	1.4	1.73	10.69	1.28	0.43
Geosmin						
Area 1	10171	10156	10306	64560	10211	67.45
Concentration (ng/L)	8.04	8.02	8.14	51	8.067	0.05
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	35	40.00	0.00
pH	6.99	7.04	6.98	7.18	6.82	0.03
Conductivity (µS/cm)	72.5	72.6	72.6	72.8	72.57	0.05
Temperature (oC)	19.7	19.8	19.9	20	19.80	0.08

Table A 92. Microcystin-LR Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11	5
1	26	13
10	176	82
20	438	204

Table A 93. Geosmin Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	Area
1	1075
5	3245
10	11843
25	29177
50	65000

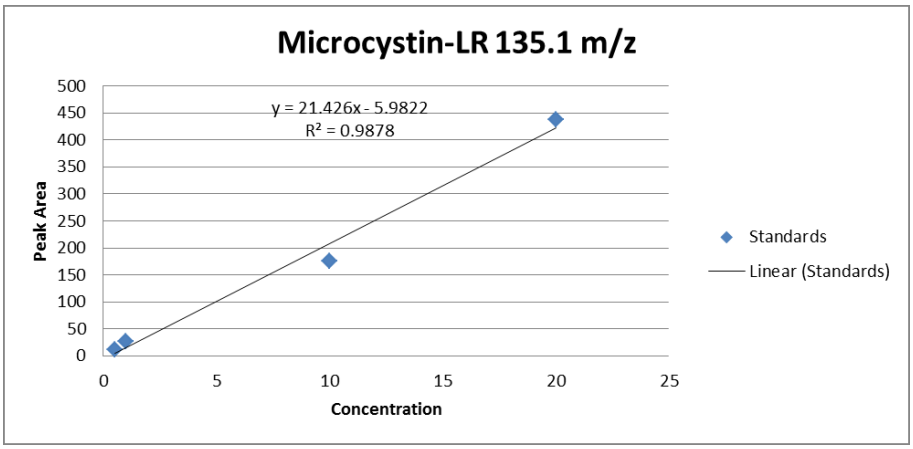


Figure A 91. Microcystin-LR 135.1 ion curve: 0,10,50,20, Oct-3

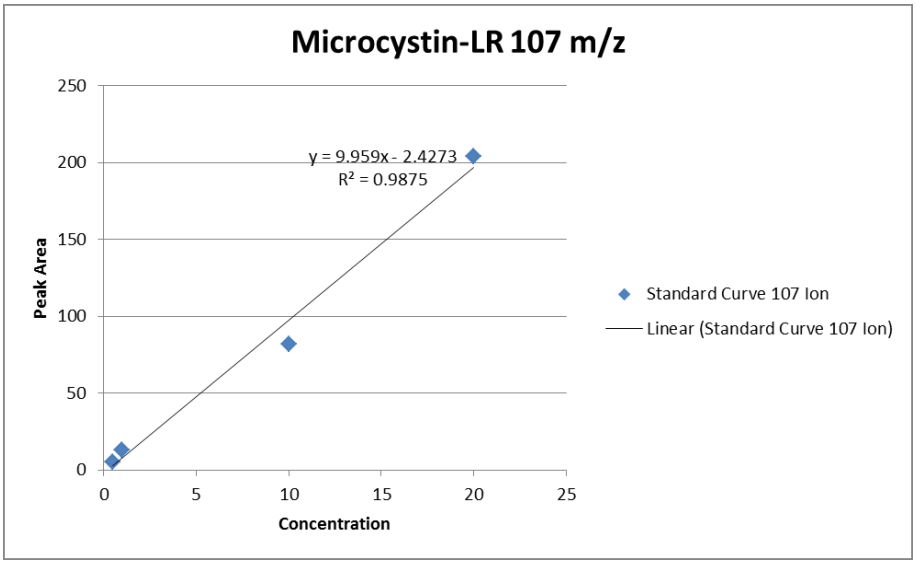


Figure A 92. Microcystin-LR 107 ion curve: 0,10,50,20, Oct-3

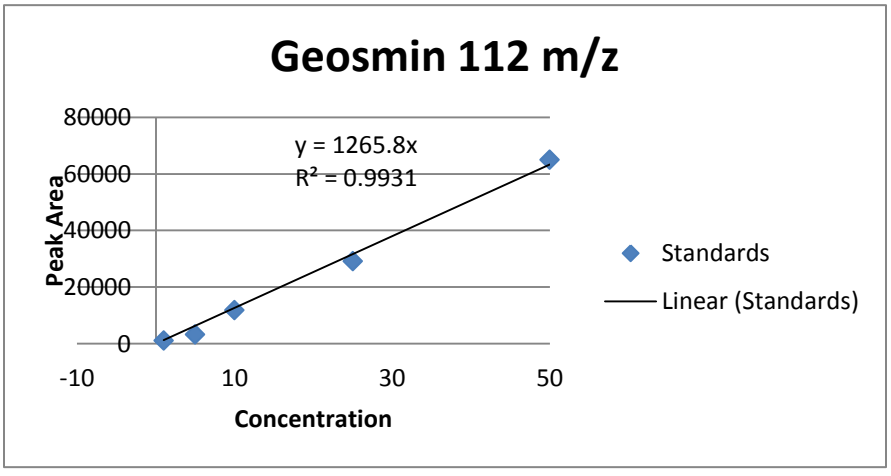


Figure A 93. Geosmin 112 ion curve: 0,10,50,20, Oct-3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**10**	**30**	**11-Jan**

Table A 94. Sample 0,2,10,30, Jan 11 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	77	41.3	51.6	3810.5	56.63	15.00
Area 2	54.7	13.2	9.7	711.2	25.87	20.44
Concentration (ug/L)	0.26	0.22	0.34	7	0.27	0.05
Geosmin						
Area 1	3712	3926	3412	6498	3683.333	210.82
Concentration (ng/L)	5.7	6.03	5.24	9.98	5.656667	0.32
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.52	7.59	7.68	7.68	7.60	0.07
Conductivity (µS/cm)	60.6	60.2	60.7	60.3	60.50	0.22
Temperature (oC)	19.5	19.5	19.8	19.5	19.60	0.14

Table A 95. Microcystin-LR Standard Curve: 0,2,10,30, Jan 11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	425.1	208.4
1	685.9	287
10	8614	3896
20	17796	7545

Table A 96. Geosmin Standard Curve: 0,2,10,30, Jan 11

Standard Conc.	Area
1	383
5	2743
10	5567
25	15507
50	33193

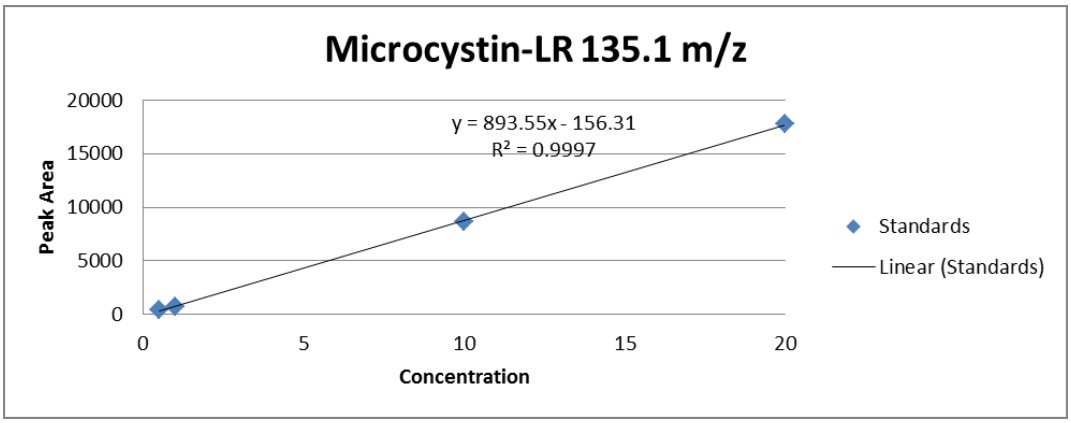


Figure A 94. Microcystin-LR 135.1 ion curve: 0,2,10,30, Jan 11

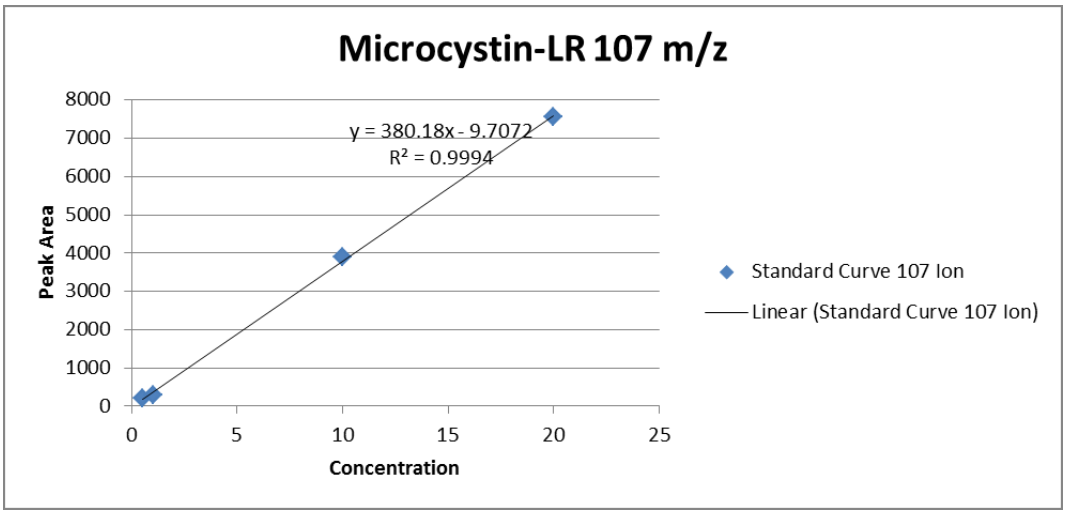


Figure A 95. Microcystin-LR 107 ion curve: 0,2,10,30, Jan 11

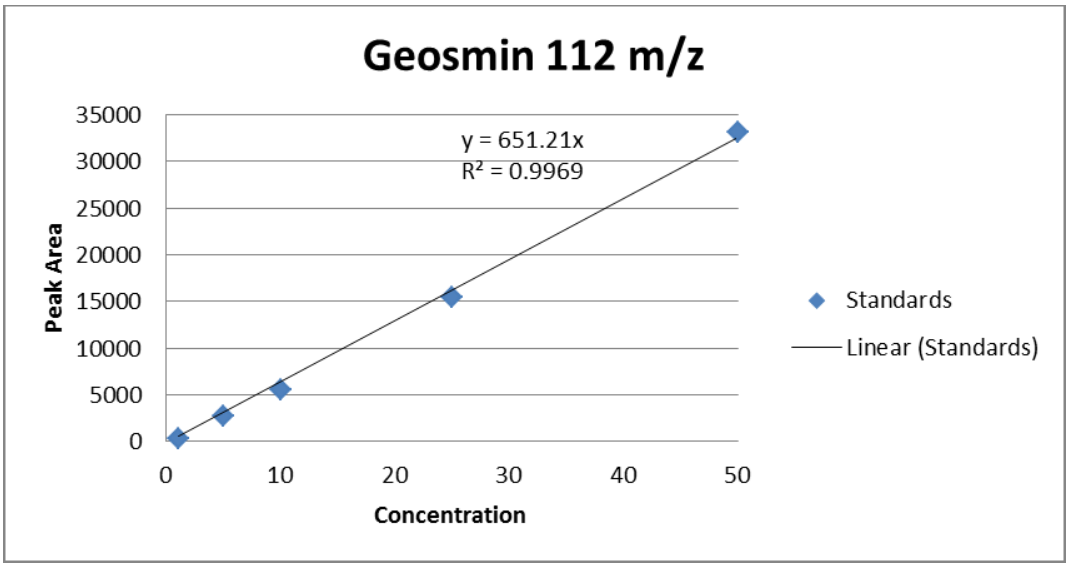


Figure A 96. Geosmin 112 ion curve: 0,2,10,30, Jan 11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**20**	**30**	**25-Jan**

Table A 97. Sample 0,2,20,30, Jan 25 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	78.6	85.2	64.5	1368.6	76.10	8.63
Area 2	36.8	40.7	50.3	600	42.60	5.67
Concentration (ug/L)	0.1	0.12	0.25	2.49	0.16	0.07
Geosmin						
Area 1	551	576	412		513	72.14
Concentration (ng/L)	0.42	0.45	0.2	29.57	0.356667	0.11
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.5	7.6	7.5	7.1	6.82	0.05
Conductivity (µS/cm)	60.8	60.6	60.3	60.4	60.57	0.21
Temperature (oC)	19.9	19.9	19.2	20.1	19.67	0.33

Table A 98. Microcystin-LR Standard Curve: 0,2,20,30, Jan 25

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0	0	0
0.5	257.2	124.7
1	506.3	280.4
10	5328	2595
20	10829	4952

Table A 99. Geosmin Standard Curve: 0,2,20,30, Jan 25

Standard Conc.	Area
1	383
5	2743
10	5567
25	15507
50	33193

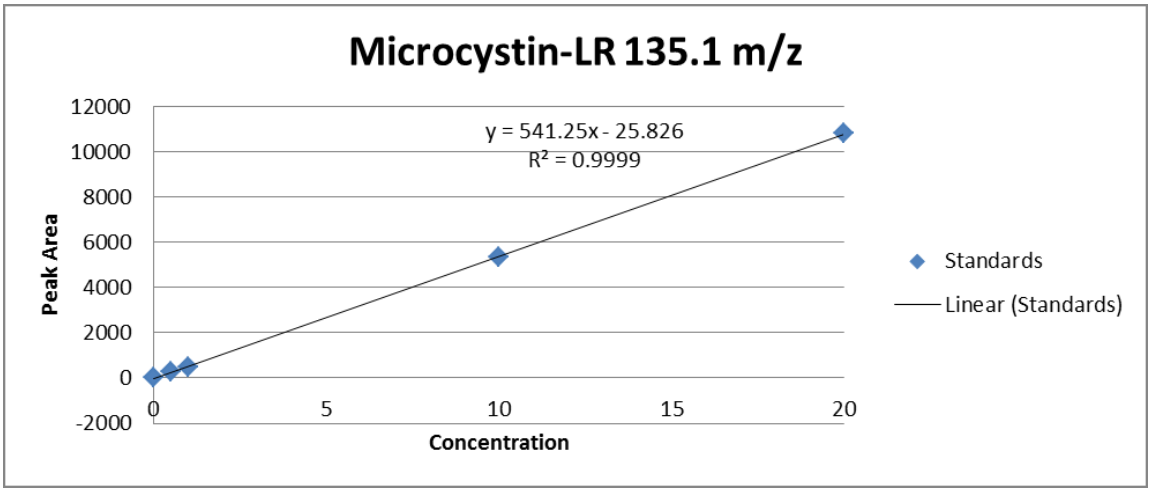


Figure A 97. Microcystin-LR 135.1 ion curve: 0,2,20,30, Jan 25

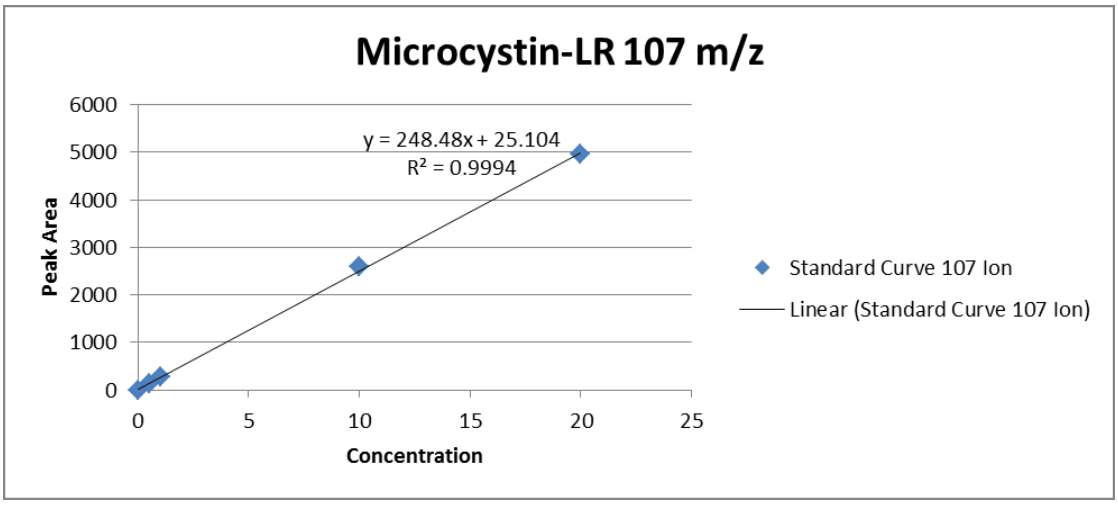


Figure A 98. Microcystin-LR 107 ion curve: 0,2,20,30, Jan 25

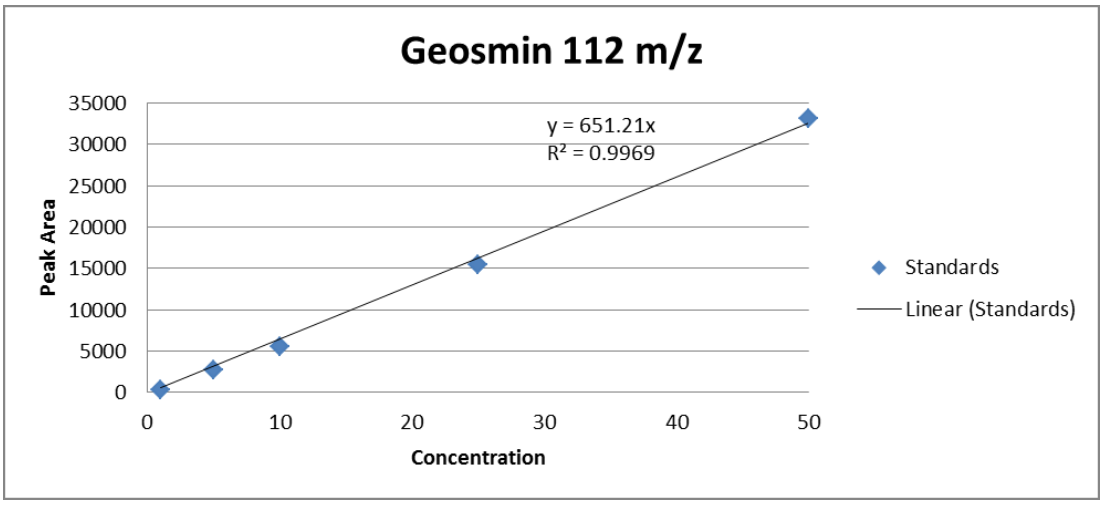


Figure A 99. Geosmin 112 ion curve: 0,2,20,30, Jan 25

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**30**	**30**	**25-Jan**

Table A 100. Sample 0,2,30,30, Jan-25 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	103.3	114.8	111.7	1368.6	109.93	4.86
Area 2	45.3	42.8	40	600	42.70	2.16
Concentration (ug/L)	0.15	0.17	0.16	2.49	0.16	0.01
Geosmin						
Area 1	3383	3460	3142	24992	3328.33	135.46
Concentration (ng/L)	4	4.09	3.72	29.57	3.94	0.16
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.5	7.6	7.6	7.1	6.82	0.05
Conductivity (µS/cm)	60.6	60.6	60.7	60.4	60.63	0.05
Temperature (oC)	19.9	19.9	19.8	20.1	19.87	0.05

Table A 101. Microcystin-LR Standard Curve: 0,2,30,30, Jan-25

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0	0	0
0.5	257.2	124.7
1	506.3	280.4
10	5328	2595
20	10829	4952

Table A 102. Geosmin Standard Curve: 0,2,30,30, Jan-25

Standard Conc.	Area
1	374
5	4701
10	6433
25	21275
50	42553

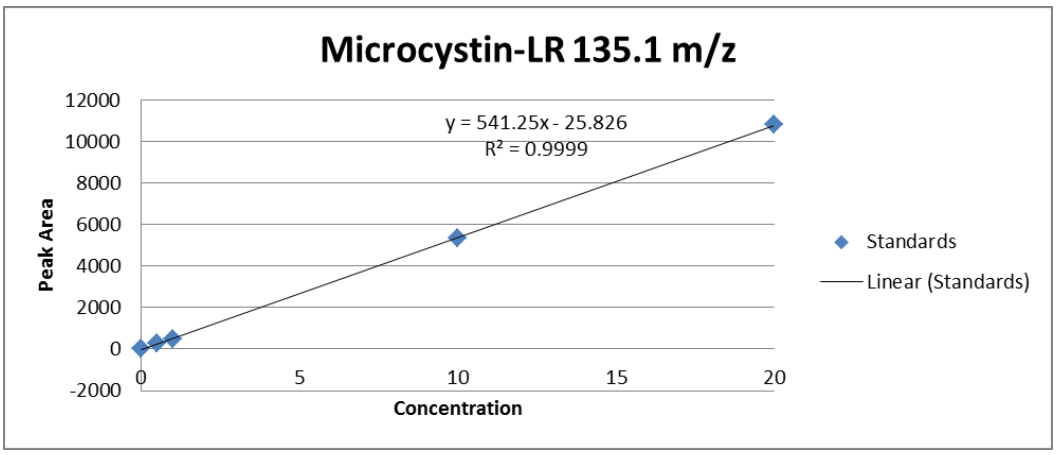


Figure A 100. Microcystin-LR 135.1 ion curve: 0,2,30,30, Jan-25

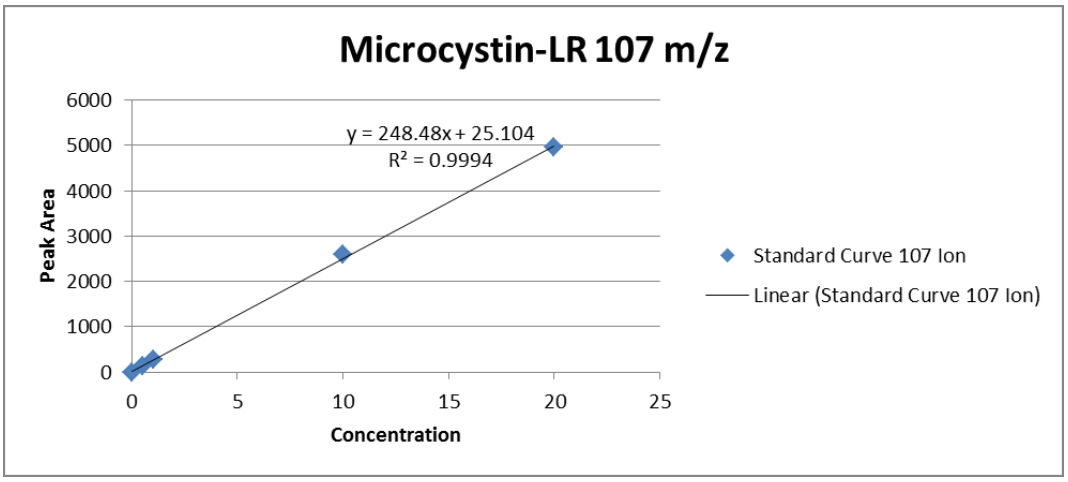


Figure A 101. Microcystin-LR 107 ion curve: 0,2,30,30, Jan-25

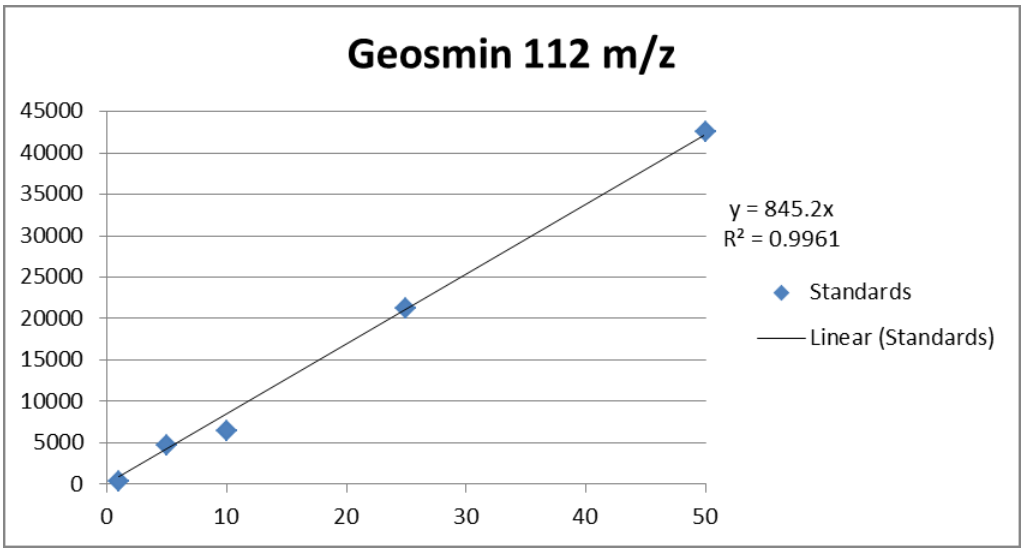


Figure A 102. Geosmin 112 ion curve: 0,2,30,30, Jan-25

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**2**	**50**	**30**	**22-Feb**

