Microcystin and *Microcystis* Destruction by Ozone in Drinking Water Treatment: Constraints and Effects

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Appropriate and effective drinking water treatment is critical to the protection of public health. Toxic cyanobacterial blooms are a globally increasing drinking water source quality-associated health risk as even very low (>1.5 parts per billion (ppb) or micrograms per litre (µg/L)) concentrations of the cyanobacteria-produced toxin microcystin can be unsafe to drink. Increased pressures on freshwater supplies as well as climate change associated factors such as alternating periods of drought and intense storms and increasing water temperature cause more nutrient runoff into water supplies and create favourable conditions for the growth of cyanobacteria.

Ozone is generally understood to effectively destroy many toxins during drinking water treatment. Its efficacy, however, can be adversely impacted by the presence of natural organic matter, often measured as dissolved organic carbon (DOC). The conditions that create favourable growth conditions for cyanobacteria, can also increase the concentrations of DOC in the source water of a drinking water treatment facility.

The objectives of this research were to determine whether ozone is an effective cyanobacterial toxin elimination technology at the conditions studied; specifically in the presence of high DOC (~10 mg/L), to determine the efficacy of ozone in the destruction of intercellular (within cells) toxin vs. extracellular (within water matrix) toxin, and to determine the extent of cell destruction by ozone.

Bench-scale experiments were conducted. Both extracted toxin and cyanobacterial cells were added to coagulated/flocculated/clarified water collected from the Mannheim Water Treatment Plant in Kitchener, Ontario. Microcystin concentrations were measured by the ELISA method and by liquid chromatography-mass spectroscopy-mass spectroscopy (LC-MS-MS).

This investigation confirmed that ozone can destroy extracellular microcystin-LR to below 1.5 µg/L (ppb) at ozone residuals above 0.3 mg O_3/L when the aqueous DOC concentration was below 5 mg/L. The relationship between required ozone residual to achieve adequate extracellular toxin destruction and DOC concentration in the water matrix was quantitatively described. Notably, when *Microcystis aeruginosa* cells were present, an amount equivalent

to less than 50% of the concentration of extracellular microcystin-LR was destroyed by ozone. This demonstrates that significant oxidative capacity is required to lyse the cells before ozone can destroy intercellular toxin. The novel contribution of this work is that this relationship was 1) demonstrated through using toxin in extracted and cellular forms and 2) maintained when all other critical operational factors (i.e., ozone residual, DOC concentration, water matrix) were the same. These results underscore the need to reassess operational requirements for ozonation for the treatment of cyanobacterial toxins when intact cells are present as opposed to extracellular toxin, which is used in most performance assessments.

Notably, as the aqueous DOC concentration increased, the proportion of live cells present following ozonation (as measured by intercellular toxin concentrations) also increased. Therefore, not only does DOC decrease the efficacy of ozone to destroy toxin, it decreases the oxidative capacity to lyse cells; moreover, the rate is not directly proportional to the aqueous DOC concentration. As a result, increases in ozone residual concentration had a minimal effect on toxin destruction in these cases. In other words, the levels of toxin destruction that would have been expected based on comparable ozone residuals in absence of DOC (or when only low levels of DOC were present) were not achieved because of the significant oxidant/ozone demand of DOC when present at high aqueous (~10 mg/L) concentrations.

Another important contribution of this work was the demonstration that not all cyanobacterial cells were destroyed following ozonation; thus, they were described as “Damaged and Potentially Viable (DAPV)” cells. These cells were present at ozone residuals less than 0.45 mg O_3/L, logically suggesting that incomplete oxidative treatment occurs at lower ozone residual concentrations. Notably, these DAPV cells may have the potential to reproduce; given this and the common assessment of treatment performance using extracellular toxin, the efficacy and operational requirements of oxidative treatment of cyanobacterial cells by ozonation may need to re-evaluated for situations in which live cells are present. These observations also underscore the need to more fully assess the significance of DAPV cells.

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Dedication

I dedicate my thesis, research, and academic work to my mother, Maggie Cunningham, who recognized and continuously encouraged my abilities – without which none of this would have been possible.