Table A 103. Sample 0,2,50,30, Feb-22 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	33	31	24	1368.6	29.33	3.86
Area 2	10	13	12	600	11.67	1.25
Concentration (ug/L)	0.15	0.17	0.16	2.49	0.16	0.01
Geosmin						
Area 1	4474	4730	4112	23191	4438.67	253.53
Concentration (ng/L)	4	4.09	3.72	29.57	3.94	0.16
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.5	7.6	7.6	7.1	6.82	0.05
Turbidity (NTU)	1.57	1.77	1.84	1.23	1.73	0.11
Conductivity (µS/cm)	60.6	60.6	60.7	60.4	60.63	0.05
Temperature (oC)	19.9	19.9	19.8	20.1	19.87	0.05

Table A 104. Microcystin-LR Standard Curve: 0,2,50,30, Feb-22

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	40	33
1	105	45
10	687	360
20	1487	698

Table A 105. Geosmin Standard Curve: 0,2,50,30, Feb-22

Standard Conc.	Area
1	576
5	4872
10	6936
25	20017
50	39099

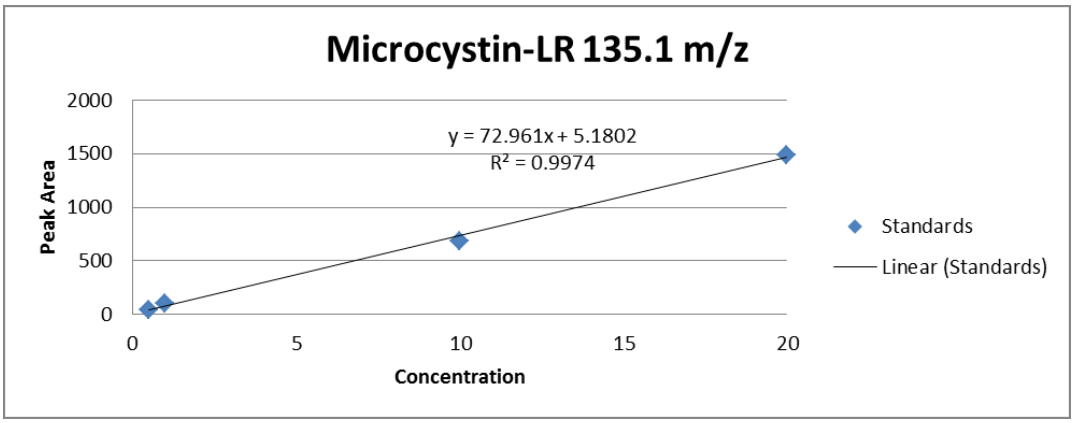


Figure A 103. Microcystin-LR 135.1 ion curve: 0,2,50,30, Feb-22

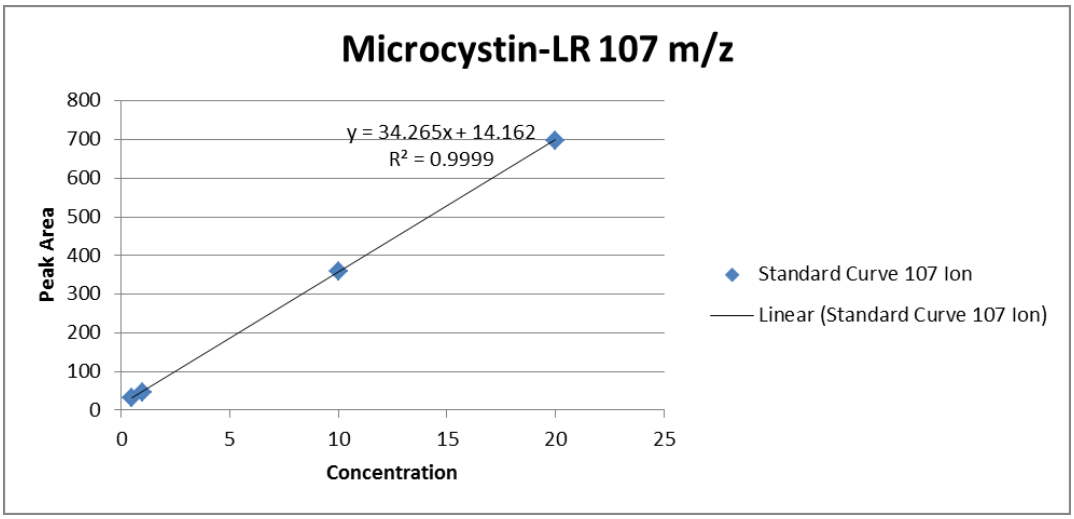


Figure A 104. Microcystin-LR 107 ion curve: 0,2,50,30, Feb-22

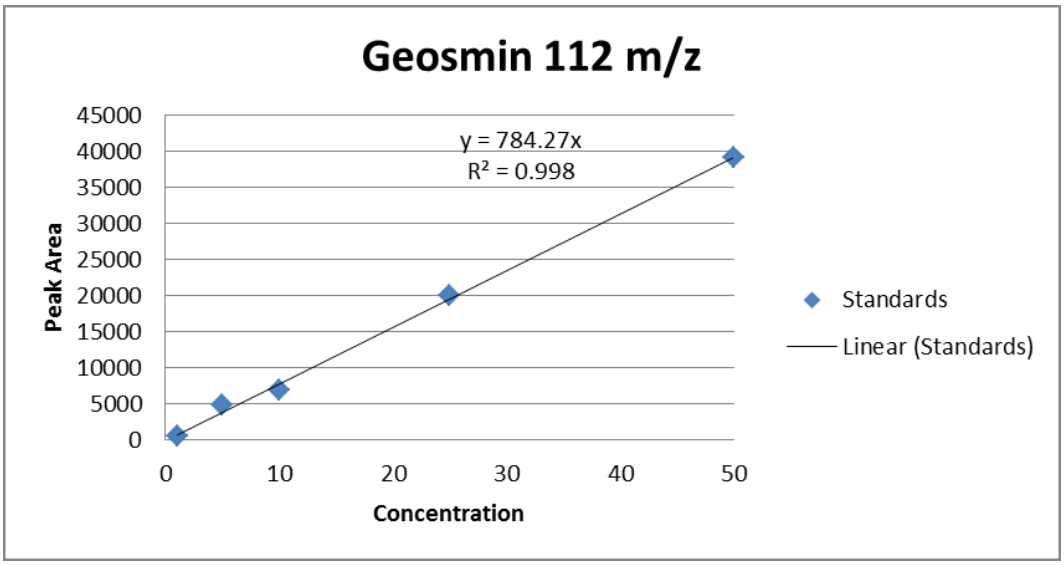


Figure A 105. Geosmin 112 ion curve: 0,2,50,30, Feb-22

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**10**	**30**	**17-Oct**

Table A 106. Sample 0,4,10,30, Oct-17 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	18	13	11	99	14.00	2.94
Area 2	7	7	5	36	6.33	0.94
Concentration (ug/L)	1.07	0.89	0.82	3.99	0.93	0.11
Geosmin						
Area 1	927	918	936	13808	927	7.35
Concentration (ng/L)	0.69	0.68	0.69	10.21	0.69	0.00
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7	7.1	7.2	6.9	6.82	0.08
Conductivity (µS/cm)	69	69.5	69.5	71.1	69.33	0.24
Temperature (oC)	19	18.7	18.8	19.5	18.83	0.12

Table A 107. Microcystin-LR Standard Curve: 0,4,10,30, Oct-17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	12	8
1	26	11
20	504	221
40	1117	487

Table A 108. Geosmin Standard Curve: 0,4,10,30, Oct-17

Standard Conc.	Area
1	1398
5	7302
10	17237
25	33405
50	67024

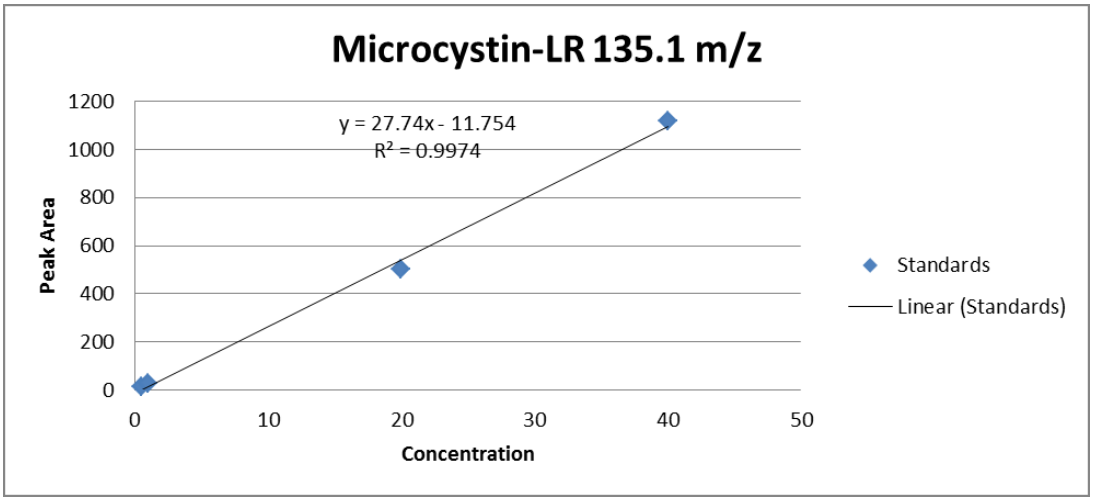


Figure A 106. Microcystin-LR 135.1 ion curve: 0,4,10,30, Oct-17

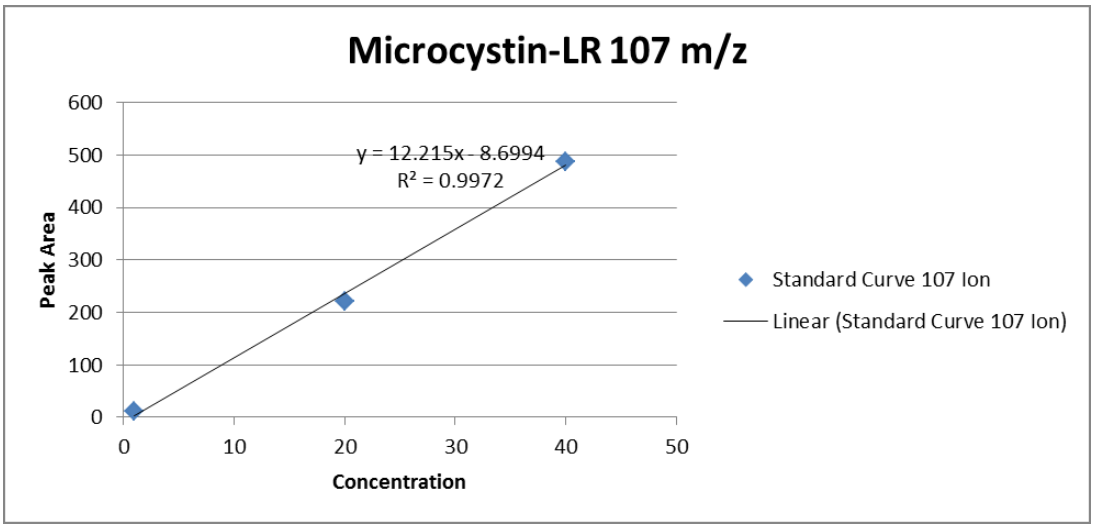


Figure A 107. Microcystin-LR 107 ion curve: 0,4,10,30, Oct-17

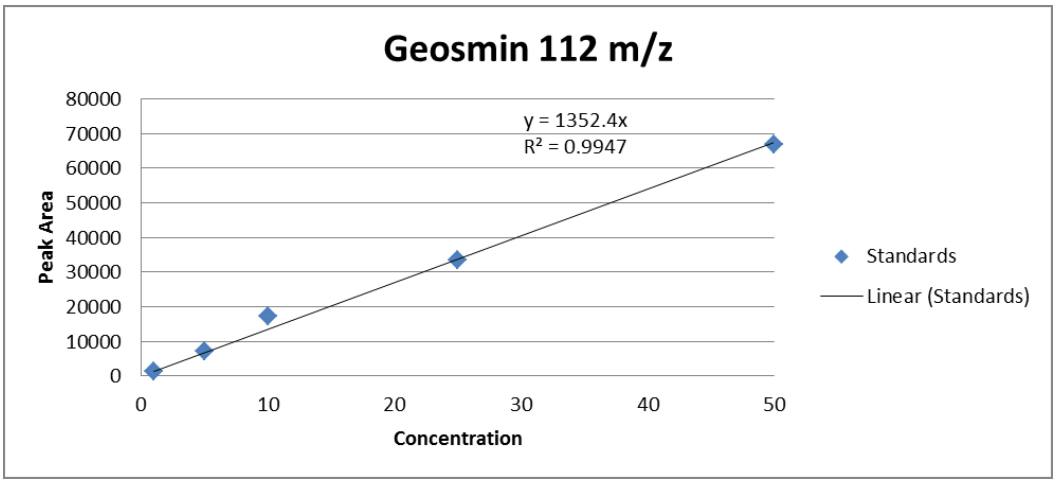


Figure A 108. Geosmin 112 ion curve: 0,4,10,30, Oct-17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**20**	**30**	**Spet 19**

Table A 109. Sample 0,4,20,30, Sept-19 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	5	7	4	99	5.33	1.25
Area 2	3	2	2	36	2.33	0.47
Concentration (ug/L)	0.18	0.28	0.13	3.99	0.20	0.06
Geosmin						
Area 1	581	554	545	31567	560	15.30
Concentration (ng/L)	0.38	3.6	0.36	20.58	1.446667	1.52
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7	7.1	7.2	6.9	6.82	0.08
Conductivity (µS/cm)	69	69.5	69.5	71.1	69.33	0.24
Temperature (oC)	19	18.7	18.8	19.5	18.83	0.12

Table A 110. Microcystin-LR Standard Curve: 0,4,20,30, Sept-19

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	10	5
1	21	10
5	106	44
10	201	90

Table A 111. Geosmin Standard Curve: 0,4,20,30, Sept-19

Standard Conc.	Area
1	1679
5	9725
10	18785
25	36552

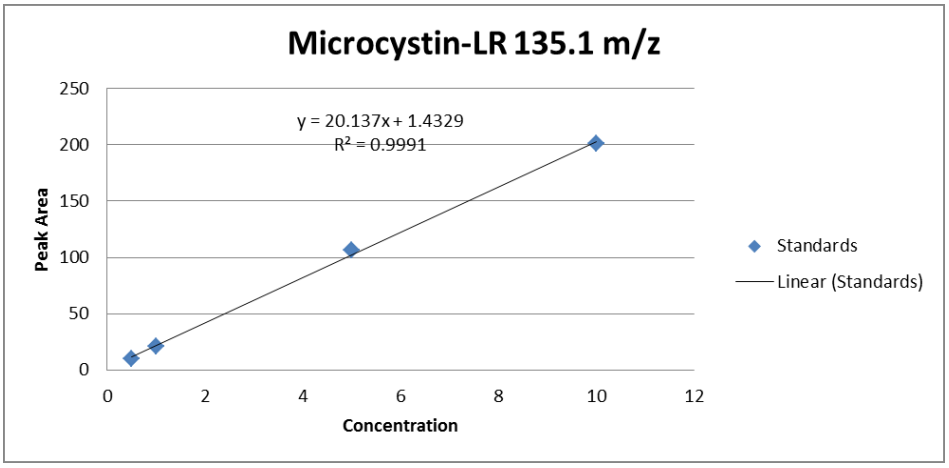


Figure A 109. Microcystin-LR 135.1 ion curve: 0,4,20,30, Sept-19

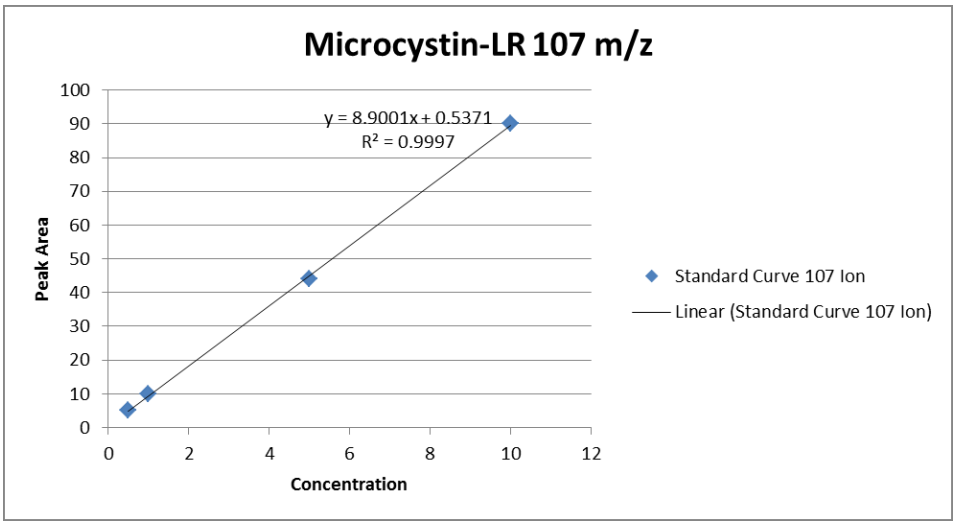


Figure A 110. Microcystin-LR 107 ion curve: 0,4,20,30, Sept-19

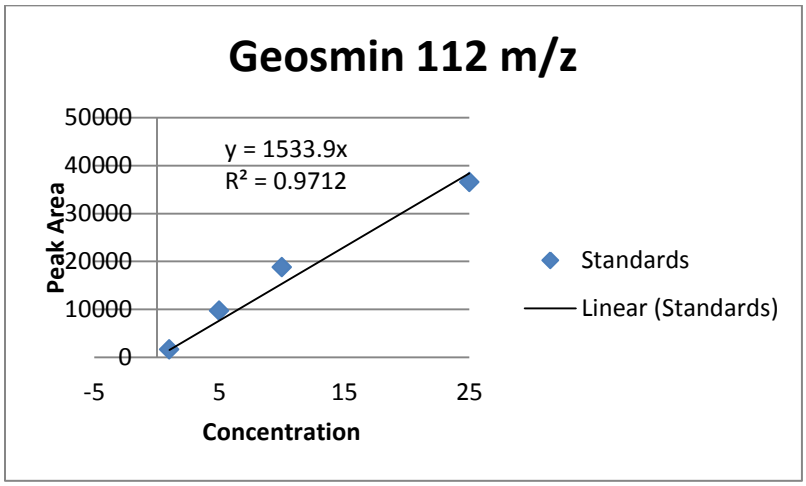


Figure A 111. Geosmin 112 ion curve: 0,4,20,30, Sept-19

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**30**	**30**	**19-Sep**

Table A 112. Sample 0,4,30,30, Sept-19 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	1	5	5	99	3.67	1.89
Area 2	0	1	4	36	1.67	1.70
Concentration (ug/L)	0	0.18	0.18	3.99	0.12	0.08
Geosmin						
Area 1	1123	1207	1087	47566	1139	50.28
Concentration (ng/L)	0.73	0.79	0.71	31.01	0.743	0.03
Water Quality						
Hardness (mg/L CaCO3)	51.3	68.4	51.3	34.2	57.00	8.06
Alkalinity (mg/L CaCO3)	45	45	40	35	43.33	2.36
pH	6.66	6.79	7.19	6.9	6.82	0.23
Conductivity (µS/cm)	68	68.3	68.5	71.1	68.27	0.21
Temperature (oC)	19.1	19.2	18.8	19.5	19.03	0.17

Table A 113. Microcystin-LR Standard Curve: 0,4,30,30, Sept-19

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	10	5
1	21	10
5	106	44
10	201	90

Table A 114. Geosmin Standard Curve: 0,4,30,30, Sept-19

Standard Conc.	Area
1	1679
5	9725
10	18785
25	36552

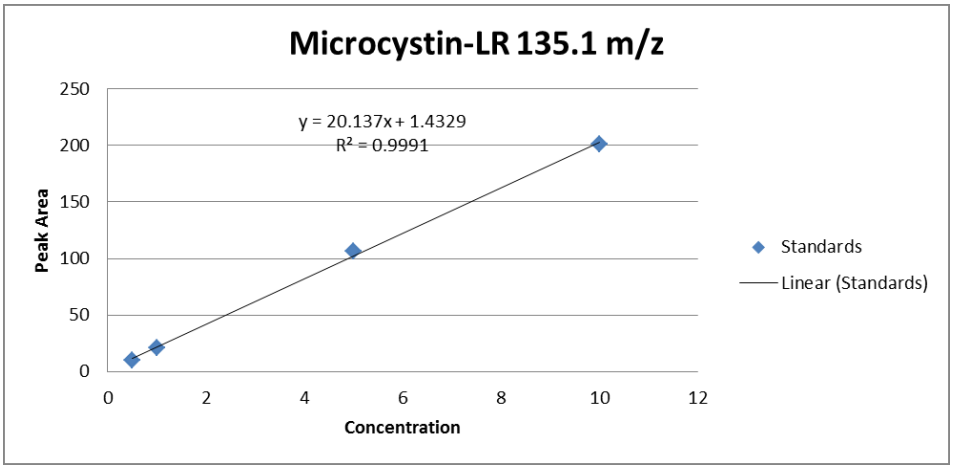


Figure A 112. Microcystin-LR 135.1 ion curve: 0,4,30,30, Sept-19

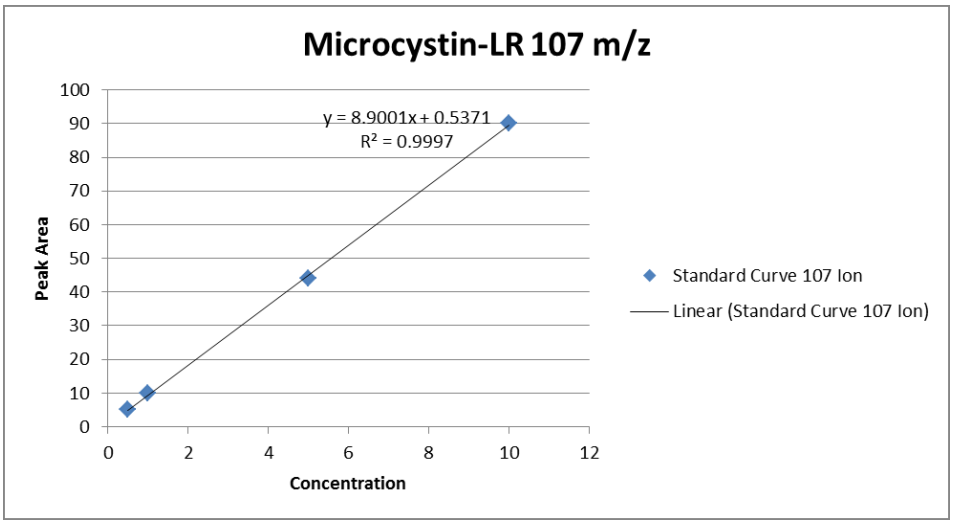


Figure A 113. Microcystin-LR 107 ion curve: 0,4,30,30, Sept-19

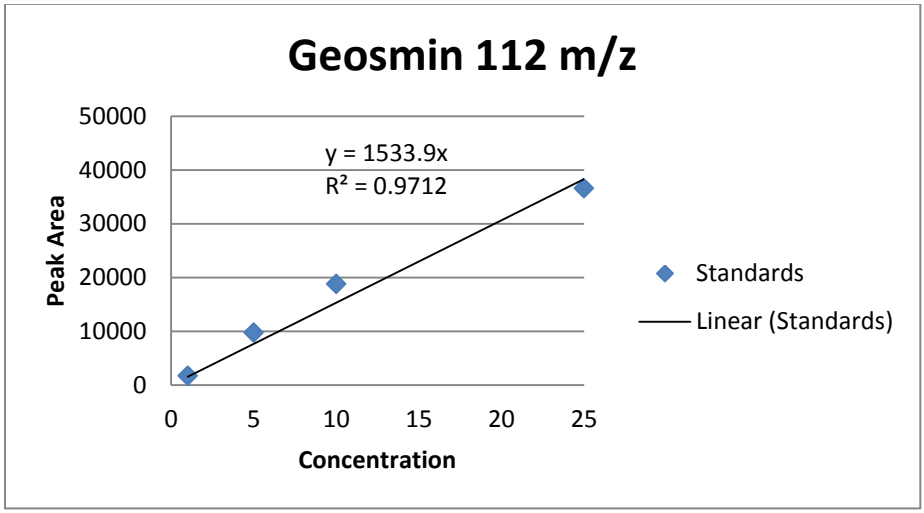


Figure A 114. Geosmin 112 ion curve: 0,4,30,30, Sept-19

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**4**	**50**	**30**	**16-Nov**

Table A 115. Sample 0,10,50,20, Oct-3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	6.4	2.5	6.3	86	5.07	1.82
Area 2	1.6	1.9	1.8	37	1.77	0.12
Concentration (ug/L)	0	0	0	3.6	0.00	0.00
Geosmin						
Area 1	3204	3204	4148	84874	3518.67	445.01
Concentration (ng/L)	2.84	2.16	2.79	49.85	2.60	0.31
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7	6.8	7	6.9	6.82	0.09
Conductivity (µS/cm)	45.4	45	45.5	65.3	45.30	0.22
Temperature (oC)	19.1	21.3	21	21.2	20.47	0.97

Table A 116. Microcystin-LR Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11.9	4.9
1	19.5	5.9
20	226.4	106.3

Table A 117. Geosmin Standard Curve: 0,10,50,20, Oct-3

1	1063
5	4184
10	15624
25	33298
50	76419

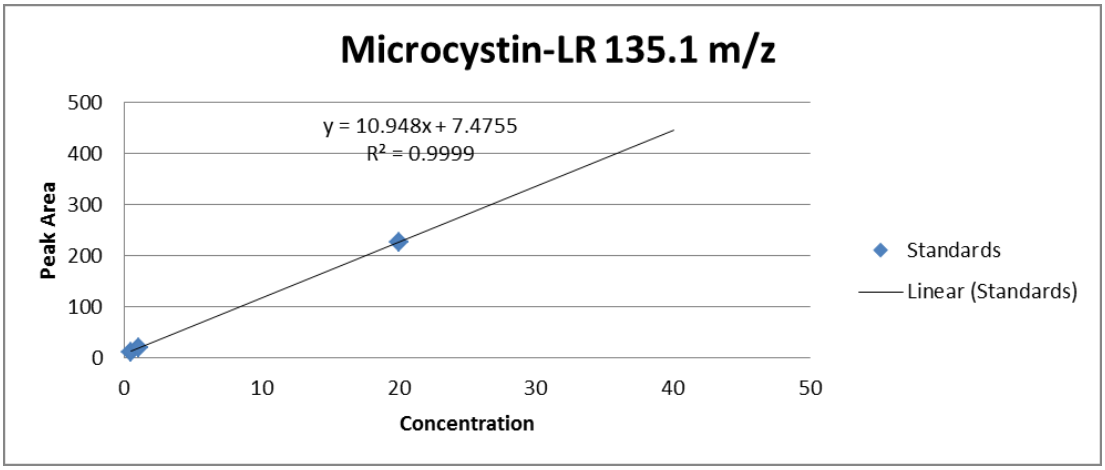


Figure A 115. Microcystin-LR 135.1 ion curve: 0,10,50,20, Oct-3

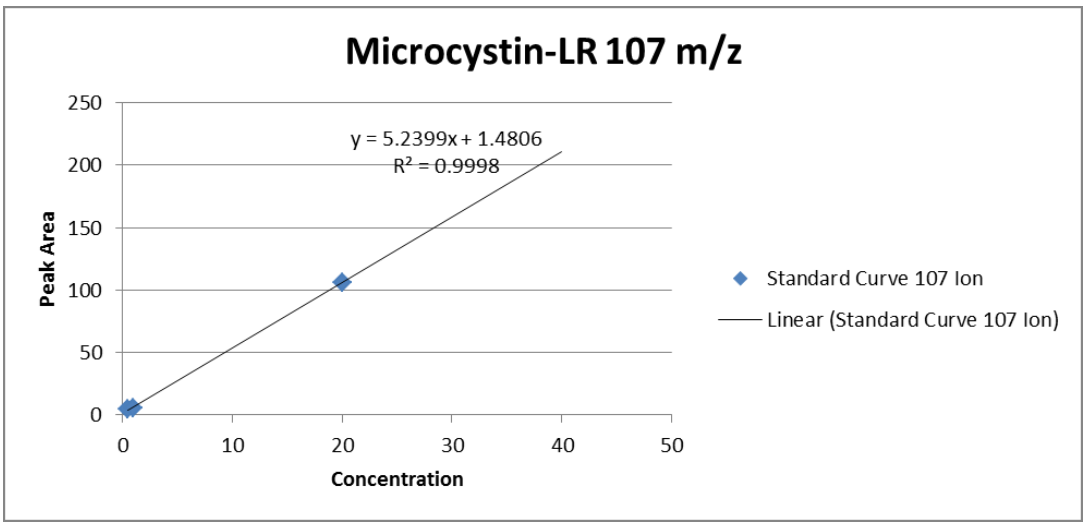


Figure A 116. Microcystin-LR 107 ion curve: 0,10,50,20, Oct-3

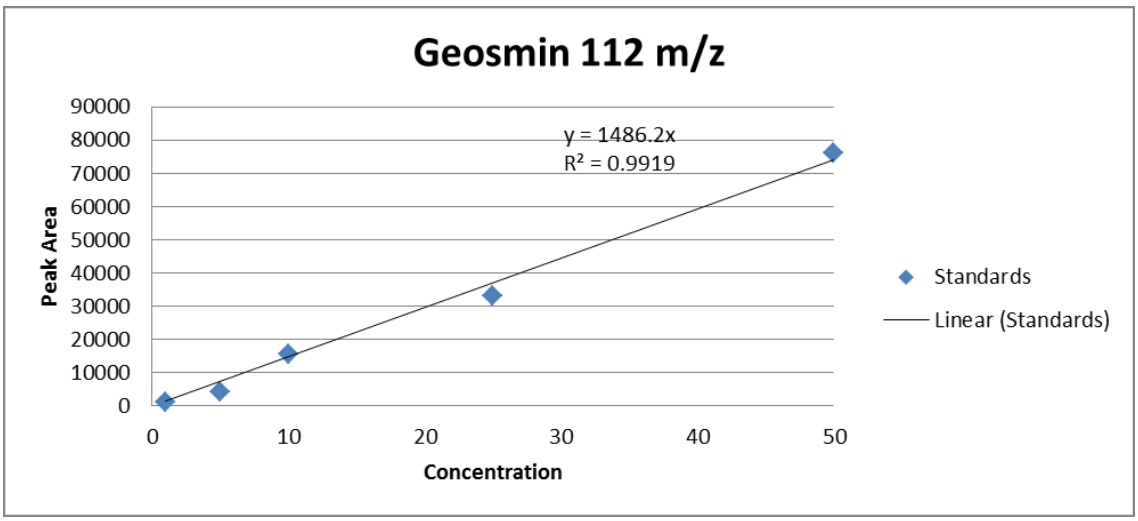


Figure A 117. Geosmin 112 ion curve: 0,10,50,20, Oct-3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**10**	**30**	**16-Nov**

Table A 118. Sample 0,6,10,30, Nov 16 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	326.2	244.2	288.3	609	286.23	33.51
Area 2	182.3	144.3	113	298.3	146.53	28.34
Concentration (ug/L)	0.21	0.2	0.12	3.6	0.18	0.04
Geosmin						
Area 1	275	270	287	9133	277.33	7.13
Concentration (ng/L)	0.31	0.31	0.33	10.41	0.32	0.01
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	30	30	30	30	30.00	0.00
pH	7.1	7.6	7	7.1	6.82	0.26
Conductivity (µS/cm)	59.6	63.5	63.6	50.1	62.23	1.86
Temperature (oC)	18	19.2	19.6	20.1	18.93	0.68

Table A 119. Microcystin-LR Standard Curve: 0,6,10,30, Nov 16

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	231.5	137.8
1	544.5	265.5
10	5406	2350
20	8847	4088

Table A 120. Geosmin Standard Curve: 0,6,10,30, Nov 16

Standard Conc.	Area
1	469
5	3664
10	6254
25	22220
50	44308

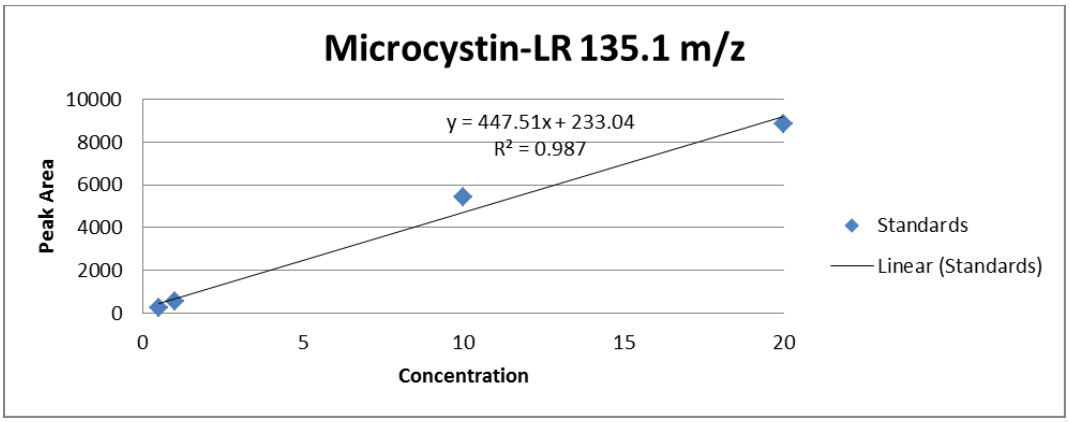


Figure A 118. Microcystin-LR 135.1 ion curve: 0,6,10,30, Nov 16

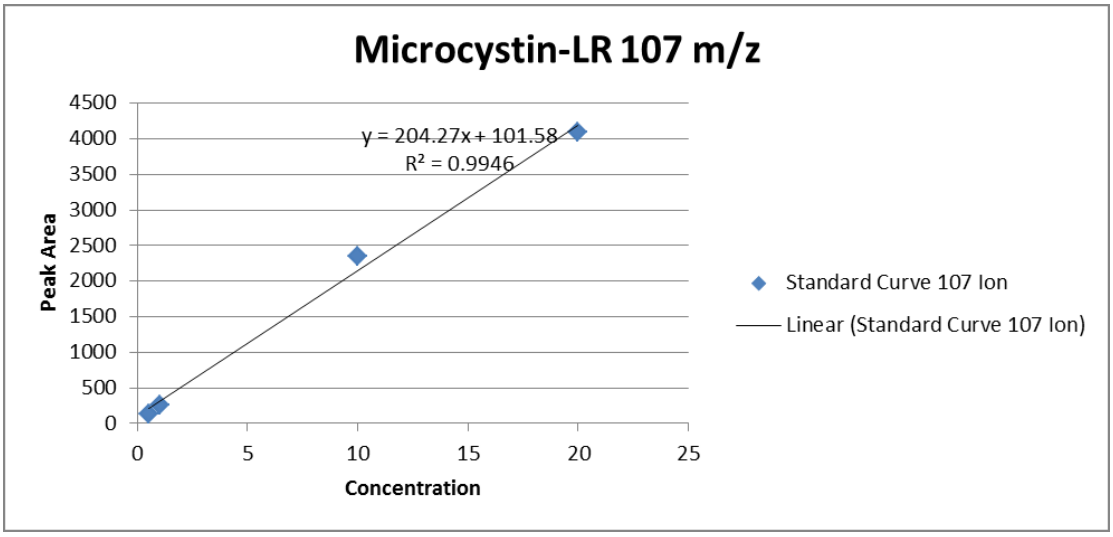


Figure A 119. Microcystin-LR 107 ion curve: 0,6,10,30, Nov 16

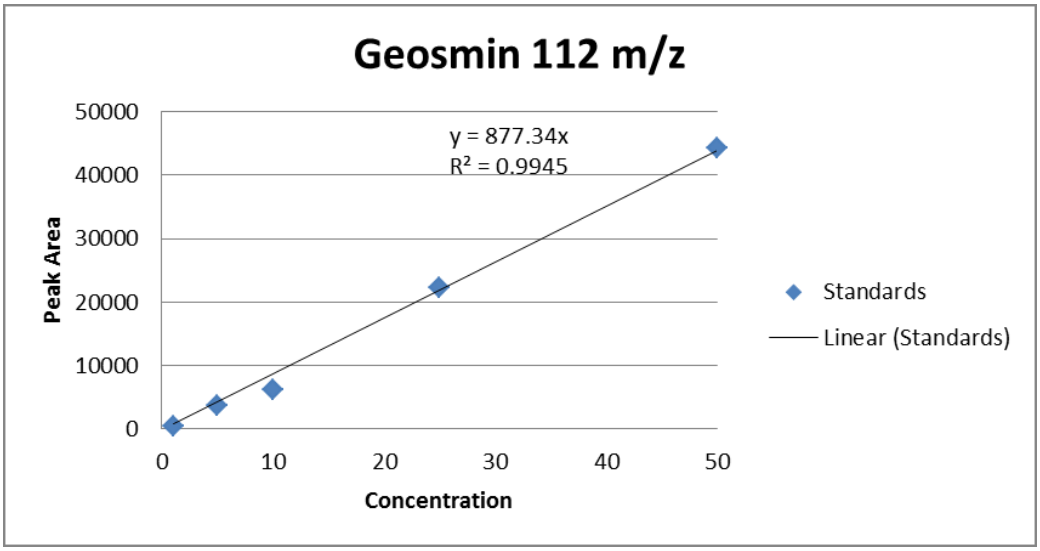


Figure A 120. Geosmin 112 ion curve: 0,6,10,30, Nov 16

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**20**	**30**	**19-Sep**

Table A 121. Sample 0,6,20,30, Sept-19 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	15	8	15	146.8	12.67	3.30
Area 2	5	3	8	63.15	5.33	2.05
Concentration (ug/L)	0.44	0.16	0.44	5.84	0.35	0.13
Geosmin						
Area 1	4106	4358	4318	20670	4260.67	110.58
Concentration (ng/L)	4	4.25	4.21	20.16	4.15	0.11
Water Quality						
Hardness (mg/L CaCO3)	51.3	34.2	51.3	51.3	45.60	8.06
Alkalinity (mg/L CaCO3)	45	40	40	40	41.67	2.36
pH	7.2	7.4	7.4	7.5	7.33	0.09
Conductivity (µS/cm)	63.3	63.5	63.5	63.6	63.43	0.09
Temperature (oC)	18.6	19.5	19.6	19.2	19.23	0.45

Table A 122. Microcystin-LR Standard Curve: 0,6,20,30, Sept-19

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	10	5
1	27	10
10	264	110
20	485	209

Table A 123. Geosmin Standard Curve: 0,6,20,30, Sept-19

Standard Conc.	Area
1	1093
5	4991
10	11755
25	28112
50	49740

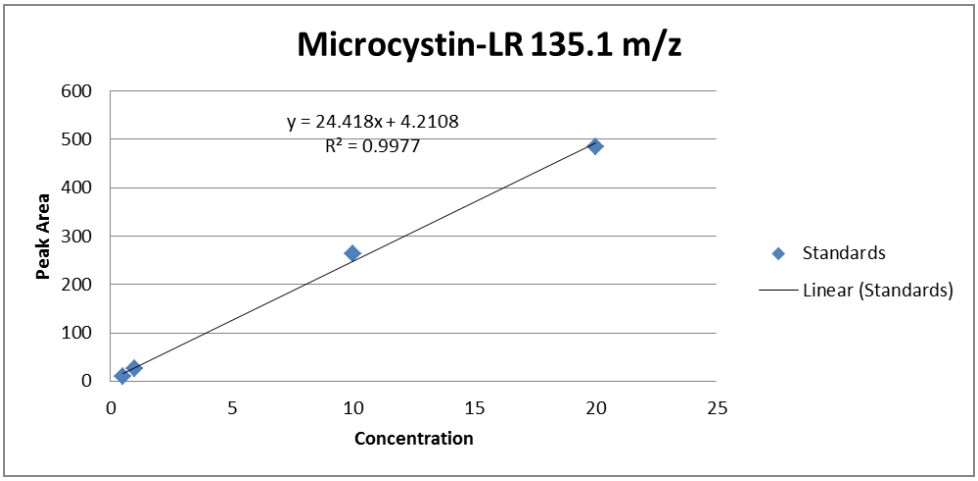


Figure A 121. Microcystin-LR 135.1 ion curve: 0,6,20,30, Sept-19

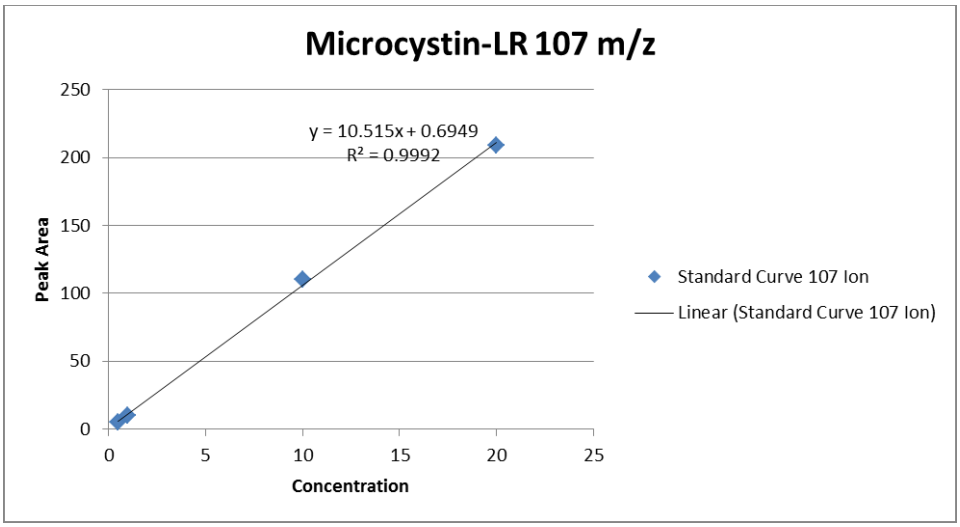


Figure A 122. Microcystin-LR 107 ion curve: 0,6,20,30, Sept-19

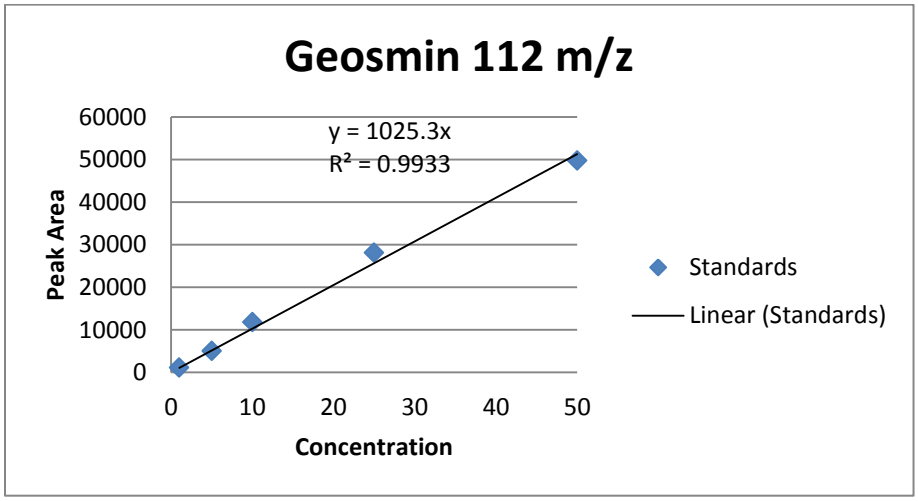


Figure A 123. Geosmin 112 ion curve: 0,6,20,30, Sept-19

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**30**	**30**	**19-Sep**

Table A 124. Sample 0,10,50,20, Oct-3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	38	31	27	153.64	32.00	4.55
Area 2	15	12	12	65.79	13.00	1.41
Concentration (ug/L)	1.38	1.1	0.93	6.12	1.14	0.19
Geosmin						
Area 1	1409	1514	1463	30841	1462	42.87
Concentration (ng/L)	1.37	1.48	1.43	30.08	1.426667	0.04
Water Quality						
Hardness (mg/L CaCO3)	51.3	34.2	34.2	34.2	39.90	8.06
Alkalinity (mg/L CaCO3)	35	40	40	40	38.33	2.36
pH	7.2	7.3	7.3	7.2	7.27	0.05
Conductivity (µS/cm)	72.5	72.6	72.6	72.8	72.57	0.05
Temperature (oC)	20.1	20.3	20.2	19.9	20.20	0.08

Table A 125. Microcystin-LR Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	10	5
1	27	10
10	264	110
20	485	209

Table A 126. Geosmin Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	Area
1	1093
5	4991
10	11755
25	28112
50	49740

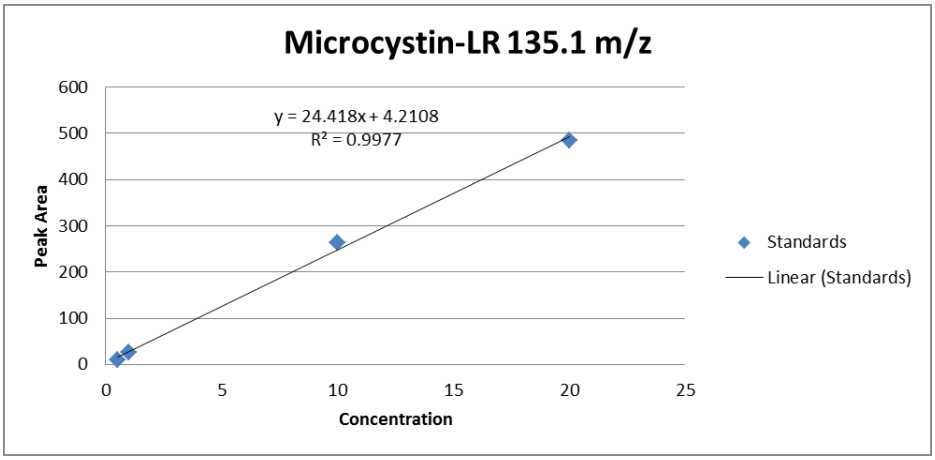


Figure A 124. Microcystin-LR 135.1 ion curve: 0,10,50,20, Oct-3

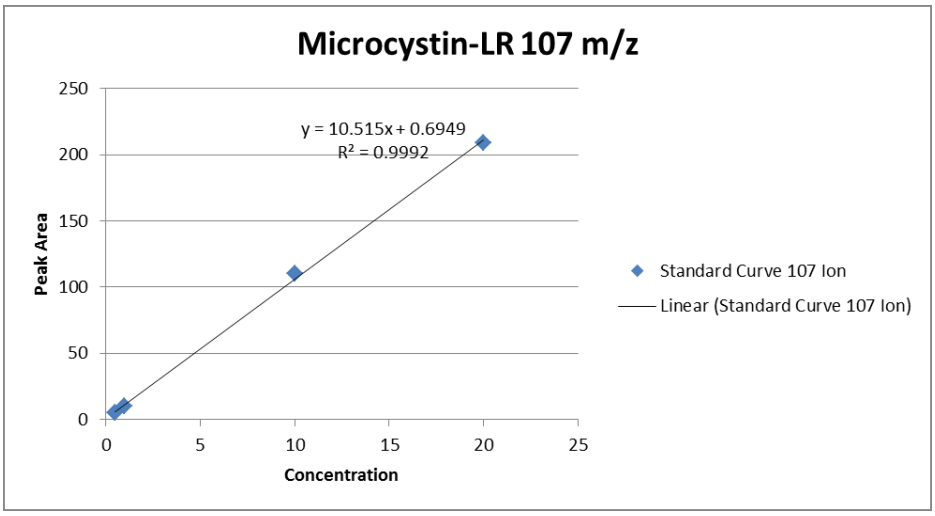


Figure A 125. Microcystin-LR 107 ion curve: 0,10,50,20, Oct-3

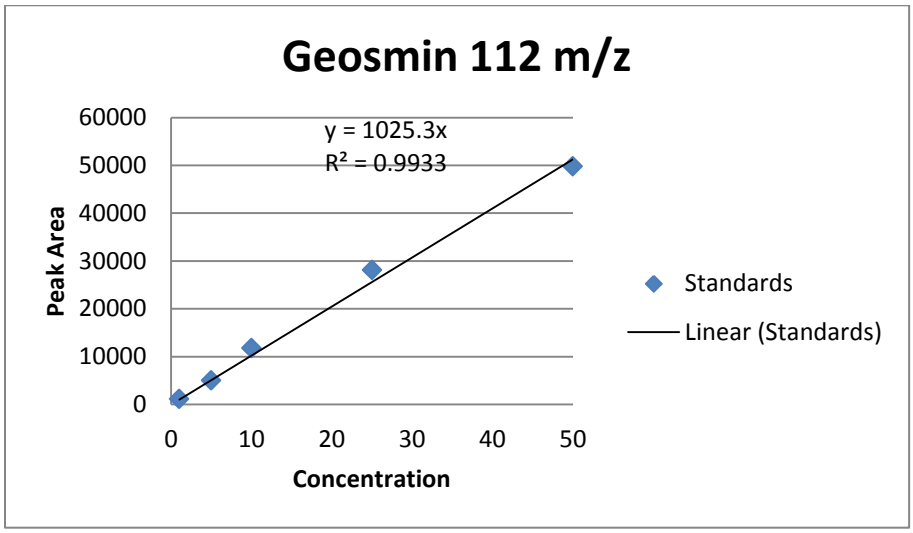


Figure A 126. Geosmin 112 ion curve: 0,10,50,20, Oct-3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**6**	**50**	**30**	**17-Oct**