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Chapter 1
Introduction

Appropriate and effective drinking water treatment is critical to the protection of public health (Gaffield et al., 2003). In North America, enforcement of drinking water treatment requirements is enabled by regulations such as the Ontario Safe Drinking Water Act (2002), Alberta's Environmental Protection and Enhancement Act: Potable Water Regulation (2003) and the United States Environmental Protection Agency (US EPA) Safe Drinking Water Act (1996). These regulations are typically source-based and it is believed that different water sources (or types) (e.g., groundwater, surface water, groundwater under the influence (GUDI) of surface water) generally have different water quality. The quantity, quality and diversity of microorganisms, solids, and organic matter in untreated water supplies will dictate the extent of treatment required for adequate public health protection (Hammes et al., 2008; Matsushita et al., 2013). The presence of toxic cyanobacterial cells often threatens the water industry's ability to protect public health because many utilities do not have the treatment infrastructure to effectively treat these toxins. Moreover, cyanobacteria themselves can be difficult to remove, thereby contributing to service disruptions should they be present in finished drinking water. Overall, the presence of toxin-bearing cyanobacteria is a significant drinking water source quality-associated health risk that is increasing in frequency globally (Merel et al., 2013; O'Neil et al., 2012). In North America, this threat was underscored by the source and treated water quality deterioration event that left 500,000 people in Toledo, Ohio in the summer of 2014 without drinking water because of the confirmed presence of unsafe levels of the cyanobacterial toxin microcystin (a hepatotoxin) in the drinking water treatment plant's finished water (Wilson, 2014).

"Cyanobacteria" and "algae" are terms that are often used interchangeably; however, cyanobacteria are prokaryotic bacteria, whereas algae are distinguished as eukaryotic organisms. Cyanobacteria are named under both Botanical and Bacteriological Codes, each having different rules of nomenclature, hence the various terminology (Palinska and Surosz, 2014). Cyanobacteria are single-celled and one of the oldest known organisms; they also form a critical part of aquatic ecosystems. Thus, they are nearly always present in surface water bodies (Svrcek and Smith, 2004). The concern over their presence in water treatment processes is with respect to the concentrations of cells and the potential for toxin production within those cells. Twelve (12) of the cyanobacteria genera are known to produce harmful

toxins (Sivonen and Jones, 1999). Cyanobacterial toxins can pose a public health risk even at very low concentrations, which is why the World Health Organization (WHO) has set a maximum acceptable microcystin-LR concentration in drinking water of 1 micrograms per litre (µg/L) (WHO, 2008). Comparably, the Ontario Drinking Water Quality Standards (ODWQS), O.Reg. 169/03 and Health Canada's Guidelines for Canadian Drinking Water Quality state a maximum of 1.5 µg/L microcystin-LR in drinking water. Heath Canada is proposing new guidelines, which include a maximum acceptable concentration of 1.5 µg/L total microcystins in drinking water. This is intended to include any of the 100 or more congeners of microcystin (Health Canada, 2016). While the US EPA has not issued maximum concentrations of microcystins in drinking water, in 2015 they set health advisory levels for microcystin-LR based on age --- 0.3 µg/L for children under six years of age and 1.6 µg/L for those six years and older (US EPA, 2015). The aspects of particular concern with these toxins, as opposed to other water quality constituents (such as metals, carbon, or even bacteria) are that:

1) Cyanotoxins require high energy treatment technologies (ozone, UV) to be destroyed (Westrick et al., 2010). "Stopgap" measures like boil water advisories that are used for other microbial contaminants such as *E. coli* are ineffectual for cyanotoxins,
2) Not all cyanobacteria blooms on surface water bodies produce toxins, and not all toxin-producing cyanobacteria are visible by the presence of a bloom (Pierce et al., 2013). This can make detection difficult and time-consuming, and
3) Cyanotoxin concentrations that cause damage or death in humans are very low and thus require reliable and effective treatment to avoid deleterious public health impacts.

Increased pressures on freshwater supplies as well as climate change associated factors, such as alternating periods of drought and intense storms and increasing water temperature, cause more nutrient runoff into water supplies and create favourable conditions for the growth of cyanobacteria (Jeppesen et al., 2011). This eutrophication of surface drinking water supplies has led to increased frequency and severity of harmful cyanobacterial blooms in the past several years (O'Neil et al., 2012). In addition to toxin concentration guidelines, there are Alert Levels for cyanobacterial cells entering water treatment processes. Water Quality Research Australia (WQRA) set a Level One Alert to between 2000 and 6500 cells/mL, a Level Two Alert at greater than 6500 cells/mL and a Level Three Alert at greater than 65 000 cells/mL (Newcombe et al., 2009). These Alert Levels are intended to prompt changes in operations within drinking water treatment processes to ensure that cells and toxin are adequately removed and absent from treated drinking water and the distribution system.