Table A 127. Sample 0,6,50,30, Oct-17 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	12	33	19	112	21.33	8.73
Area 2	6	16	10	51	10.67	4.11
Concentration (ug/L)	0.86	1.61	1.11	5.65	1.19	0.31
Geosmin						
Area 1	4276	3937	4164	68269	4125.667	141.03
Concentration (ng/L)	3.16	2.91	3.08	50.48	3.05	0.10
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7.1	6.7	6.9	6.9	6.90	0.16
Turbidity (NTU)	6.2	5.7	3.3	1.9	5.07	1.27
Conductivity (µS/cm)	70.8	70.6	70.7	72.2	70.70	0.08
Temperature (oC)	19.4	19.7	19.9	19.1	19.67	0.21

Table A 128. Microcystin-LR Standard Curve: 0,6,50,30, Oct-17

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	12	8
1	26	11
20	504	221
40	1117	487

Table A 129. Geosmin Standard Curve: 0,6,50,30, Oct-17

Standard Conc.	Area
1	1398
5	7302
10	17237
25	33405
50	67024

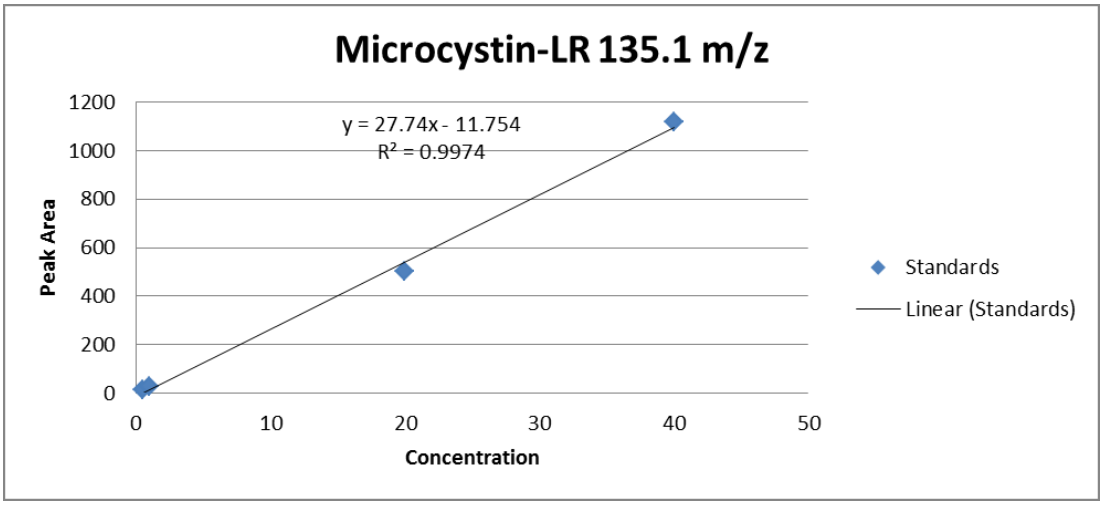


Figure A 127. Microcystin-LR 135.1 ion curve: 0,6,50,30, Oct-17

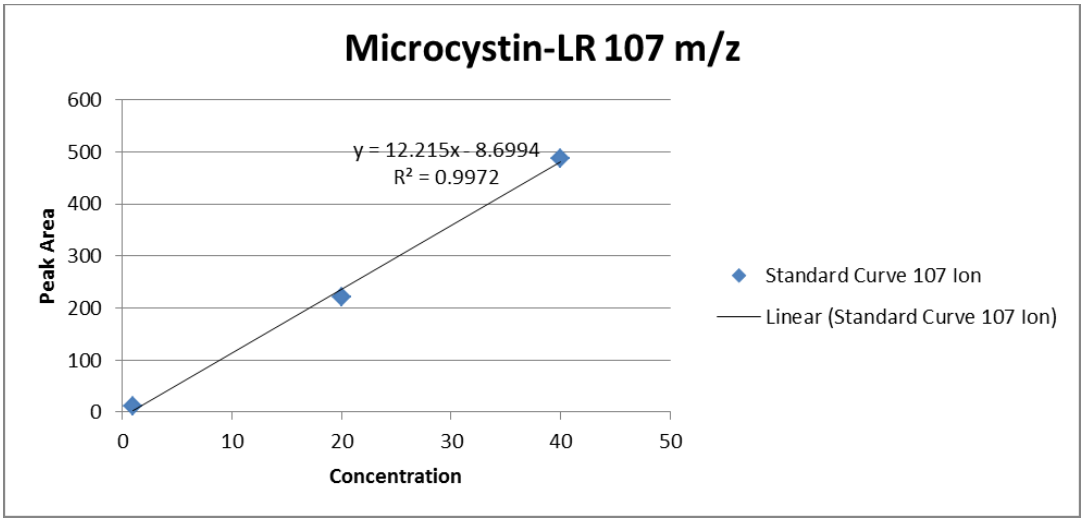


Figure A 128. Microcystin-LR 107 ion curve: 0,6,50,30, Oct-17

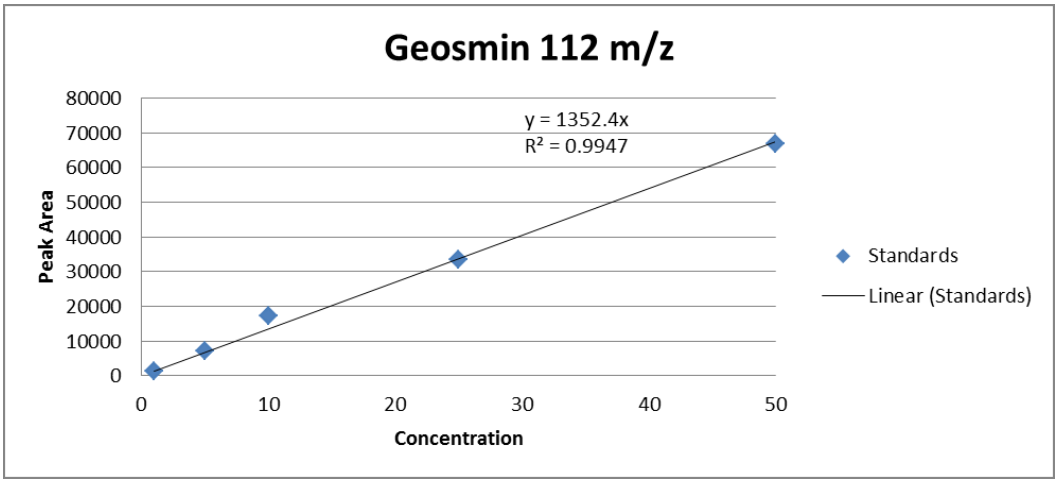


Figure A 129. Geosmin 112 ion curve: 0,6,50,30, Oct-17

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**10**	**30**	**11-Jan**

Table A 130. Sample 0,10,10,30, Jan-11 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	638.9	638.4	471.8	7077.9	583.03	78.65
Area 2	236.9	328.9	203.8	2993.5	256.53	52.93
Concentration (ug/L)	0.89	0.89	0.7	8.1	0.83	0.09
Geosmin						
Area 1	993	864	990	8451	949	60.12
Concentration (ng/L)	1.27	1.1	1.26	10.78	1.21	0.08
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7.4	7.4	7.5	7.2	7.43	0.05
Conductivity (µS/cm)	64.3	64.6	64.6	60.6	64.50	0.14
Temperature (oC)	21.1	21.2	21.1	21.3	21.13	0.05

Table A 131. Microcystin-LR Standard Curve: 0,10,10,30, Jan-11

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	425.1	208.4
1	685.9	287
10	8614	3896
20	17796	7545

Table A 132. Geosmin Standard Curve: 0,10,10,30, Jan-11

Standard Conc.	Area
1	576
5	4872
10	6936
25	20017
50	39099

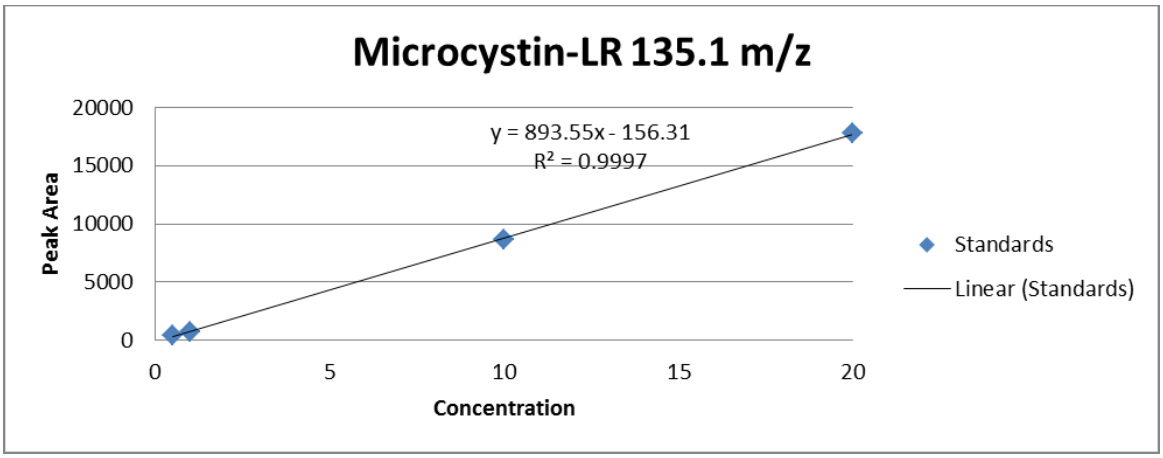


Figure A 130. Microcystin-LR 135.1 ion curve: 0,10,10,30, Jan-11

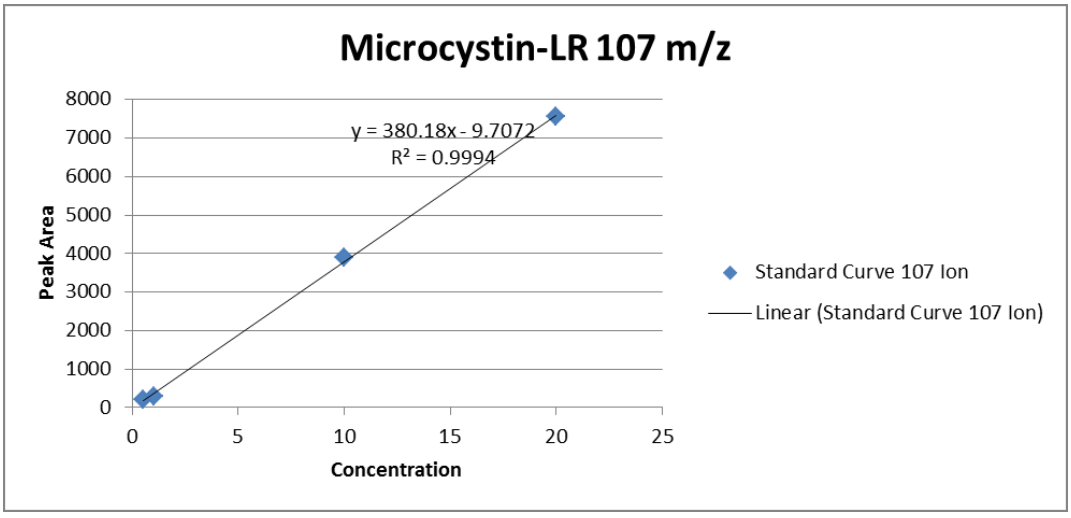


Figure A 131. Microcystin-LR 107 ion curve: 0,10,10,30, Jan-11

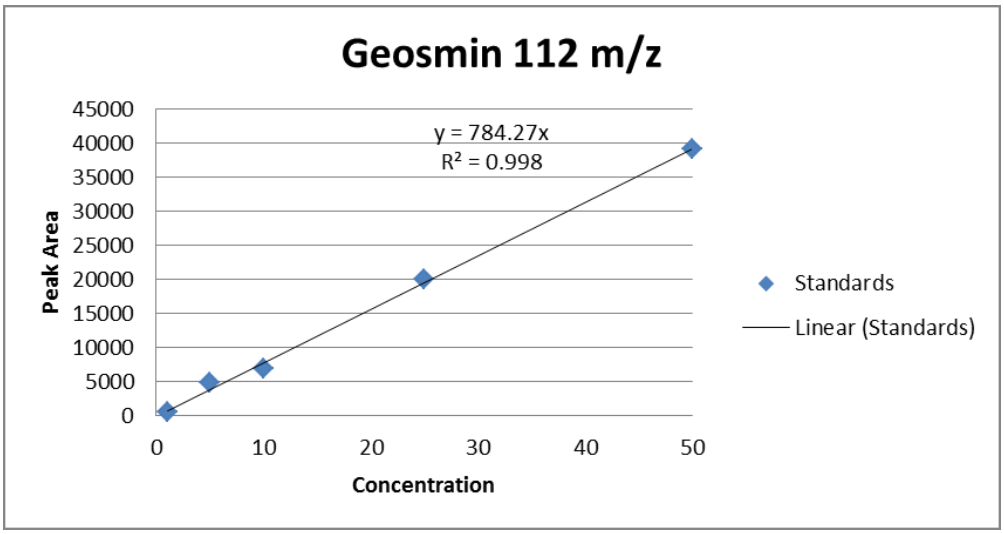


Figure A 132. Geosmin 112 ion curve: 0,10,10,30, Jan-11

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**20**	**30**	**3-Oct**

Table A 133. Sample 0,10,20,30, Oct-3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	8	4	38	236	16.67	15.17
Area 2	3	2	18	109	7.67	7.32
Concentration (ug/L)	0	0	1.56	12.5	0.52	0.74
Geosmin						
Area 1	4581	4231	4567	32952	4459.667	161.79
Concentration (ng/L)	3.22	2.98	3.21	23.19	3.136667	0.11
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	35	35	35	35	35.00	0.00
pH	7.02	7.12	7.18	7.2	7.11	0.07
Conductivity (µS/cm)	71.9	72.1	72	71.9	72.00	0.08
Temperature (oC)	20.1	20.1	20.3	20.4	20.17	0.09

Table A 134. Microcystin-LR Standard Curve: 0,10,20,30, Oct-3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	1	1
1	19	9
10	242	104
20	347	159

Table A 135. Geosmin Standard Curve: 0,10,20,30, Oct-3

Standard Conc.	Area
1	959
5	5374
10	12644
25	32248
50	73188

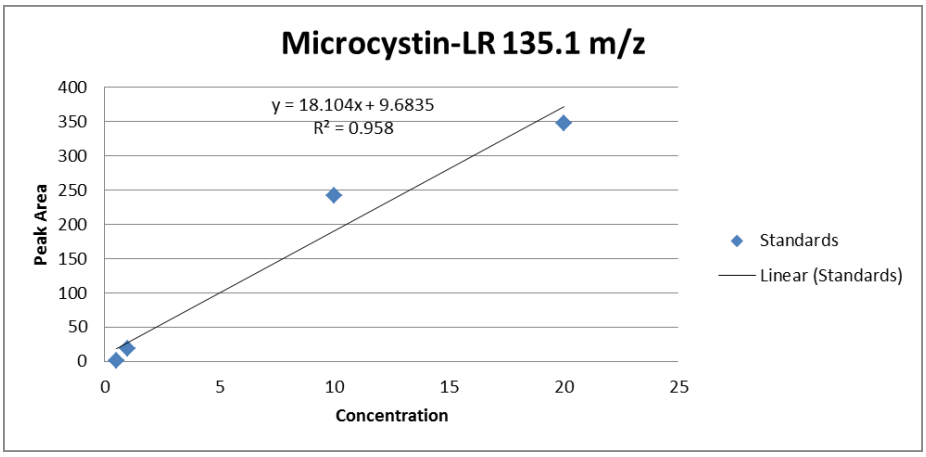


Figure A 133. Microcystin-LR 135.1 ion curve: 0,10,20,30, Oct-3

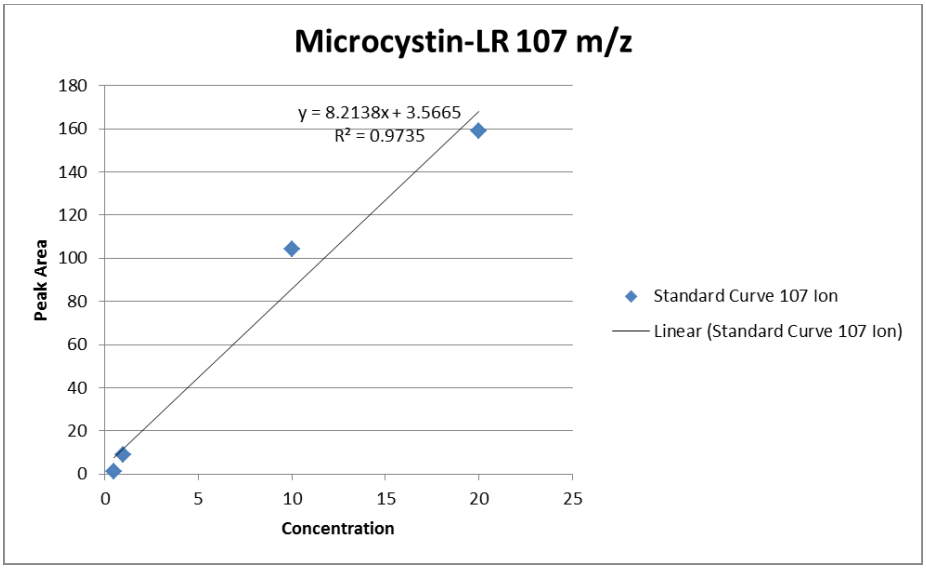


Figure A 134. Microcystin-LR 107 ion curve: 0,10,20,30, Oct-3

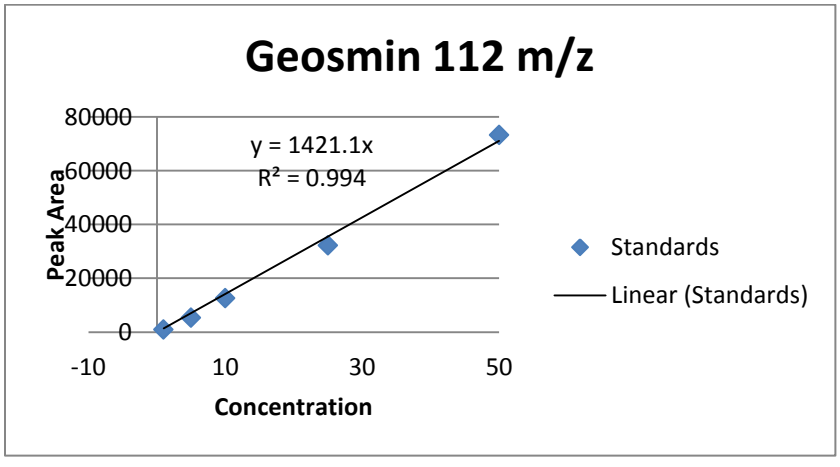


Figure A 135. Geosmin 112 ion curve: 0,10,20,30, Oct-3

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**30**	**30**	**9-Aug**

Table A 136. Sample 0,10,30,30, Aug-9 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	52	48	51	239	50.33	1.70
Area 2	20	28	20	108	22.67	3.77
Concentration (ug/L)	1.16	1.08	1.14	11.43	1.13	0.03
Geosmin						
Area 1	531	420	433	13582	461.3333	49.55
Concentration (ng/L)	1.31	1.03	1.06	33.39	9.1975	0.13
Water Quality						
Hardness (mg/L CaCO3)	51.3	51.3	51.3	34.2	51.30	0.00
Alkalinity (mg/L CaCO3)	40	40	40	35	40.00	0.00
pH	7.1	7	6.9	7.2	7.00	0.08
Conductivity (µS/cm)	63.5	63.8	63.6	72.4	63.63	0.12
Temperature (oC)	19.1	19.1	18.7	20.9	18.97	0.19

Table A 137. Microcystin-LR Standard Curve: 0,10,30,30, Aug-9

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	24	11
1	54	53
10	498	209
20	1095	467

Table A 138. Geosmin Standard Curve: 0,10,30,30, Aug-9

Standard Curve	Area
5	1425
10	3610
25	10474

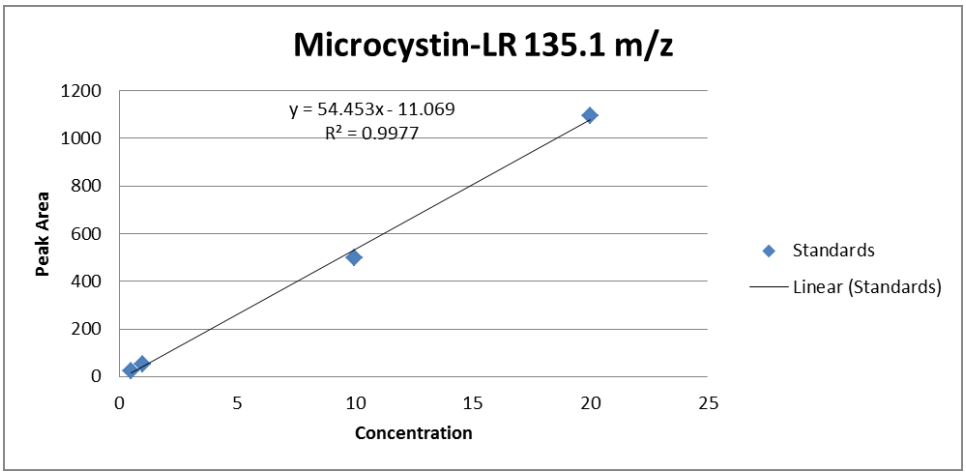


Figure A 136. Microcystin-LR 135.1 ion curve: 0,10,30,30, Aug-9

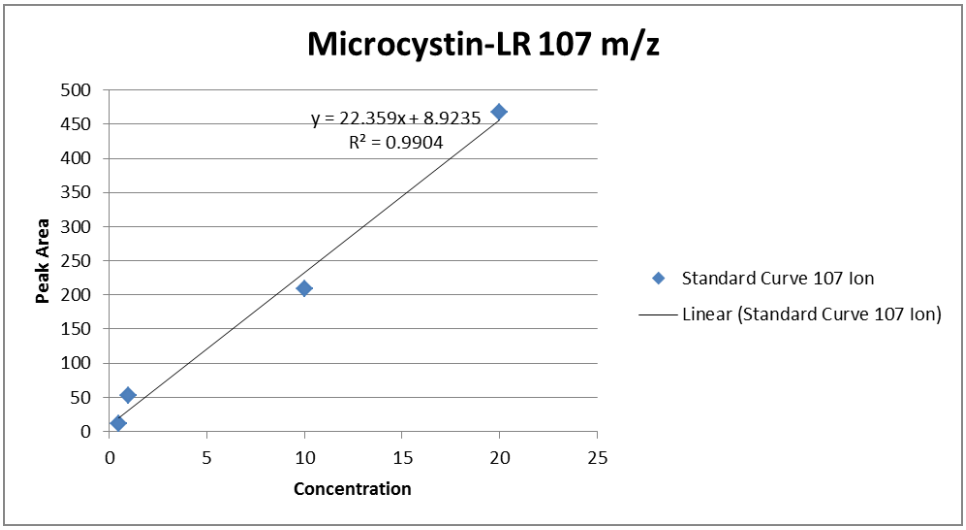


Figure A 137. Microcystin-LR 107 ion curve: 0,10,30,30, Aug-9

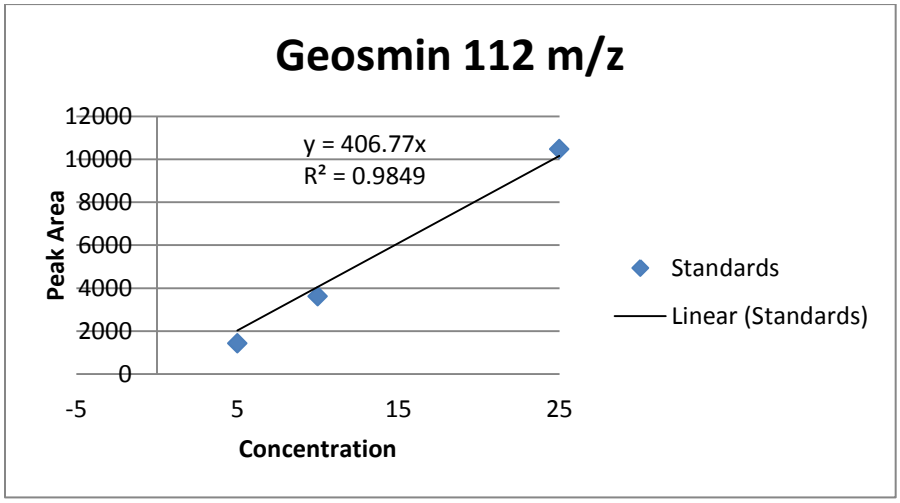


Figure A 138. Geosmin 112 ion curve: 0,10,30,30, Aug-9

Sample ID				
Moringa Coagulant (mg/L)	Microcystin-LR (ug/L)	Geosmin (ng/L)	PAC (mg/L)	Water Sample (month-date)
0	**10**	**50**	**30**	**25-Jan**

Table A 139. Sample 0,10,50,20, Oct-3 results

	Replicate 1	Replicate 2	Replicate 3	Control	Average	Std. Dev
Microcystin-LR						
Area 1	29	25	22	223	25.33	2.87
Area 2	13	9	11	97	11.00	1.63
Concentration (ug/L)	1.63	1.45	1.31	10.69	1.46	0.13
Geosmin						
Area 1	913	875	881	33286	889.6667	16.68
Concentration (ng/L)	1.4	1.34	1.35	51	1.363333	0.03
Water Quality						
Hardness (mg/L CaCO3)	34.2	34.2	34.2	34.2	34.20	0.00
Alkalinity (mg/L CaCO3)	40	40	40	35	40.00	0.00
pH	7	7	6.9	7.18	6.97	0.05
Conductivity (µS/cm)	72.3	72.4	78.3	72.8	74.33	2.81
Temperature (oC)	19.7	20.4	21.6	20	20.57	0.78

Table A 140. Microcystin-LR Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	135.1 m/z	107 m/z
(ug/L)	Area A	Area B
0.5	11	5
1	26	13
10	176	82
20	438	204

Table A 141. Geosmin Standard Curve: 0,10,50,20, Oct-3

Standard Conc.	Area
1	410
5	2172
10	5178
25	15968
50	33191

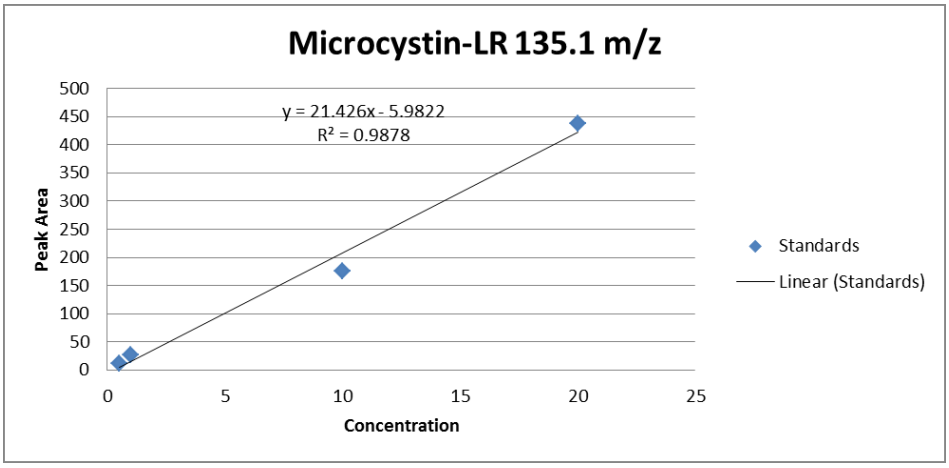


Figure A 139. Microcystin-LR 135.1 ion curve: 0,10,50,20, Oct-3

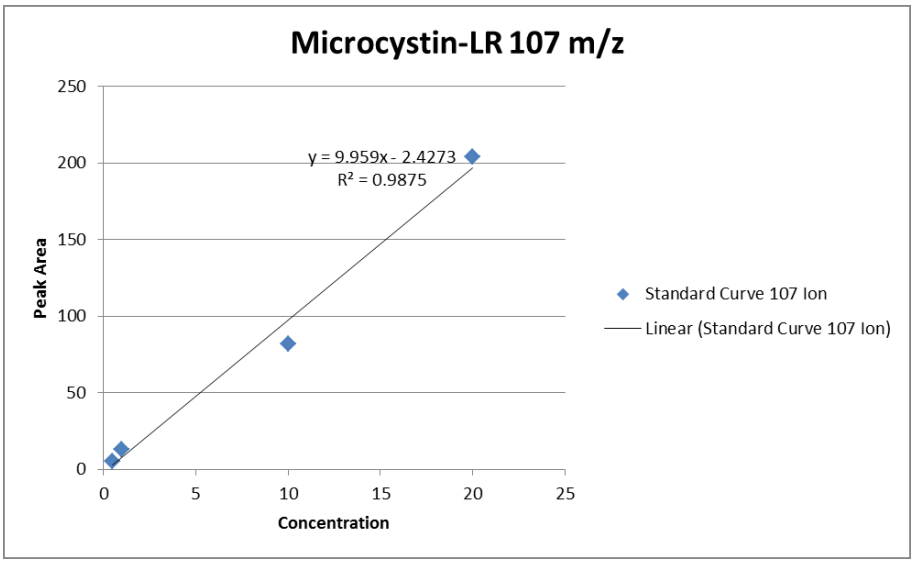


Figure A 140. Microcystin-LR 107 ion curve: 0,10,50,20, Oct-3

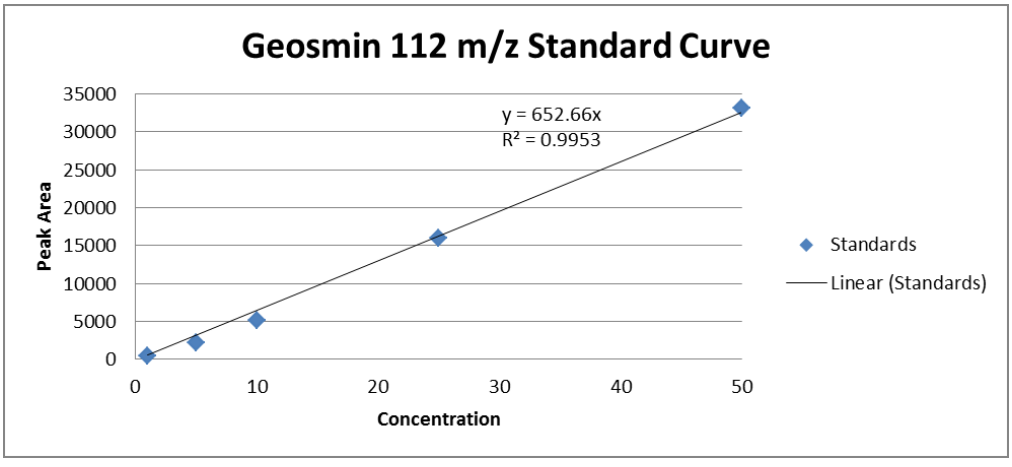
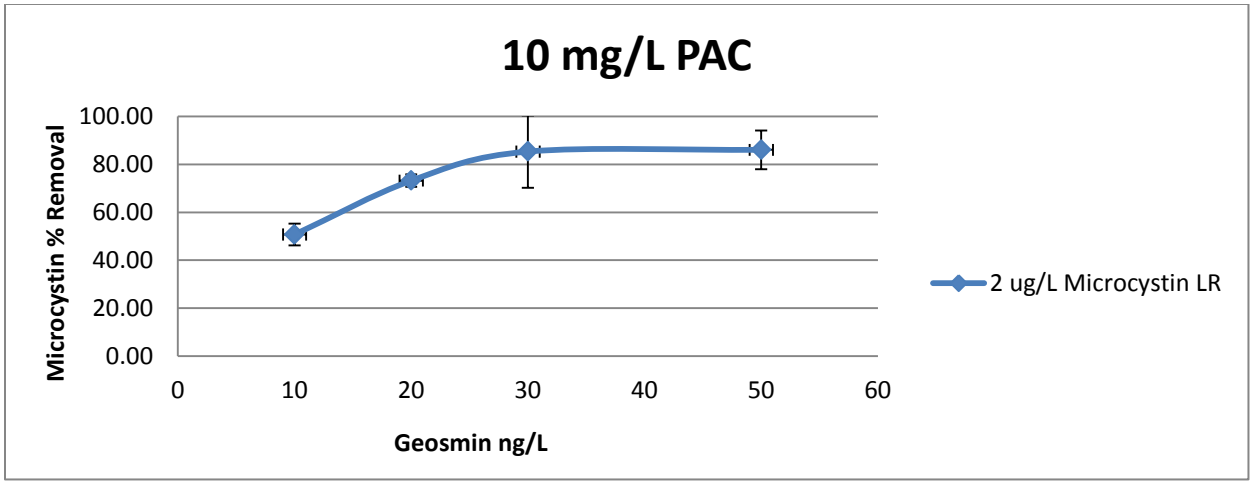
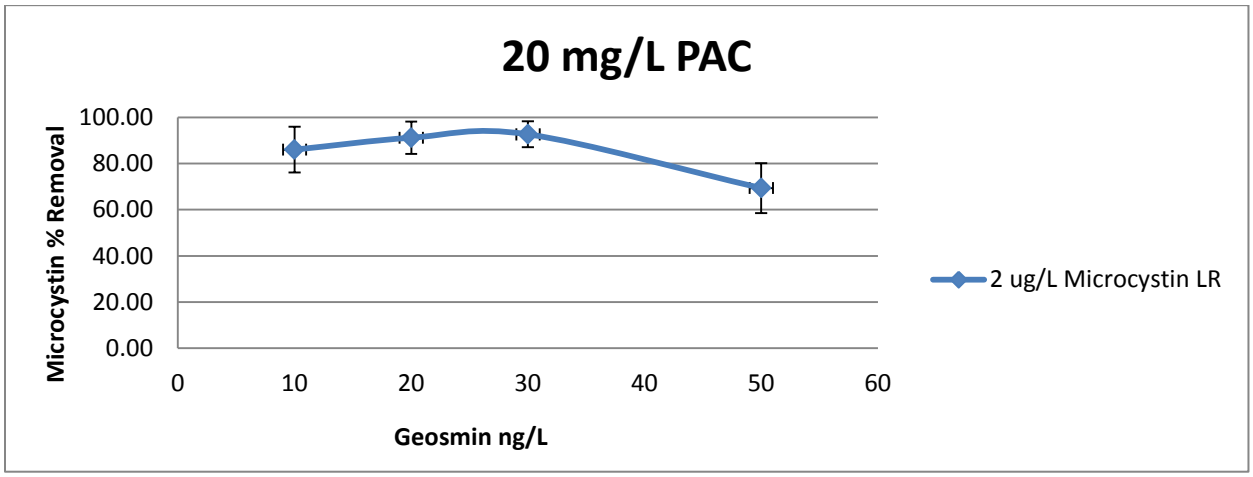


Figure A 141. Geosmin 112 ion curve: 0,10,50,20, Oct-3

Appendix B. Jar Test Results Graphs

B.1 Microcystin-LR Removal





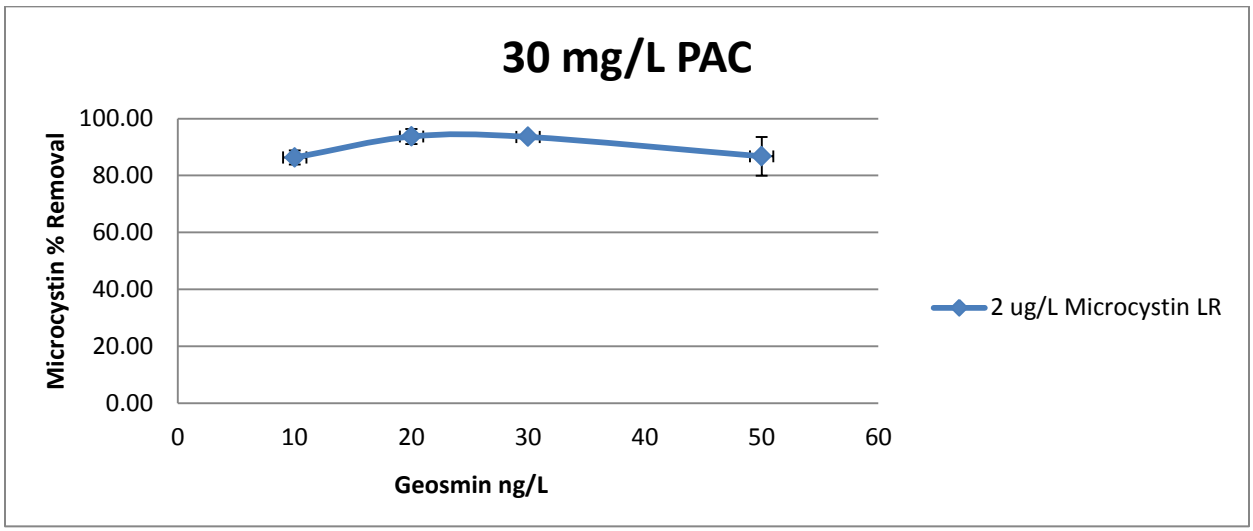


Figure B 1. Graphs of Removal of 2 ug/L Microcystin-LR

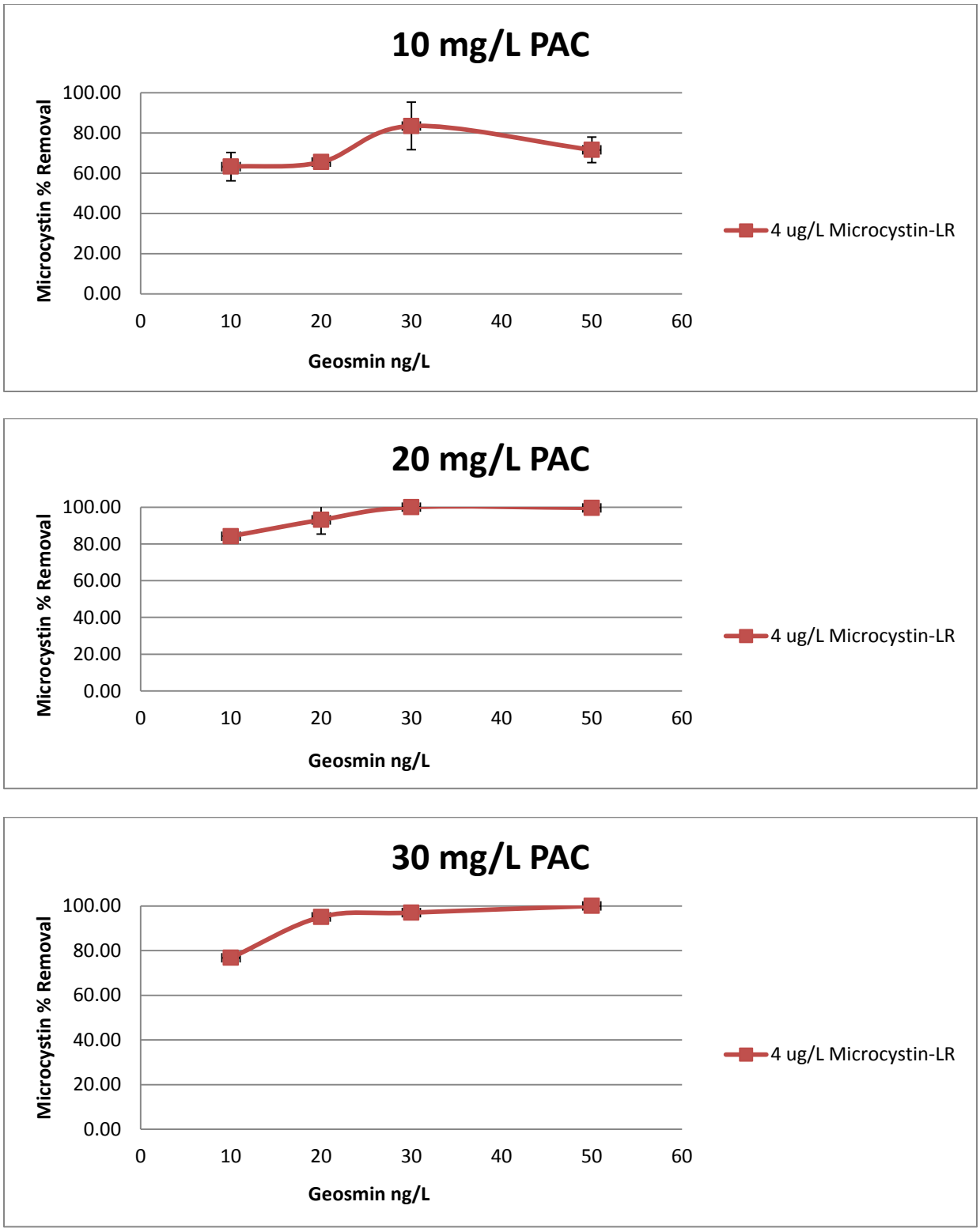
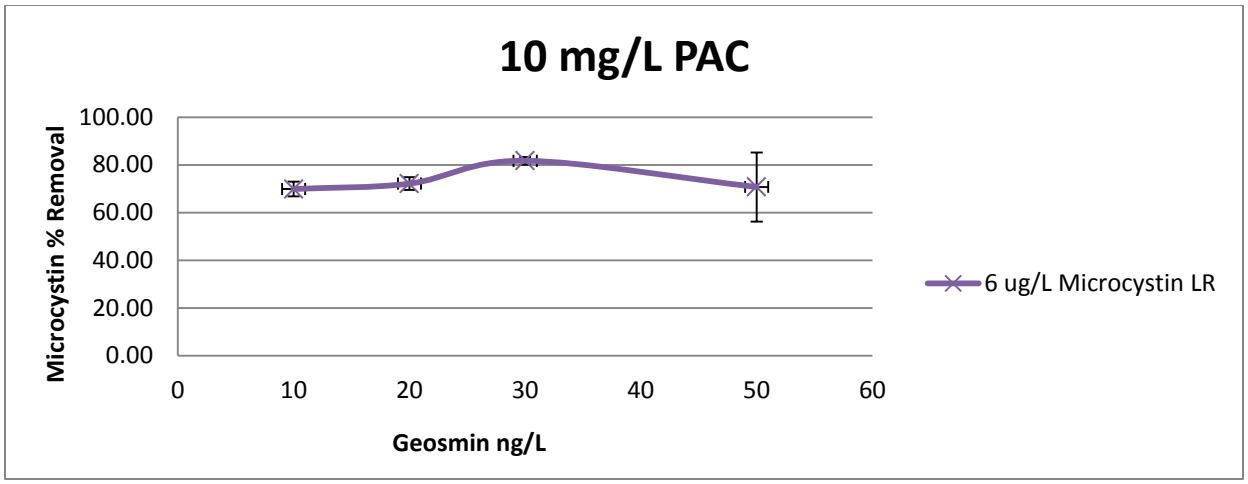
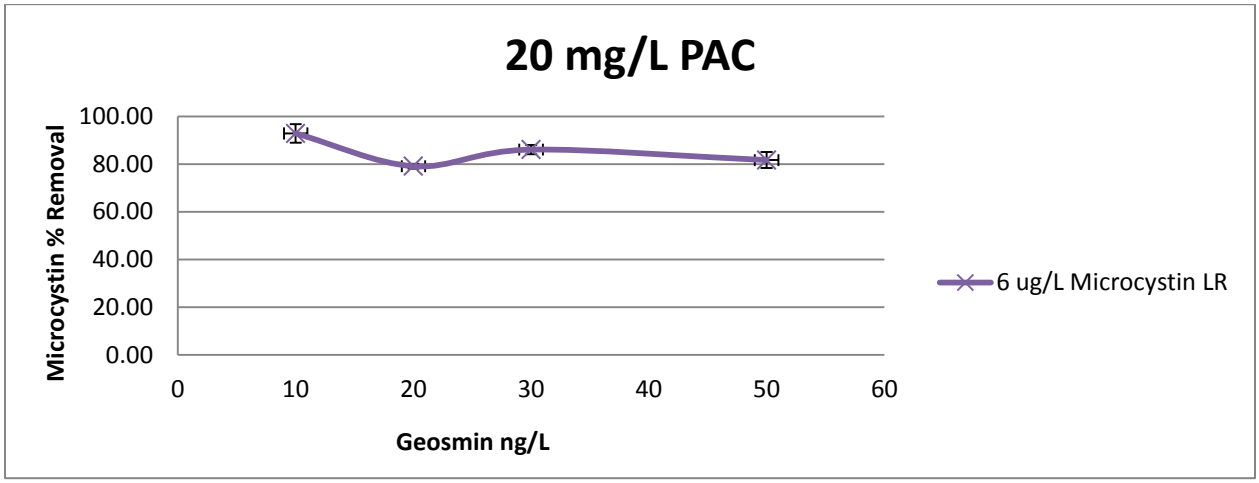


Figure B 2. Graphs of Removal of 4 ug/L Microcystin-LR





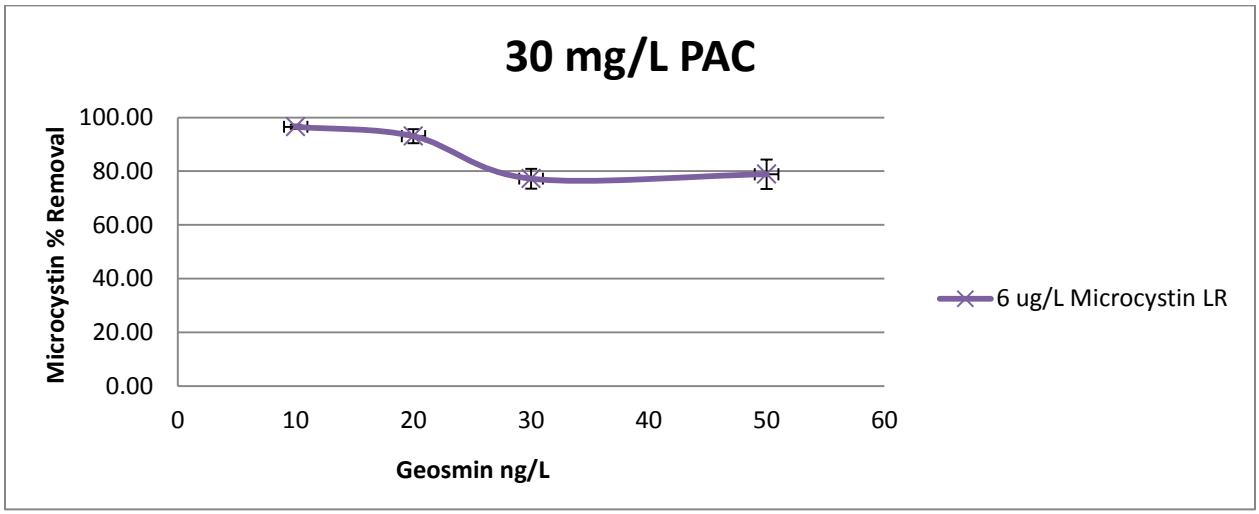
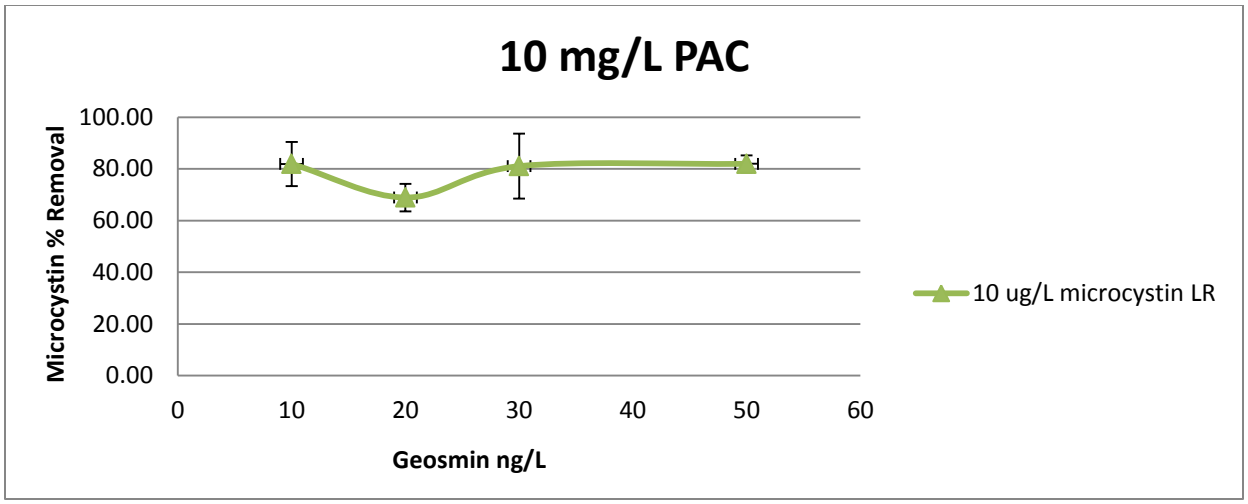
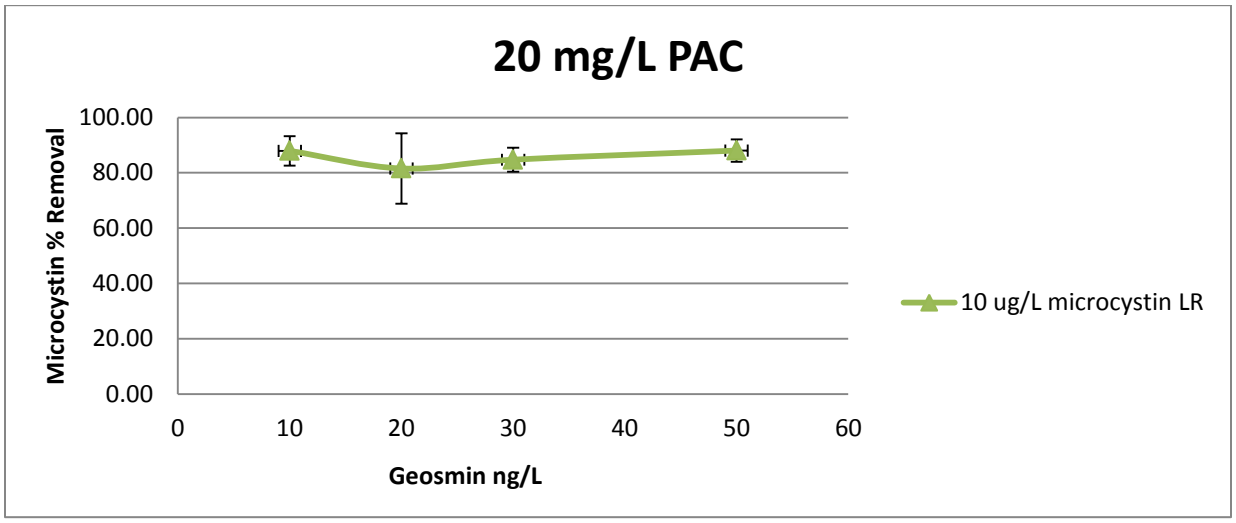