Several technologies are employed to remove cyanobacterial cells and toxin during drinking water treatment. Among them are coagulation and filtration, UV irradiation, and oxidation (with ozone, chlorine, permanganate, or hydrogen peroxide [usually combined with another technology such as ozone]) (Svrcek and Smith, 2004). Ozone is commonly utilized for treatment of cyanotoxins due to its rapid reaction kinetics and efficacy in destroying cyanotoxin concentrations to below regulatory limits (Shawwa and Smith, 2001; Hoeger et al., 2002). It does not produce potentially harmful, regulated disinfection by-products that result from the use of other technologies such as chlorination (Pressman et al., 2012). However, oxidation by ozone causes cell lysis, which results in the release of toxins into the water matrix and the subsequent need for toxin elimination (Fan et al., 2014). Source water quality—specifically, organic carbon levels—can reduce the efficacy of cyanobacterial cell and toxin treatment by ozonation (Onstad et al., 2007); however, this relationship is not well understood.

Natural organic matter (NOM) is present in surface water from the metabolic activities of organisms and the dissolution of soil (Awad et al., 2016). NOM is a complex mixture of compounds, normally measured in terms of the carbon-containing molecules (Pressman et al., 2012). The components of NOM are very source water specific and changes in the source water quality include or can be the result of changes in organic matter (Xue et al., 2014). Dissolved organic carbon (DOC) is the most common water quality metric used as a surrogate indicator for NOM during drinking water treatment.

Most surface source waters contain low or moderate DOC concentrations, under 5 milligrams per litre (mg/L) and 10 mg/L, respectively (Crittenden et al., 2012). However, there also are source waters with DOC concentrations, averaging ~15 mg/L and higher (Ledesma et al., 2012). High DOC concentrations present challenges to treatment processes because they exert significant coagulant demand, form disinfection by-products when organics react with chemical disinfectants, and increase microbial growth in distribution systems (Awad et al., 2016); thereby resulting in relatively high coagulation and filtration costs. Increases in DOC concentrations have been attributed to changes in climate/more extreme weather events (Emelko et al., 2011). Water treatment facilities are designed to treat influent water quality over a certain range. Should that range shift over time, the technologies employed and processes designed can be stressed and less effective at producing drinking water of satisfactory quality. This may result in decreased capacity or service disruptions. Parts of the Eastern United States, Eastern Canada, United Kingdom, and Nordic countries have seen consistent DOC increases of 0.02 to >0.15 mg/L/yr in source water from 1990 to 2004 (Monteith et al., 2007).

Thus, over this period, drinking water source concentrations of DOC have increased by >2.1 mg/L on average in many of these areas. Combined with seasonal changes or increases due to discrete natural events (floods, fires), (Emelko et al., 2011) the implications for treatment operations are considerable and potentially catastrophic in some cases. For example, changes in the carbon character (molecular weight, origin) have been observed in the influent water to treatment facilities during periods of droughts and floods (Fabris et al., 2015). Drought-impacted NOM was more recalcitrant to coagulation, and floods introduced more NOM of terrestrial origin. During the Calgary, Alberta flood of 2013, the water treatment plant experienced raw water turbidity values above 4000 NTU (normally less than 40 NTU) (Kundert et al., 2014).

The alterations in watersheds due to drought, flood, water temperature, or other significant effects that produce changes in the NOM character of the water are also the same factors that promote increases in cyanobacteria populations. This combination of factors further challenges cyanobacterial toxin destruction technologies in treatment plants as it requires changes to our collective understanding of the necessary operations of the processes and also requires understanding of the limitations the various technologies employed.

The general goal of this research was to examine the relationship between ozone, DOC, cyanobacteria, and the toxins they produce to establish the limitations of ozone in drinking water treatment. This research also aimed to determine key water quality conditions at which effective toxin elimination may be compromised. To address these goals, the specific objectives of the research were:

1. To assess the efficacy of ozone as an effective cyanobacterial toxin elimination technology,
2. To determine the efficacy of ozone in the destruction of both intercellular (within cells) toxin and extracellular (within water matrix) toxin,
3. To determine the limits of ozone efficacy in the presence of DOC, and
4. To determine the extent of cell destruction by ozone.