Figure B 3. Graphs of Removal of 6 ug/L Microcystin-LR





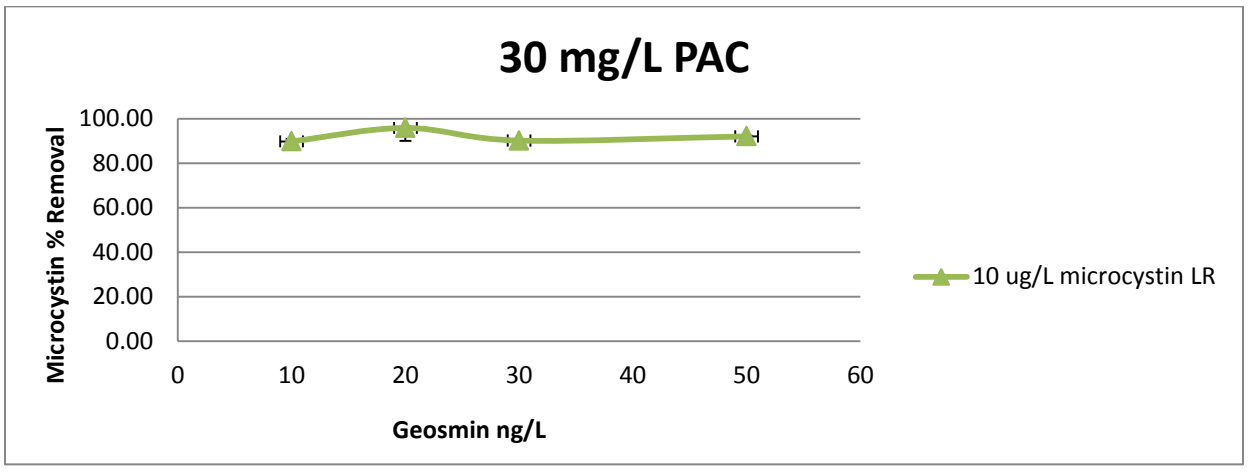
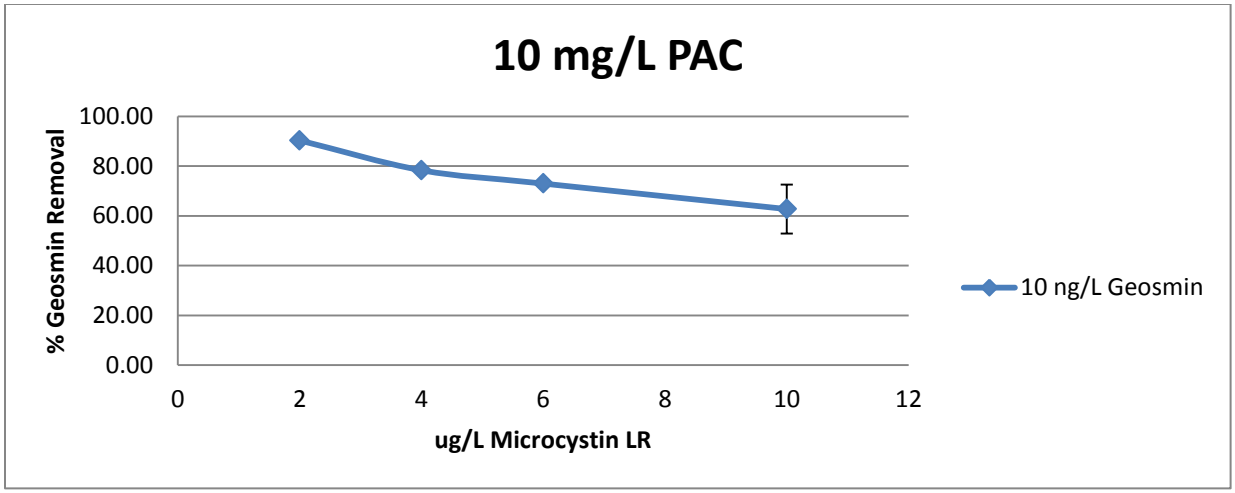
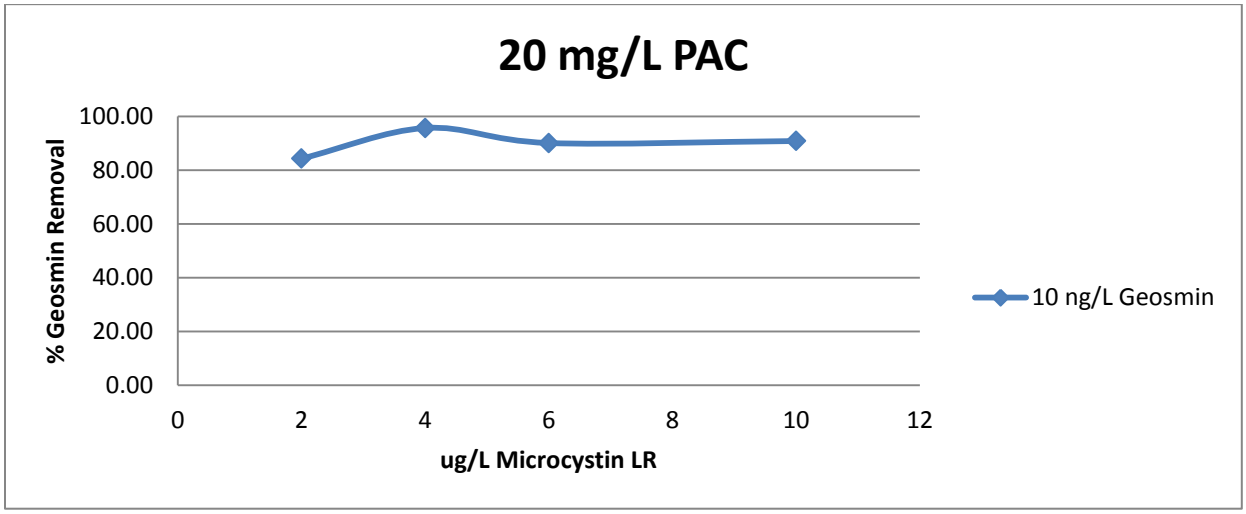


Figure B 4. Graphs of Removal of 10 ug/L Microcystin-LR

B.2 Geosmin Removal





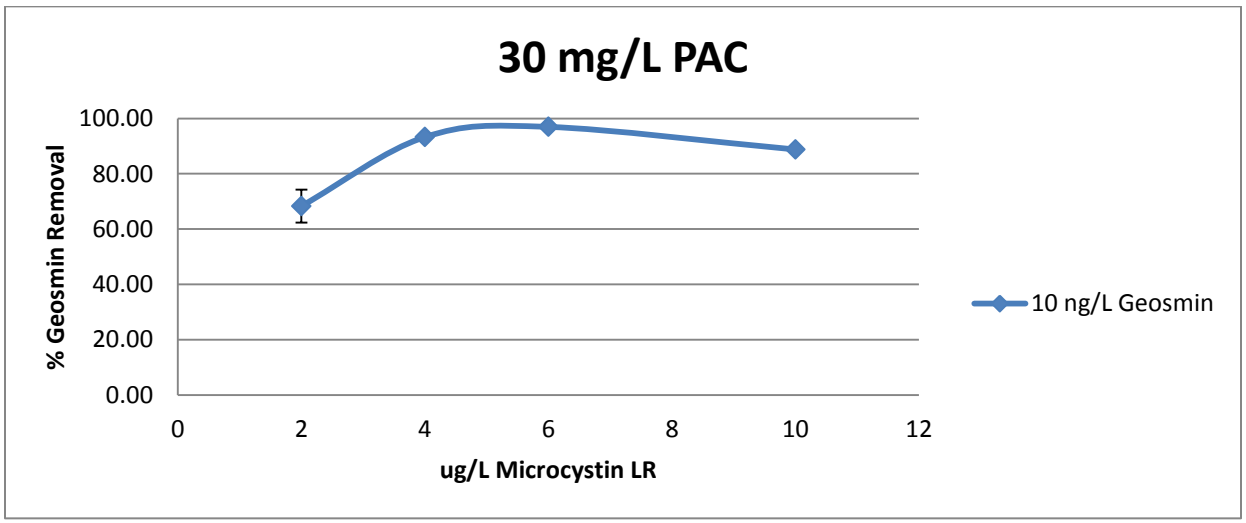


Figure B 5. Graphs of Removal of 10 ng/L Geosmin

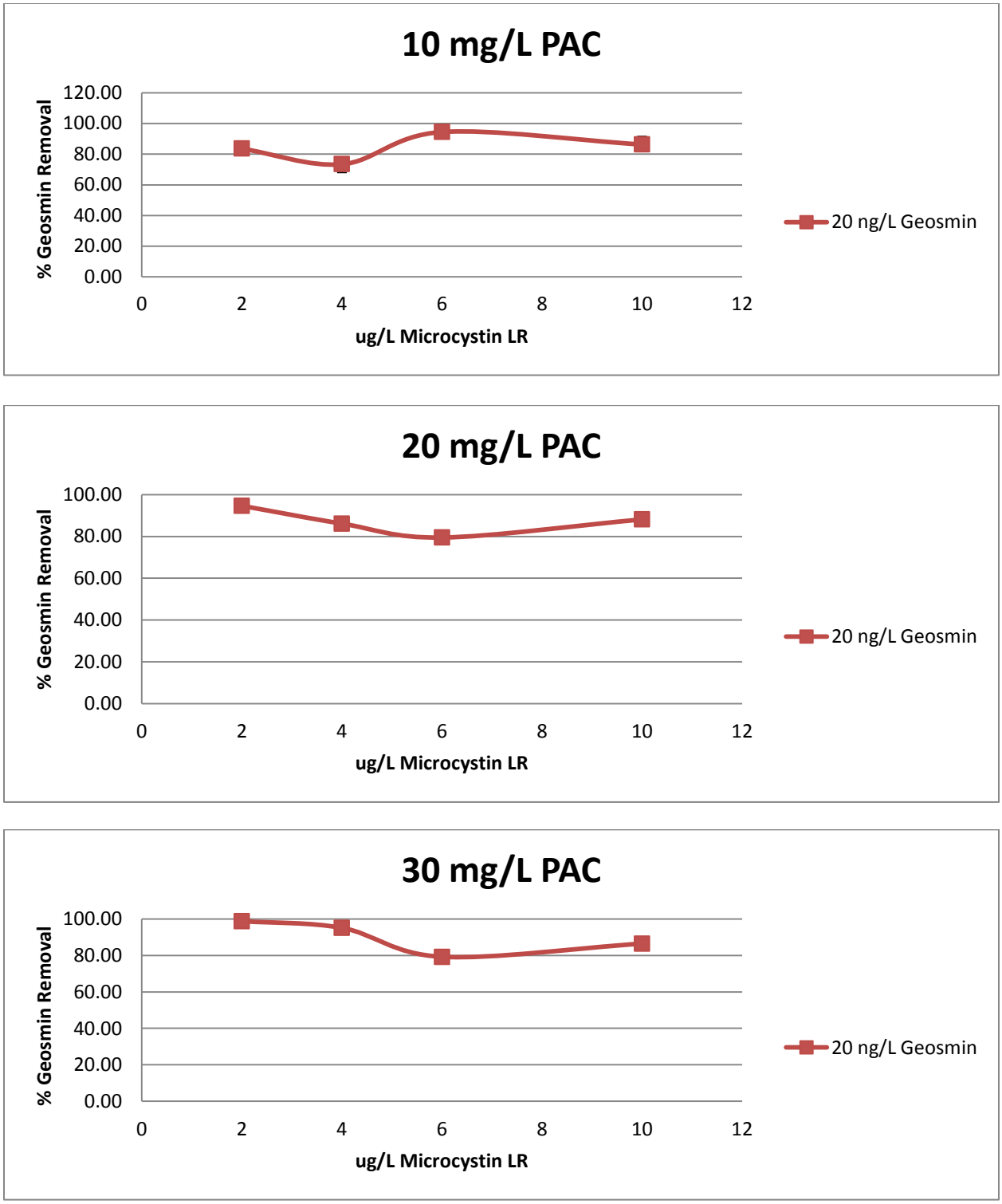


Figure B 6. Graphs of Removal of 20 ng/L Geosmin

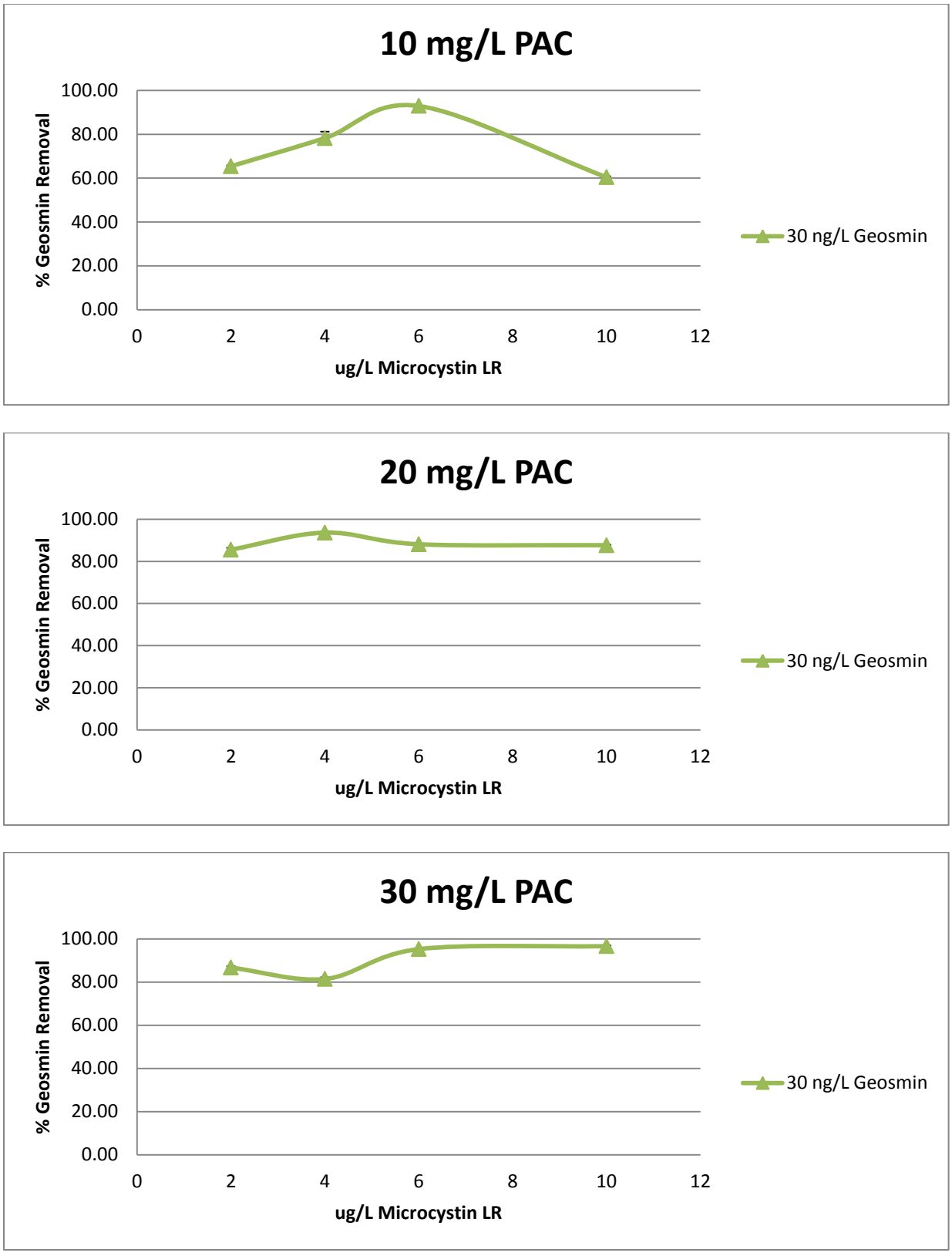


Figure B 7. Graphs of Removal of 30 ng/L Geosmin

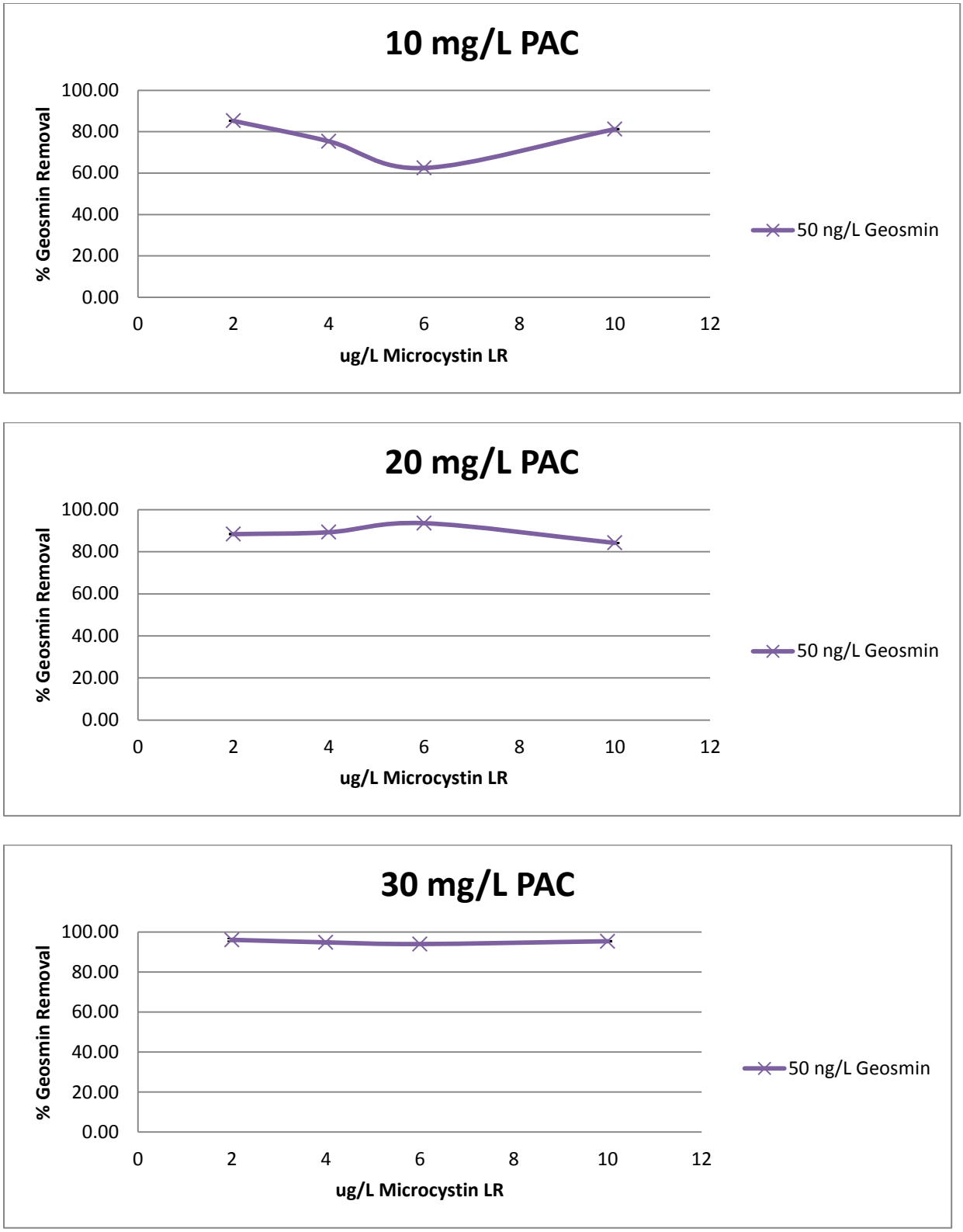


Figure B 8. Graphs of Removal of 50 ng/L Geosmin

Appendix. C. Environmental Samples

C.1 Horsetooth Water Quality.

Table C 1. Horsetooth Water Quality from raw water samples

Sample Date	Notes	Hardness	Alkalinity	pH	Turbidity	TOC	DO
Day-Month		(mg/L CaCO3)	(mg/L CaCO3)		NTU	(mg/L)	(mg/L)
11-Jun		51.3	35	7.1	3.29	3.3	N/A
22-Jun		34.2	35	7.41	3.64	3.68	7.43
7-Jul		42.75	35	7.34	3.29	3.77	6.9
19-Jul		34.2	35	7.5	3.04	3.5	7.84
9-Aug		34.2	38	7.37	2.57	3.77	6.76
17-Sep		100.6	70	7.4	1.96	3.61	N/A
	Diluted	51.3	35	7.14	1.5	N/A	N/A
19-Sep		34.2	45	7.19	2.52	3.82	4.56
3-Oct		34.2	40	7.18	1.8	3.68	4.01
17-Oct		34.2	35	7.3	2.69	3.9	3.39
26-Oct		34.2	35	7.38	0.94	3.88	8.77
16-Nov		34.2	30	7.5	2.31	3.54	8.82
11-Jan		34.2	35	7.69	1.71	3.86	10.3
25-Jan		34.2	35	7.57	2.35	4	10.6
22-Feb		34.2	35	7.5	1.94	3.8	10.8
	Average	36.17	36.00	7.39	2.47	3.73	7.52
	std dev	6.14	3.24	0.17	0.74	0.18	2.42

Figure C 1. Horsetooth Water Samples Collected in 4L Amber Jugs

C.2. Northern Colorado Grab Sampling

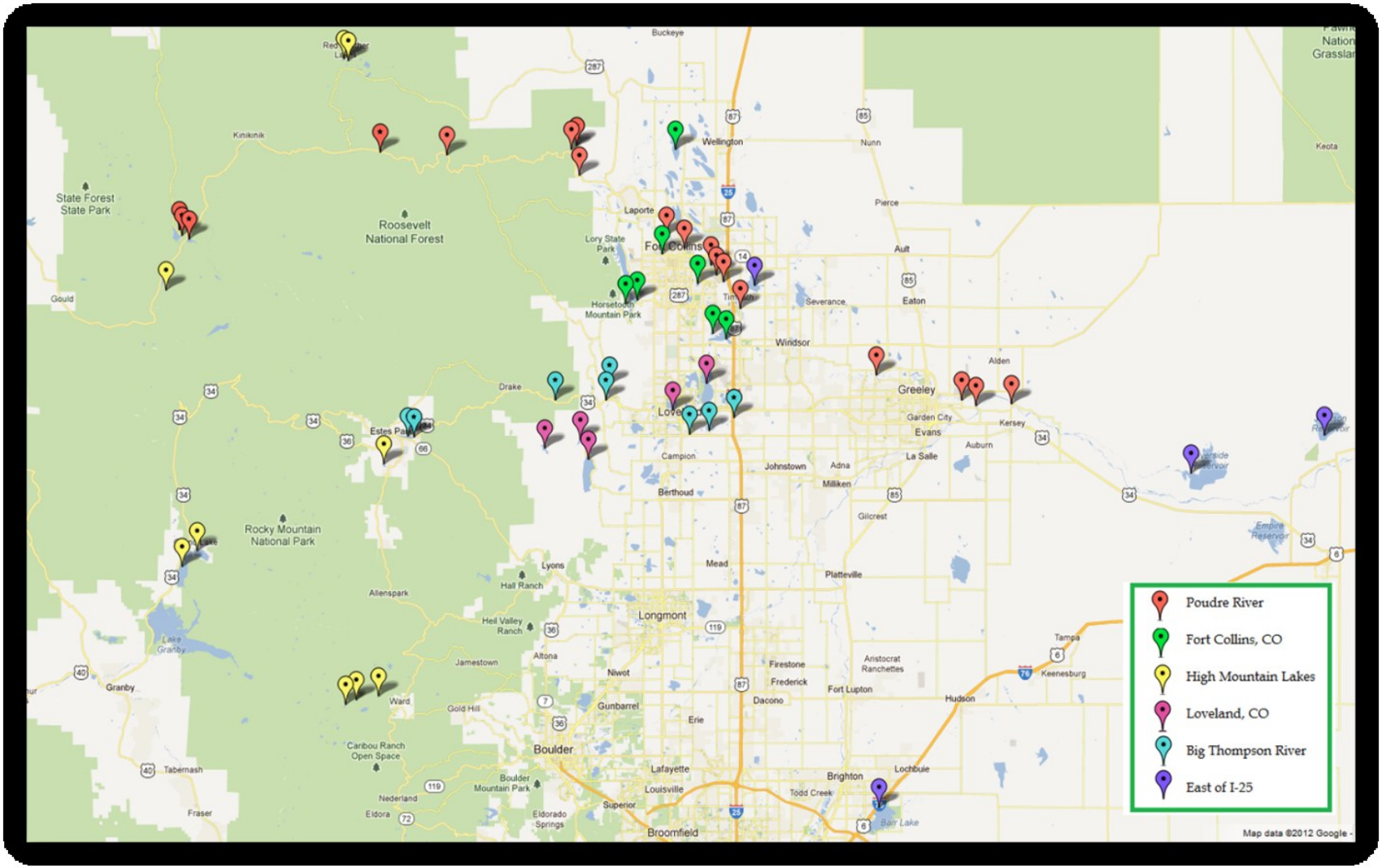


Figure C 2. Sampling sites from 2011

Table C 2. Locations of sampling sites

Site Name	Site Category	Coordinates
BT @ 9E	Big Thompson	-105.184303,40.434177,0.000000
BT @ I25 M150	Big Thompson	-104.992393,40.397419,0.000000
BT 160	Big Thompson	-105.267609,40.417431,0.000000
BT M130	Big Thompson	-105.060699,40.378788,0.000000
BT M140	Big Thompson	-105.030960,40.383301,0.000000
BT M40	Big Thompson	-105.486557,40.375748,0.000000
BT M70	Big Thompson	-105.189461,40.417187,0.000000
Barr Lake	East I-25	-104.768372,39.956070,0.000000
Greeley West	East I-25	-104.772835,40.445511,0.000000
Jackson Reservoir	East I-25	-104.081039,40.378460,0.000000
Riverside Reservoir	East I-25	-104.285660,40.334507,0.000000
Timath Reservoir	East I-25	-104.959946,40.546940,0.000000
City Park Lake	Fort Collins	-105.103622,40.582344,0.000000
Douglass Lake	Fort Collins	-105.082512,40.700684,0.000000
Fossil Creek	Fort Collins	-105.003975,40.487106,0.000000
Fossil Creek Pond	Fort Collins	-105.025002,40.492981,0.000000
Horsetooth Dam	Fort Collins	-105.142593,40.529980,0.000000
Horsetooth Inlet	Fort Collins	-105.159714,40.526295,0.000000
Sherwood Lake	Fort Collins	-105.048569,40.548912,0.000000
Brainard Lake	High Mountain	-105.575356,40.077942,0.000000
Grand Lake	High Mountain	-105.821686,40.247040,0.000000

Hiawatha Lake	High Mountain	-105.595695,40.801987,0.000000
Lake Estes	High Mountain	-105.496559,40.377022,0.000000
Lake Ramona	High Mountain	-105.588783,40.799973,0.000000
Long Lake	High Mountain	-105.592346,40.072357,0.000000
Mary's Lake	High Mountain	-105.532951,40.345104,0.000000
Red Rock	High Mountain	-105.541275,40.082012,0.000000
SMR (Adj to Grand Lake)	High Mountain	-105.845032,40.229218,0.000000
Zimmerman Lake	High Mountain	-105.869148,40.541656,0.000000
Boyd Lake	Loveland	-105.034966,40.436886,0.000000
Carter Lake	Loveland	-105.217094,40.350731,0.000000
Flatiron Reservoir	Loveland	-105.230484,40.372772,0.000000
Lake Loveland	Loveland	-105.086975,40.405914,0.000000
Pinewood Lake	Loveland	-105.284302,40.363483,0.000000
022 Fern	Poudre	-104.640053,40.418137,0.000000
Barnes Meadow	Poudre	-105.834450,40.599232,0.000000
Chambers Reservoir	Poudre	-105.845078,40.603230,0.000000
Dadd Gulch	Poudre	-105.539436,40.697205,0.000000
LCR 5	Poudre	-104.982475,40.521336,0.000000
Lost Lake	Poudre	-105.849365,40.609467,0.000000
N. Fork Poudre Gateway	Poudre	-105.234604,40.704262,0.000000
Picnic Rock	Poudre	-105.231598,40.670746,0.000000
Poudre @ Boxelder	Poudre	-105.008377,40.551262,0.000000
Poudre @ campgrounds	Poudre	-105.435318,40.694275,0.000000

Poudre @ Drake WWTP	Poudre	-105.020203,40.559181,0.000000
Poudre @ Lincoln St	Poudre	-105.069763,40.588600,0.000000
Poudre @ Shields	Poudre	-105.096588,40.603268,0.000000
Poudre at Prospect	Poudre	-105.027580,40.569794,0.000000
Poudre N. Fork Confluence	Poudre	-105.243141,40.700111,0.000000
S. Platte & Poudre (58th)	Poudre	-104.618340,40.411144,0.000000
S. Platte @ 18th	Poudre	-104.564262,40.413105,0.000000

Table C 3. Northern Colorado Environmental Sampling Results May-Oct 2011

Name	Area Description	Microcystin Screen 1 May-July (ug/L)	1) Microcystin LR (ug/L)	Microcystin Screen 2 Aug-Oct (ug/L)	2) Microcystin LR (ug/L)	1) Geosmin May - June 2011 (ng/L)	2) Geosmin July 2011 (ng/L)	3) Geosmin August 2011 (ng/L)	4) Geosmin Sept-Oct 2011 (ng/L)
022 Fern	Poudre	0	0	0	0	1.41	0	0	0
Barnes Meadow	Poudre	0	0	0.0512	0	0	0	0	0
Barr Lake	East I-25	0.8265	1.32	1.4328	0.61	1.1	0	0	0
Boyd Lake	Loveland	0	0	0	0	2.2	2.3	1.1	0
Brainard Lake	High Mountain	0	0	0	0	0	0	0	0
BT @ 9E	Big Thompson	0	0	0	0	0	0	0	0
BT @ I25 M150	Big Thompson	0	0	0	0	0.79	0	0	0
BT 160	Big Thompson	0	0	0	0	0	0	0	0
BT M130	Big Thompson	0	0	0	0	0	0	0	0
BT M140	Big Thompson	0	0	0	0	0	0	0	0
BT M40	Big Thompson	0	0	0	0	0	0	0	0
BT M70	Big Thompson	0	0	0	0	0	0	0	0
Carter Lake	Loveland	0	0	0	0	0.82	0	0	0
Chambers Reservoir	Poudre	0	0	0	0	0	0	0	0
City Park Lake	Fort Collins	0.309	0	0	0	0.71	0.7	0	0
Dadd Gulch	Poudre	0	0	0	0	2.64	2.5	0	0
Douglass Lake	Fort Collins	0	0	0	0	1.4	0	0	0
Flatiron Reservoir	Loveland	0	0	0	0	0	0	0	0
Fossil Creek	Fort Collins	0.1117	0	0	0	8.8	12.57	13.34	3.82
Fossil Creek Pond	Fort Collins	0.111	0	0	0	0.9	1.78	0	0
Grand Lake	High Mountain	0	0	0	0	0	0	0	0
Greeley West	East I-25	0	0	0.056	0	0	0	0	0
Hiawatha Lake	High Mountain	0	0	0	0	0	0	0	0
Horsetooth Dam	Fort Collins	0.1264	0	0	0	0	0	0	0
Horsetooth Inlet	Fort Collins	0.1367	0	0	0	0	0	0	0
Jackson Reservoir	East I-25	0.5636	0	0.07875	0	2.5	4.3	2.21	1.58
Lake Estes	High Mountain	0	0	0	0	0	0	0	0
Lake Loveland	Loveland	0	0	0	0	0.8	7.4	0	0
Lake Ramona	High Mountain	0	0	0	0	0	0	0	0
LCR 5	Poudre	0	0	0	0	19.95	0	0	0
Long Lake	High Mountain	0	0	0	0	0	0	0	0
Lost Lake	Poudre	0	0	0	0	3.22	3.38	0	0
Mary's Lake	High Mountain	0	0	0	0	0.89	1.6	0	0
N. Fork Poudre Gateway	Poudre	0	0	0	0	0	0	0	0
Picnic Rock	Poudre	0	0	0	0	0.72	0	0	0
Pinewood Lake	Loveland	0.7498	0	0	0	1.1	0	0	0
Poudre @ Boxelder	Poudre	0	0	0	0	0	0	0	0
Poudre @ campgrounds	Poudre	0	0	0	0	0	0	0	0
Poudre @ Drake WWTP	Poudre	0	0	0	0	0	0	0	0
Poudre @ Lincoln St	Poudre	0.9552	0	0	0	18.9	0.71	1.29	1.4
Poudre @ Shields	Poudre	0	0	0	0	2.89	0	0	0
Poudre at Prospect	Poudre	0	0	0	0	0	0	0	0
Poudre N. Fork Confluence	Poudre	0	0	0	0	0.96	0	0	0
Red Rock	High Mountain	0.5461	0	0	0	0	0	0	0
Riverside Reservoir	East I-25	0	0	0	0	0	0	0	0
S. Platte & Poudre (58th)	Poudre	0	0	0	0	12.32	1.89	1.89	0
S. Platte @ 18th	Poudre	3.636	0	0	0	0	0.82	0	0
Sherwood Lake	Fort Collins	0.6689	0	0.0578	0	1.1	1.6	0	0
SMR (Adj to Grand Lake)	High Mountain	0	0	0	0	0	0	0	0
Timath Reservoir	East I-25	0.1065	0	0	0	0.7	0	0	0
Zimmerman Lake	High Mountain	0	0	0.0618	0	0	0	0	0

Appendix D. Toxicity Analysis

D.1 Toxicity Analysis Results

Table D 1. Averaged SRB Raw results from plate reader

M-LR dose ug/L	150	100	75	50	25	12.5	6.25	3.125	1.563	0.781	Control Meo	Control
100,000 Cells	1.02	0.78	0.82	0.74	0.60	0.56	0.53	0.52	0.55	0.80	1.07	0.97
Std Dev.	0.11	0.13	0.17	0.06	0.12	0.13	0.21	0.10	0.16	0.03	0.23	0.04
50,000 Cells	0.91	0.86	0.92	0.95	0.83	0.70	0.65	0.66	0.65	0.78	0.75	0.85
Std Dev.	0.04	0.06	0.08	0.08	0.07	0.05	0.11	0.10	0.09	0.12	0.16	0.07

Table D 2. Averaged SRB Quantified results

M-LR dose ug/L	150	100	75	50	25	12.5	6.25	3.125	1.563	0.781	Control Meo	Control
100,000 Cells	105106	88247	83118	76185	53786	49901	42013	47039	46767	82352	109403	100000
Std Dev. Cells	15341	309	24295	5716	850	5068	2960	481	4172	3254	22720	3508
50,000 Cells	2125	3161	5407	4148	3528	2392	5700	5033	4293	2828	2509	3266
Std Dev. Cells	107	101	108	112	98	83	77	78	76	99	78	100

D.2 Hematoxylin and Eosin Staining Procedure:

(http://www.ihcworld.com/_protocols/special_stains/HE_Mayer.htm):

Eosin Y Solution:

Eosin Y Stock Solution (1%):

Eosin Y ---------- 10 g
Distilled water ---------- 200 ml
95% Ethanol ---------- 800 ml
Mix to dissolve and store at room temperature.

Eosin Y Working Solution (0.25%):

Eosin Y stock solution ---------- 250 ml
80% Ethanol ---------- 750 ml
Glacial acetic acid (concentrated) ------- 5 ml
Mix well and store at room temperature.

Hematoxylin Solution (Mayer):

Potassium or ammonium (alum) -------- 50 g
Hematoxylin ---------- 1 g
Sodium iodate ---------- 0.2 g
Citric acid ---------- 1 g
Distilled water ---------- 1000 ml

Stir to dissolve the chemicals in the order listed above. For example, dissolve alum in 1000 ml distilled water first. When alum is completely dissolved, add hematoxylin. When hematoxylin is completely dissolved, add sodium iodate, etc.

Procedure:

1. Deparaffinize sections, 2 changes of xylene, 10 minutes each.
2. Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.
3. 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.
4. Wash briefly in distilled water.
5. Stain in Mayer hematoxylin solution for 8 minutes.
6. Wash in warm running tap water for 10 minutes.
7. Rinse in distilled water.
8. Rinse in 95% alcohol, 10 dips.
9. Counterstain in eosin-phloxine B solution (or eosin Y solution) for 30 seconds to 1 minute.
10. Dehydrate through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.
11. Clear in 2 changes of xylene, 5 minutes each.
12. Mount with xylene based mounting medium.

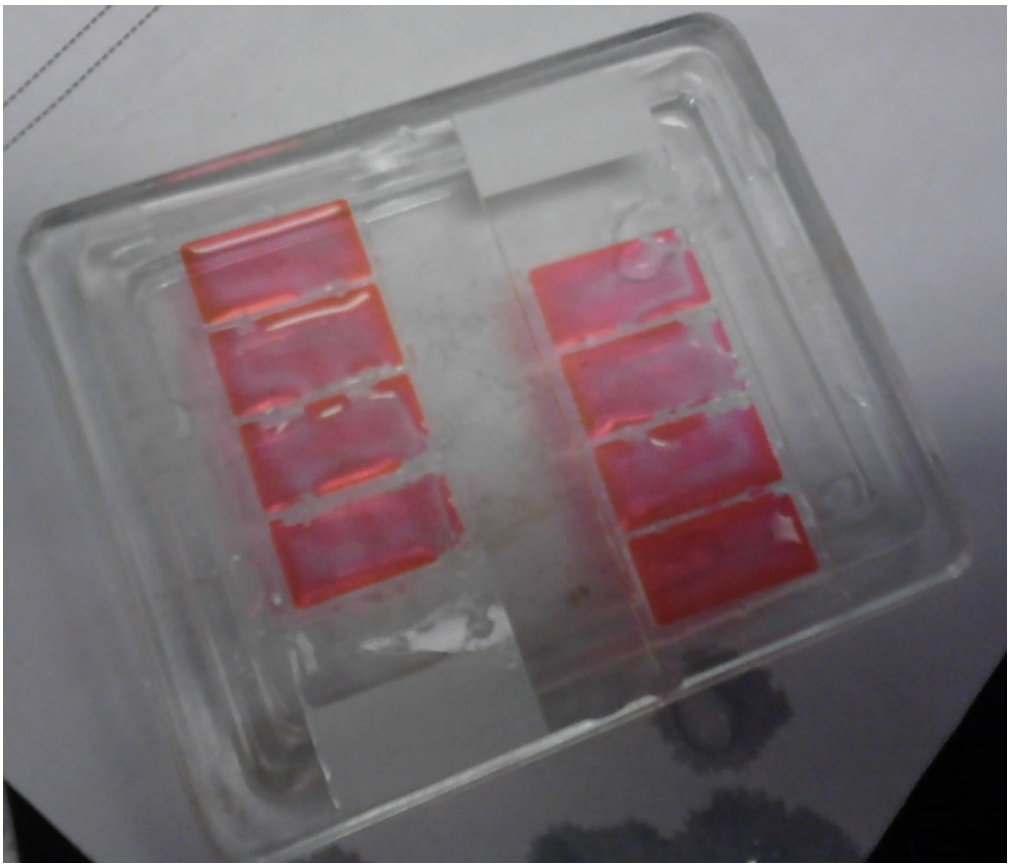


Figure D 1. Eosin Stain on H4IIE Cells.

D.3 SRB Protocol

1. Treat cells with microcystin-LR toxin with respective dosages and incubation periods.
2. When ready to begin assay, remove media and fix cells with 50 µl of 10% TCA (10 ml TCA, 90 ml Mili Q water).
3. Remove TCA and wash 5x with tap water to remove TCA, growth medium and low molecular weight metabolites.
4. Allow plates to dry.
5. Add 0.4 % SRB (w/v) dissolved in 1% acetic acid to TCA fixed cells and allow to sit for 30 minutes.
    a. SRB- 1 ml acetic acid, 99 ml Mili Q water, 0.4 g SRB.
6. Remove SRB, then wash quickly 4x 1% acetic acid to remove the dye.
7. Air dry until no standing moisture is visible.
8. Solubilize dye with 50 mM Tris base (pH 10.5) with 50 µl per well.
9. Place plate on an orbital shaker for 5 minutes.
10. Read plate on a plate reader at 560 nm.

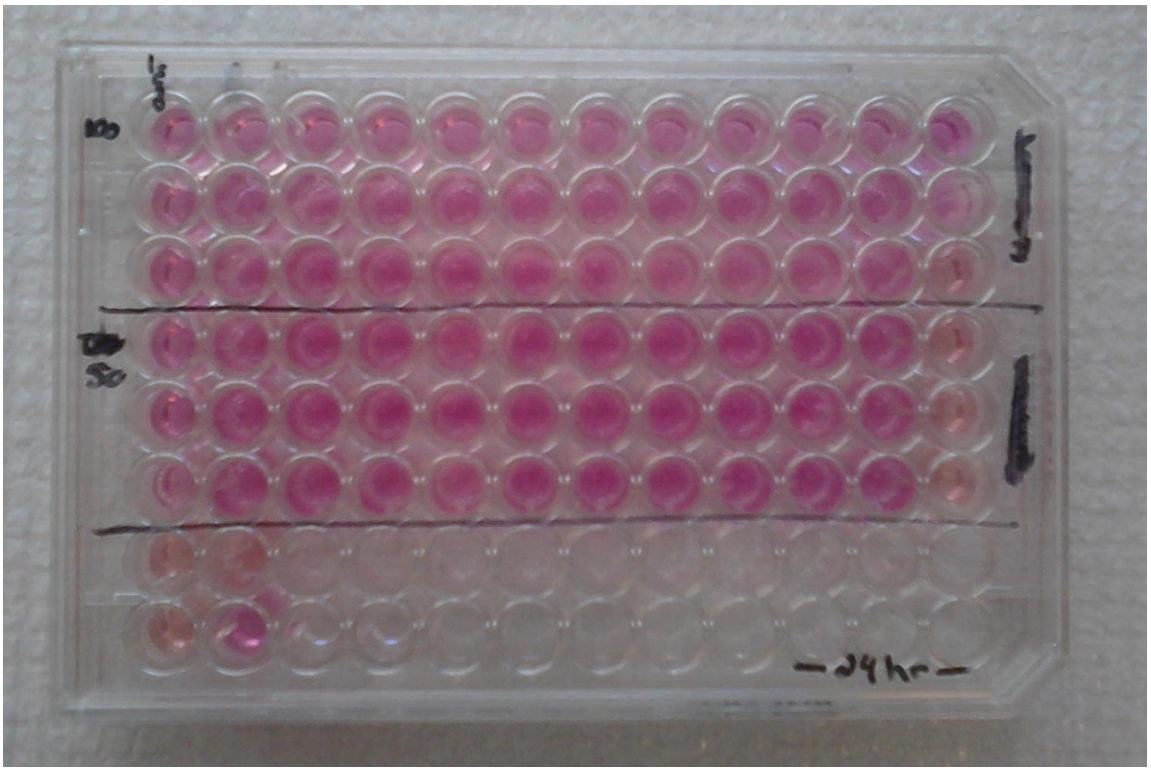


Figure D 2. H4IIE Cells under SRB Staining

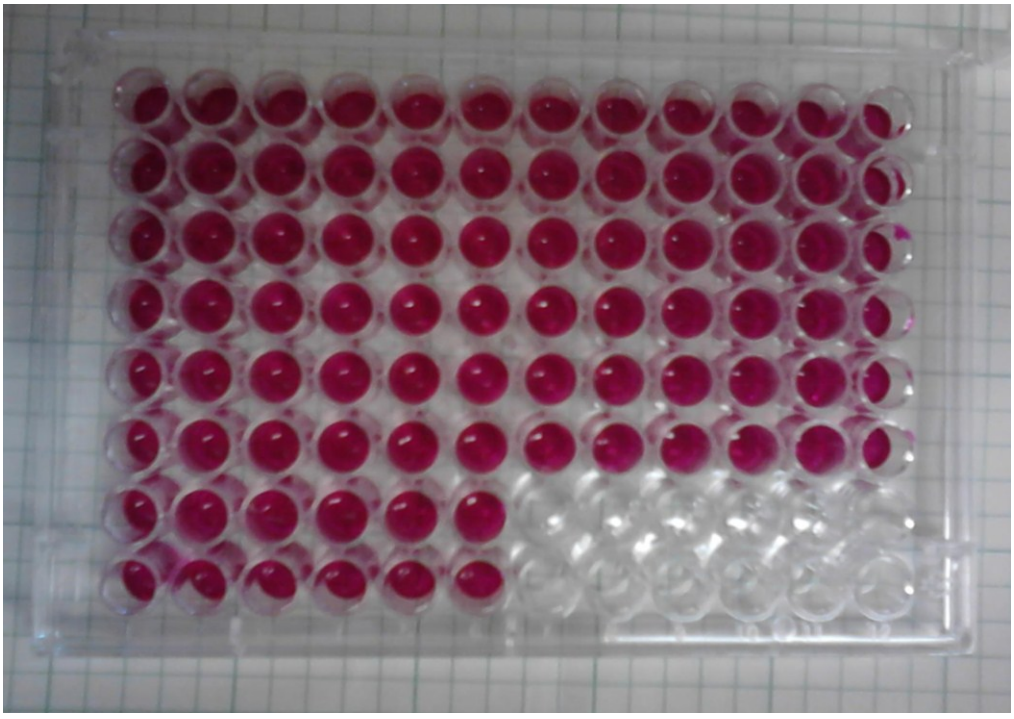


Figure D 3. Solubilized H4IIE cells ready for plate reader.

D.4 Enzyme Linked Immunosorbent Assay for Microcystins

The 96 well plates are lined with anti-rabbit IgG polyclonal antibodies that are raised to bind microcystins and a microcystin-enzyme conjugate. There is the same number of antibody binding sites in each well allowing the same number of microcystin-enzyme conjugate enzyme molecules to bind to the antibodies. Once the conjugate binds and the reaction proceeds, a blue color should appear. Conversely, if there were a high concentration of microcystins, there will be fewer microcystin-enzyme conjugate molecules bound to the antibodies thus a lighter blue color would indicate the presence of microcystins. Cross reactivity could occur if variants of microcystins and nodularins are present. The detection limit of the assay is 0.1 ug/L of Microcystin-LR. The ELISA testing kit is a good, quick and inexpensive way to screen many samples at once for microcystin-LR before confirmation and quantification with more advanced methods. Environmental samples were filtered through a 0.45 um glass filter so that only dissolved cyanotoxin concentrations would be analyzed.