Chapter 2
Literature Review

2.1 Surface Water Environment

Surface water source quality is inherently variable; changing seasonally and across longer time scales in response to pressures such as development, agriculture, and climate change (Ramaker et al., 2005). Changes to the surrounding landscape, such as densification or increased agricultural activity have profound effects on surface water bodies and water quality; thus, they can also have significant effects on drinking water treatment (Jeppesen et al., 2011). Because the treatment processes employed at a given facility are selected and designed based on present and anticipated water quality (Crittenden et al., 2012), a treatment plant's ability to concurrently meet demands and deliver safe, treated drinking water in compliance with regulatory criteria can be compromised if there are sudden and/or significant changes to that water quality, especially if such fluctuations occur frequently (Emelko et al., 2011; Emelko and Sham, 2014).

Climate change can significantly impact water quality, quantity, and treatability in source watersheds (Yuo et al., 2013; Emelko et al., 2011). Drought, forest fires, less glacier melt, floods, warmer water temperatures, and changes in precipitation amounts have all had far reaching consequences for downstream communities, both challenging treatment processes and sometimes resulting in service disruptions (Ritson et al., 2014; Writer et al., 2014; Emelko et al., 2011). In some environments such as the sediment-rich regions of Western Canada, disturbance effects on water quality can last for decades or longer (Emelko et al., 2016). Notably, many such climate-change associated land disturbances have also been linked with increased occurrences of cyanobacterial bloom events (Brookes and Carey, 2011; Silins et al., 2014; Emelko et al., 2016).

There are numerous environmental conditions that contribute to the reproduction of cyanobacteria including: hydro-climatic and physiographic setting, water body morphometry, nutrient availability, light availability, competition with other cyanobacteria or algae, and grazing (AWWA, 2010; Mur et al., 1999; Torres et al., 2016; Nicklisch et al., 2008). In temperate climates, like Canada, cyanobacteria are most prevalent in late summer (Figure 2.1). The exact combination of environmental factors that result in bloom events is not well understood (Mur et al., 1999). Most importantly for drinking water treatment, the conditions at which cyanobacteria

produce toxins are even less understood. Thus, the presence of a cyanobacterial bloom in source watersheds can be a benign nuisance or—in stark contrast—a small number of cells can produce substantial amounts of toxin and pose a significant public health threat if not properly treated.

Figure 2.1: Satellite image of cyanobacterial bloom in Lake Erie, 2011 (NOAA, 2011)

Nitrogen and phosphorus are the main nutrients that commonly limit the growth of cyanobacteria in freshwater (Salmaso, 2011). Notably, cyanobacteria, as compared to algae, have the ability to store phosphorus (Oliver and Ganf, 2000). This, combined with the ability to move to phosphorus-rich regions of the water column by regulating buoyancy, makes cyanobacteria such as *Microcystis* sp. very adaptable; and contributes to it being one of the most prevalent toxin-producing cyanobacteria in surface waters, globally (AWWA, 2010; Mur et al., 1999).

Microcystis species are freshwater cyanobacteria that can produce cyclic heptapeptide toxins that attack the liver in mammals. These toxins were first isolated in *Microcystis aeruginosa* and thus, were called microcystins (Carmichael et al., 1988). Cyclic microcystins are among the most toxic of the cyanobacterial toxins produced and can be produced by a variety of cyanobacteria (*Planktothrix, Anabaena, Oscillatoria* and *Nostoc* genera); they also are environmentally ubiquitous (Sivonen et Jones, 1999). The toxin strain microcystin-LR is the

only cyanobacterial toxin for which the maximum drinking water concentration is specified in the WHO Guidelines for Drinking-Water Quality (WHO, 2008).

While it is possible to prevent some cyanobacteria from entering drinking water intakes simply by increasing intake depth, this approach does not work well for *Microcystis* sp. because of its ability to move through and survive at various depths in the water column (McQuaid et al., 2011). *Microcystis* sp. are further resilient because they are adaptable to light. Although they prefer brighter environments, like other cyanobacteria, they can alter the size and number of their photosynthetic units based on available light intensity (Torres et al., 2016).