50 µl of the enzyme conjugate was pipetted into each well. Next, 50 µl of homogenized environmental samples and calibration solutions were pipetted to the appropriate wells making sure a clean pipet was used for each solution to avoid cross contamination. 50 µl of the antibody was also pipetted into each well before the plate was covered with parafilm and put on an orbital shaker to continuously mix the solution during a 30 minute incubation period. Afterwards the wells were washed five times with a wash solution before 100 µl of the indicator substrate was pipetted into each well. The wells were covered and incubated for another 30 minutes. After incubation 100 µl of stop solution was pipetted into each well and after processing the samples according to the Beacon ELISA kit, the resulting plate was read on a FLUOstar Omega Plate Reader at 450 nm.

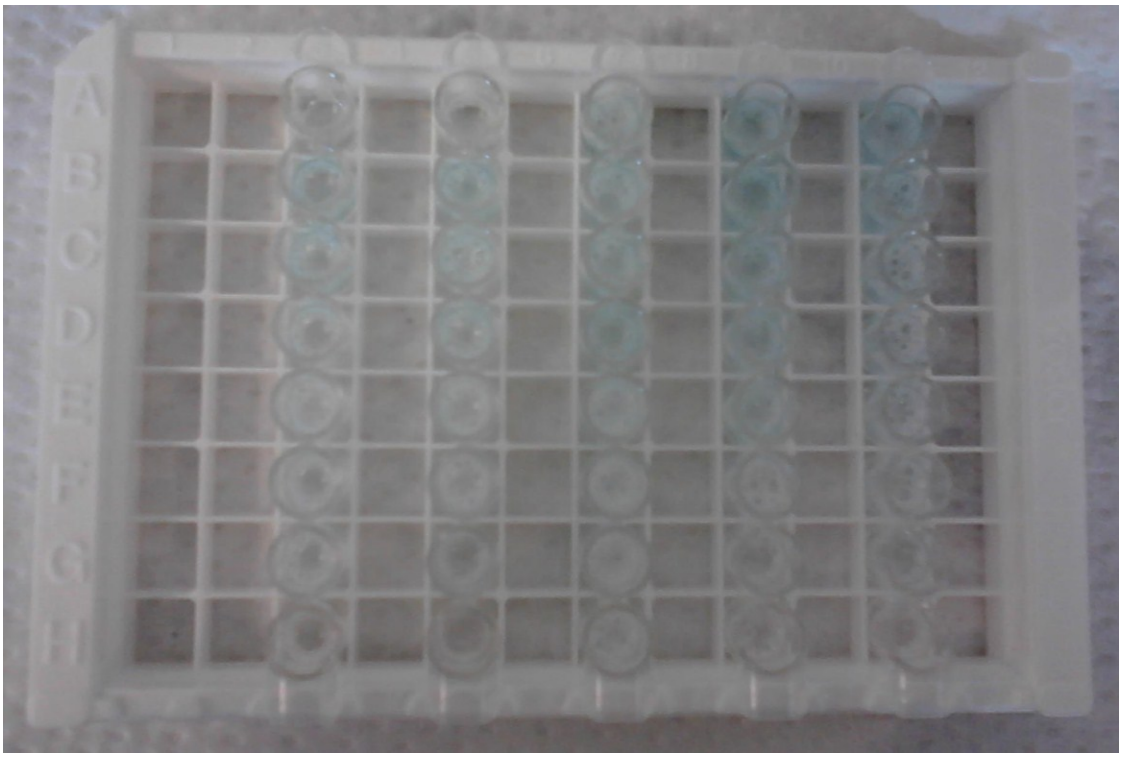


Figure D 4. Beacon Microcystin-LR ELISA after 30 seconds

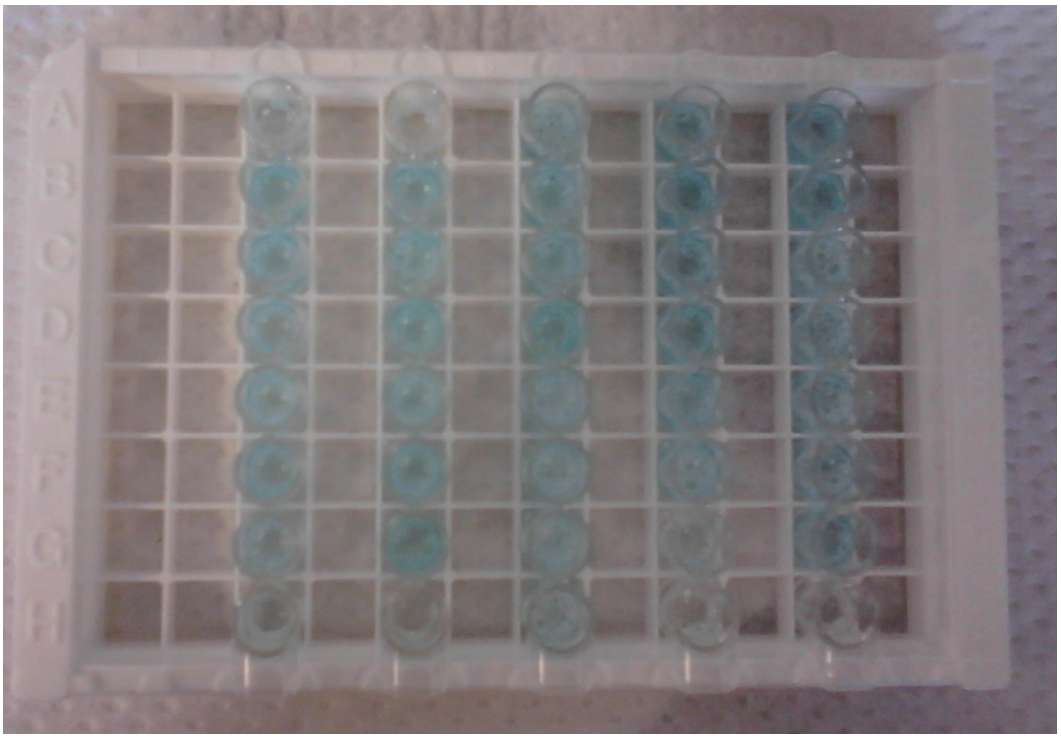


Figure D 5. Beacon Microcystin-LR ELISA after 60 seconds

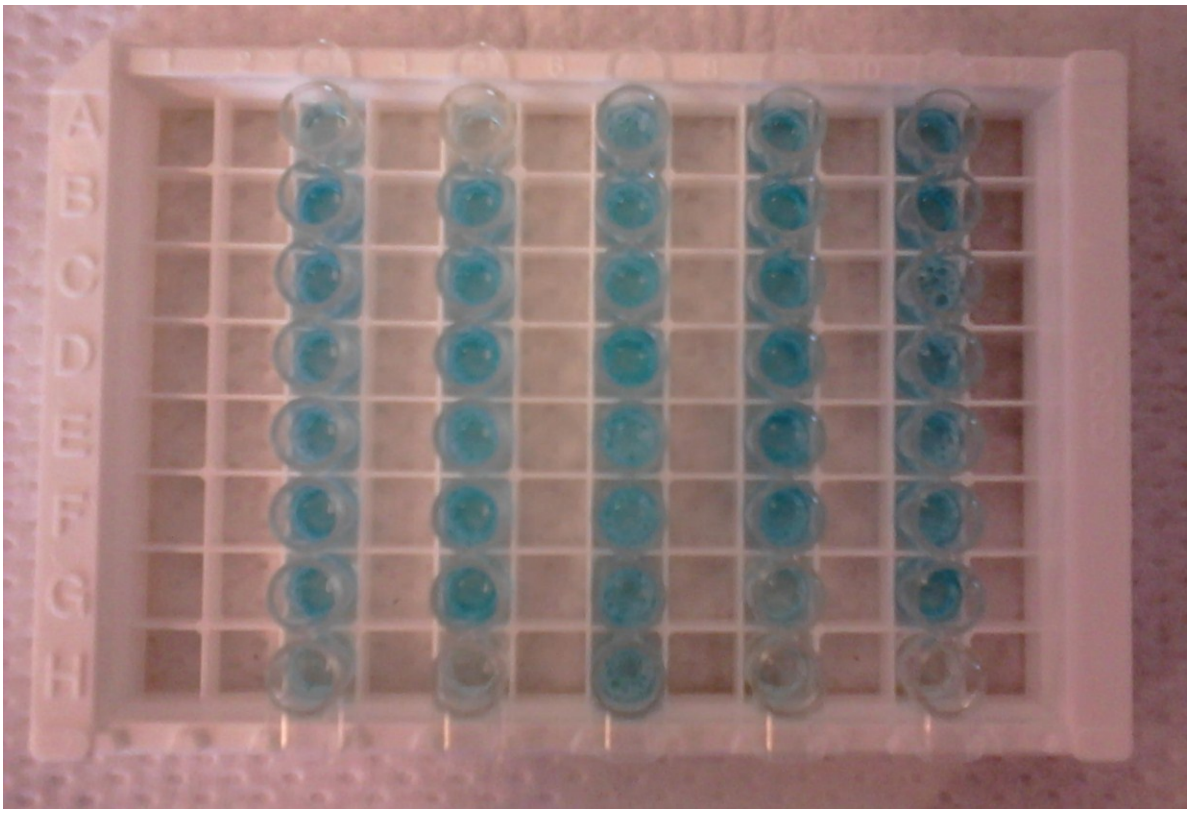


Figure D 6. Beacon Microcystin-LR ELISA after 60 seconds

D.5 Histological observations

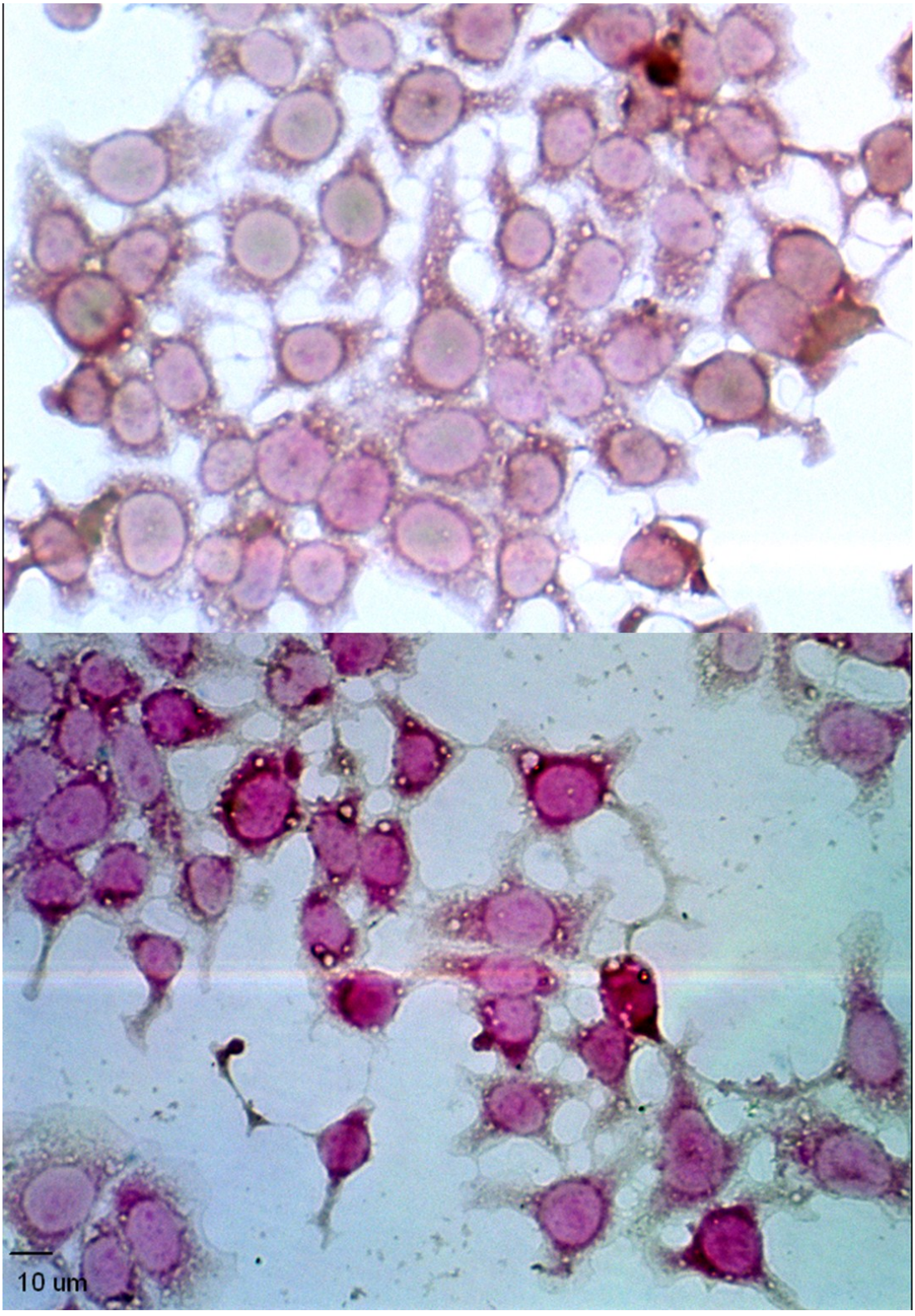


Figure D 7. Histological view of control cells.

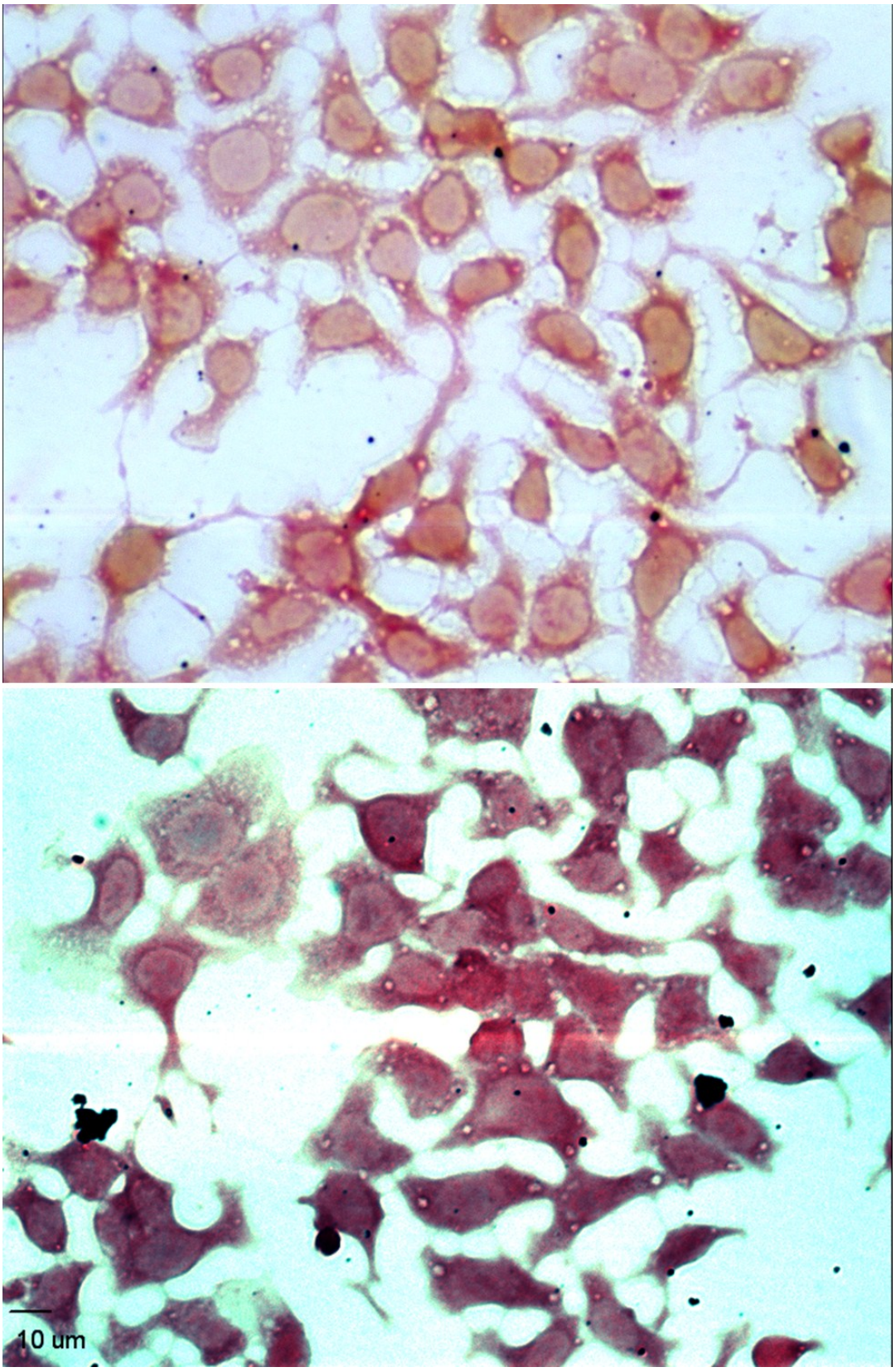


Figure D 8. Histological view of cells under 24 hr exposure to 25 µg/L microcystin-LR

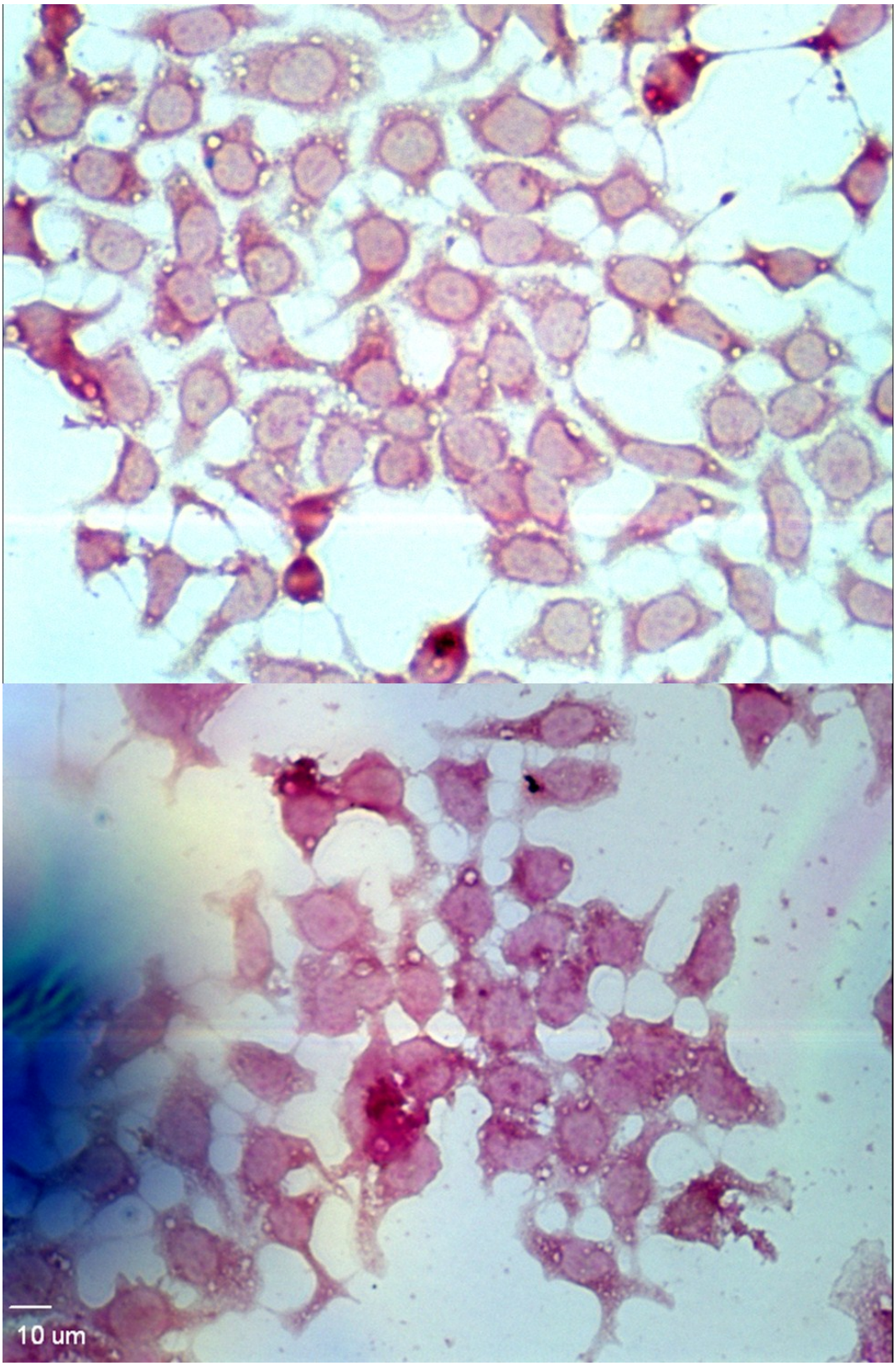


Figure D 9. Histological view of cells under 24 hr exposure to 50 µg/L microcystin-LR

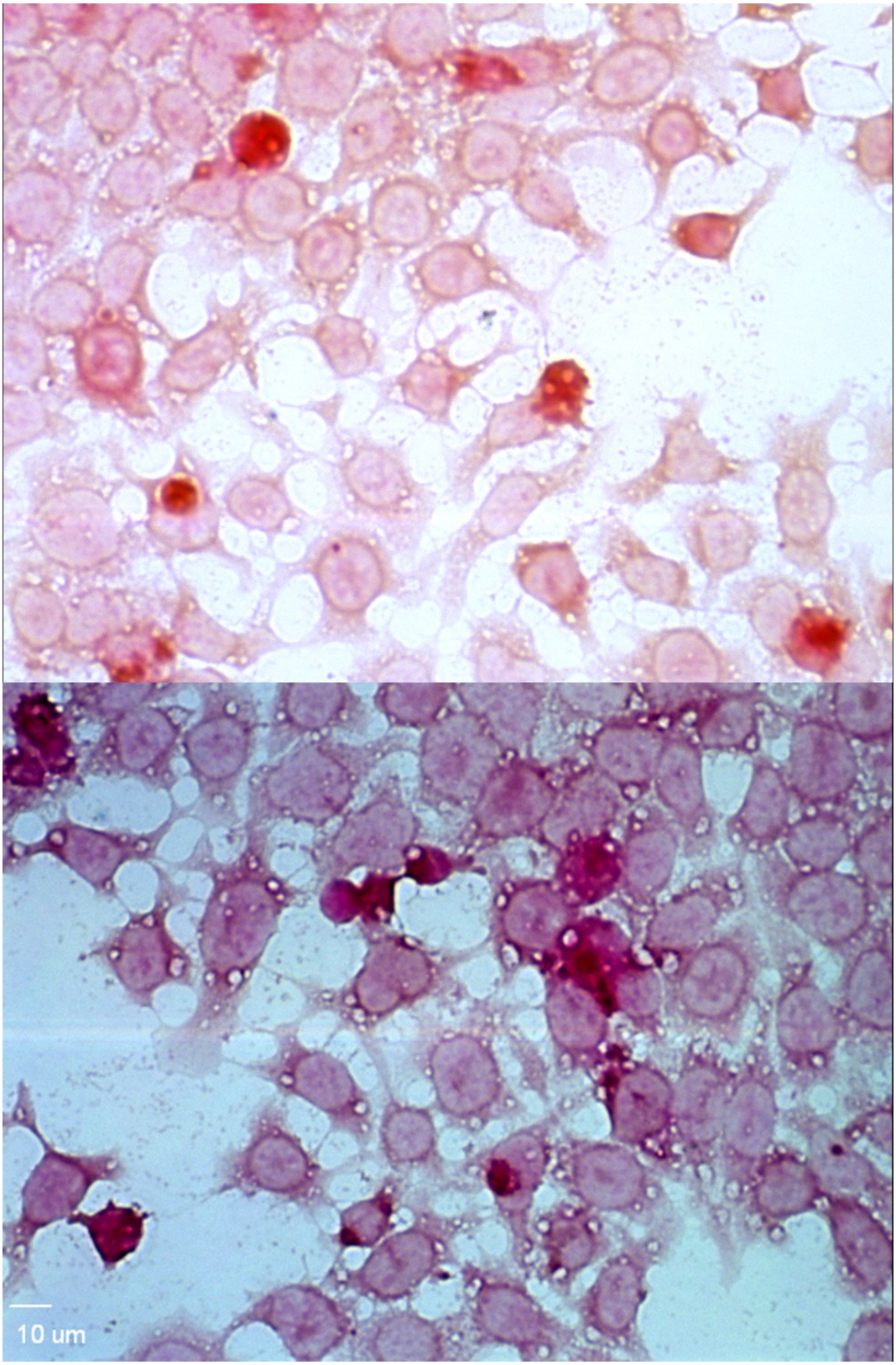


Figure D 10. Histological view of cells under 24 hr exposure to 100 µg/L microcystin-LR