The environmental resilience of cyanobacteria such as *Microcystis* sp. combined with climate related changes in water quality that could favour its growth make it a strain of cyanobacteria that will likely continue to increase in significance to drinking water treatment providers. While source water management is the most effective way to deal with cyanobacteria and potential toxins, cells will inevitably continue to enter drinking water treatment streams (Jetoo et al., 2015). Additionally, the high-level, multi-jurisdictional cooperation required for enacting and enforcing proactive source watershed protection measures can be difficult to achieve; thus, treatment of toxins and protection of public health may continue to ultimately fall to individual water treatment facilities.

2.2 *Microcystis* sp. and Microcystin: Relevance and Characteristics

Microcystis sp. are cyanobacteria; a group of photosynthetic prokaryotes. Cyanobacteria are single-celled organisms that are prevalent in a variety of environments and can be found in both aquatic and terrestrial habitats, although *Microcystis* sp. are predominately found on the surface of freshwater bodies (Crittenden et al., 2012). *Microcystis* sp., like other planktonic surface bloom-forming cyanobacteria, are aerotopes that consist of gas vesicles. These vesicles are hollow with cell walls that allow for the passage of gas, but not water, and contribute to the ability of the cells to float (Oliver and Ganf, 2000). The resilience and prevalence of *Microcystis* sp., such that they can produce large surface blooms through a combination of rapid division and buoyancy regulation, and their dominant presence in eutrophic waters enables them to out-compete other phytoplankton for light (Torres et al., 2016).

An understanding of the morphology and structure of cyanobacteria such as *Microcystis* sp. informs the design of strategies for cell and toxin destruction during drinking water treatment. As prokaryotes, cyanobacteria do not have a nuclear envelope or a nucleus. Instead, their DNA

is located freely in the liquid component of the cytoplasm (Mur et al., 1999). The components of the cyanobacterial cell are shown below (Figure 2.2).

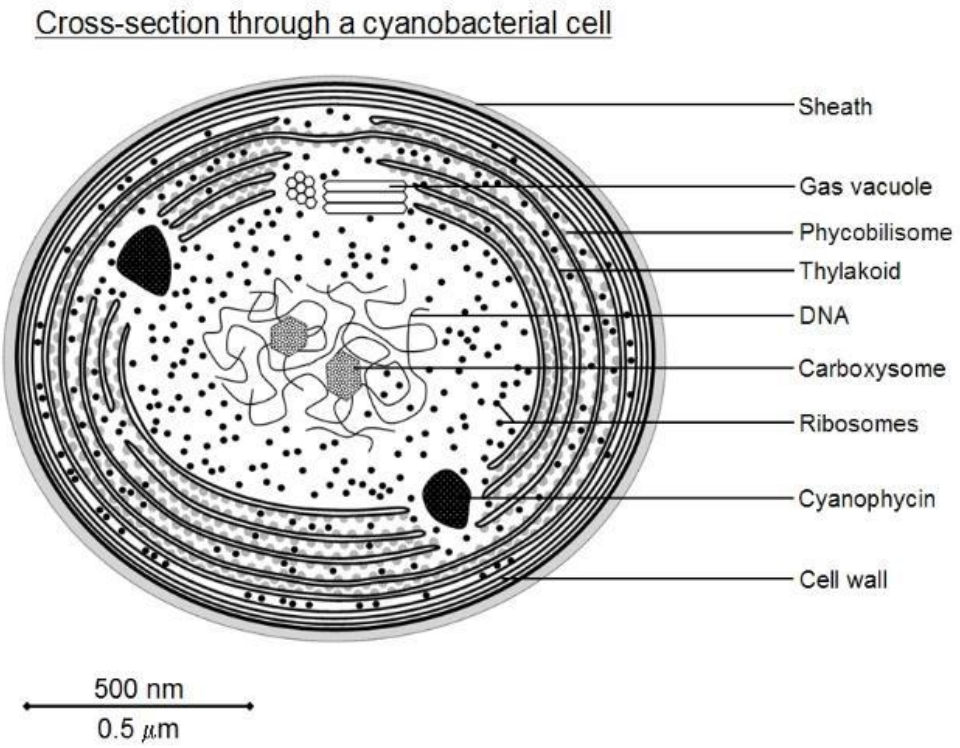

Figure 2.2: Cyanobacteria cell components (cronodon.com/BioTech/cyanobacteria.html)

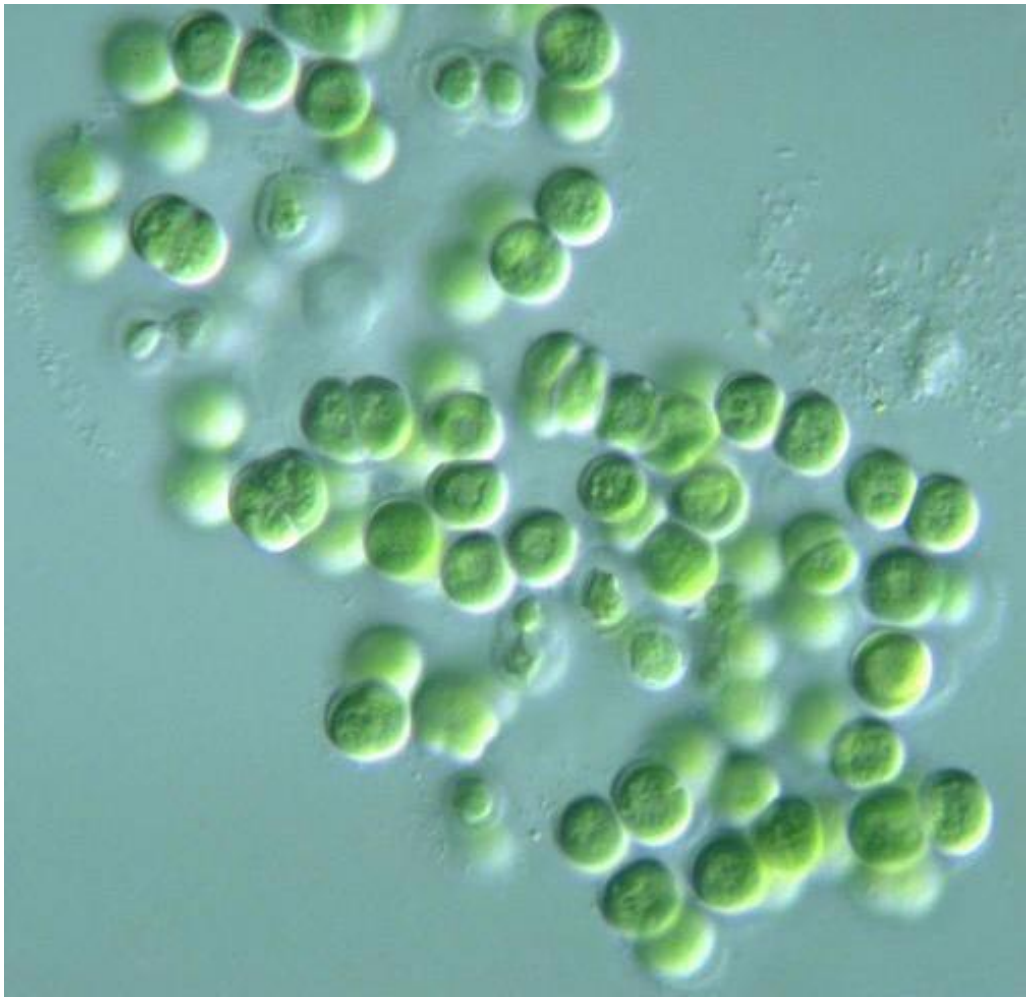

Figure 2.3: Microcystis species at 640x magnification (Tsukii, 2001)

Cyanobacteria are Gram negative bacteria. A micrograph of *Microcystis* sp. is presented in Figure 2.3; it was obtained using optical microscopy. The cell wall of *Microcystis* sp. is comprised of peptidoglycan and lipopolysaccharide, similar to other cyanobacteria (Graham and Wilcox, 2000). On top of the outer membrane is an S-layer of proteins, which itself is covered by an oscillin layer that is responsible for the gliding movement of the cells (Crittenden et al., 2012; Mur et al. 1999; AWWA 2010). The structure of a cyanobacterial cell envelope is provided in Figure 2.4.

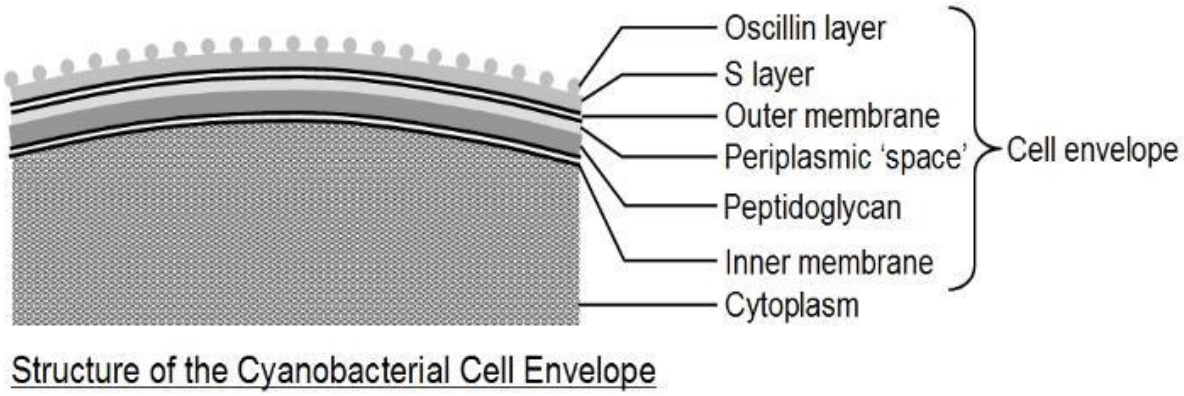

Figure 2.4: Cyanobacteria cell envelope (cronodon.com/biotech/cyanobacteria.html)

Individual *Microcystis* sp. cells are ovoid and spherical in shape and range in size from 2.6 µm to 5.4 µm (Kim et al, 1997; Figure 2.3). The organisms exist as discrete, individual cells in both laboratory and natural environments; however, they are more prone to aggregation in natural environments. This is believed to be due to the threat of predation (Yang et al., 2006; Yang et al., 2009). As a result of this threat (from predators such as *Daphnia*), they typically form gelatinous colonies (Sommer et al., 2003).

Predation of *Microcystis* sp. affects toxin production. In one study, microcystin production increased by up to five times with exposure to *Daphnia* zooplankton; feeding inhibition and increased mortality were also observed among *Daphnia* zooplankton in the presence of toxin producing *Microcystis* sp. (Jang et al., 2003). Other factors also influence toxin production and its concentration in cells. For example, cell toxicity and toxin production rate can increase with light intensity by up to 40 microeinsteins m^{-2} s^{-1} (Utkilen and Gjolme, 1992); thus, accurate prediction of toxin production and toxicity is not presently possible for most natural environments.

The potent hepatotoxin microcystin is of particular interest for the drinking water treatment industry. Microcystin is a cyclic peptide hepatotoxin estimated to be between 1.2 and 2.6 nm (Figure 2.5; Donati et al., 1994). There are approximately 100 variants of the toxin, although the most commonly produced by *Microcystis* sp. are MC-LR, MC-RR, and MC-YR (Mazur-Marzec et al., 2010; Sivonen et Jones, 1999; Pekar et al., 2016). As these toxins inhibit eukaryotic protein phosphatases 1 and 2A, they can cause liver damage and tumours, and gastroenteritis (MacKintosh et al., 1990). A non-ribosomal enzyme is responsible for the synthesis of microcystin. This enzyme complex is encoded by the gene cluster mcy, which has been found

in all toxin containing *Microcystis* strains. Despite this, toxic and non-toxic strains are 99% genetically similar and it is still not possible to determine the toxicity of *Microcystis* cells from genetic analysis (Mazur-Marzec et al., 2010).

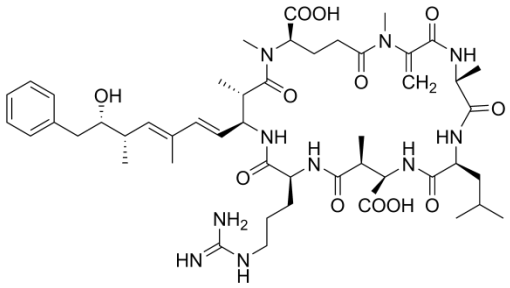

Figure 2.5: Microcystin-LR (Wikipedia/MacKintosh et al., 1990)

Cyanobacterial cells can pass through all of the processes within a conventional drinking water treatment plant and remain intact; notably, the removal of intact cells is greatest during coagulation/filtration/clarification (Zamyadi et al., 2012). The cyanobacterial cell removal performance of these processes is highly dependent upon their cell concentration, because they can substantially challenge and disrupt coagulation efficacy and clog filters (Zamyadi et al., 2012). Pre-ozonation (i.e., ozonation applied at the beginning of the treatment process, prior to coagulation) can destroy cyanobacterial cells, but in doing so it can also release any toxins present in the cells. That toxin can further pass through the treatment system if not sufficiently destroyed by ozonation. Ozone applied toward the end of the treatment process is often relied upon to destroy remaining cells and toxin (Crittenden et al., 2012). Its efficacy is affected by the presence of organic matter (Hoeger et al., 2002); however, this relationship has not been extensively investigated. **Thus, describing the relationship between organic matter, *Microcystis* cells, and microcystin toxin in a controlled and quantitative manner is a key goal of this thesis research.**

While the release of metabolites such as MIB and geosmin by cyanobacteria during lysis has been studied (Hoeger et al., 2002), the extent of damage to cyanobacterial cell integrity caused by ozonation has been reported only more recently. Loss of chlorophyll and alterations in cell structure after ozonation using BacLight® and Scanning Electron Microscope (SEM) microphotography was reported by Coral et al. (2013). The structure of healthy cells was spherical and osmotic pressure was visible. Cells following ozonation had dimples and distortions in cell shape and appeared taut and shrunken. Although an ozone dose of 0.5 to 4.0 mg O_3/L was applied, no lysed cells were observed. In stark contrast, Miao and Tao (2009)

measured a 91% reduction in chlorophyll following ozonation at concentrations of ozone similar to those used by Coral et al. (2013) and reported extensive cell lysis with 3 mg O_3/L. To build on such work, the impacts of ozone on cell lysis and toxin elimination in the absence of DOC were investigated in this thesis research, and the altered structure of the cells remaining post-ozonation was assessed. Corresponding experiments were conducted with varied levels of DOC.

2.3 Ozone

Ozone treatment is increasingly common in drinking water treatment plants; it typically is situated in either the early phase (pre-ozonation) or late phase (post-sedimentation) of the treatment process (Crittenden et al., 2012). Ozone has been shown to be a relatively quick and effective method of eliminating several cyanobacterial toxins, including microcystin-LR (Rositano et al., 1998). Ozone concentrations as low as 1.5 mg/L can quickly (often less than 5 minutes) reduce toxin concentrations to less than 1.0 µg/L. (Jurczak et al, 2005; Rositano et al, 2001). Kinetic studies often describe oxidation by molecular ozone, but during drinking water treatment contaminant degradation is often through the generation of hydroxyl radicals, which can degrade some organic micropollutants more effectively than ozone (von Gunten, 2003). Particularly, the predominant kinetic mechanism of toxin destruction is believed to be by the hydroxyl radicals formed rather than the ozone itself (Shawwa and Smith, 2001). Hydroxyl radical reactions are promoted by higher pH and by the presence of natural organics in water, however, the reactions occur less at higher water alkalinity (Rositano et al, 1998). Also, the ·OH molecules can be scavenged by other aqueous species, resulting in reduced toxin elimination. Dissolved organic carbon (DOC) is a known ·OH scavenger at higher pH; in this case, ozone more readily dissociates into oxygen and water, forming fewer ·OH molecules (Pocostales et al., 2010). Additionally, ozonation causes cell lysis, thereby increasing aqueous DOC concentrations, as well as releasing toxins and taste and odour compounds such as MIB and geosmin (Wert et al., 2014).

Cyanobacterial destruction during drinking water treatment has, to-date, focused on processes at the front end of typical treatment systems. Several studies have examined pre-coagulation oxidation for toxin elimination (Hoeger et al., 2002; Chen et al., 2009); several others have investigated the efficacy of ozonation for the destruction of toxins present in raw water (Rodriguez et al., 2007; Pietsch et al., 2001). Substantially less research has focused on the efficacy of oxidation of clarified water for toxin destruction. Compared to untreated source

water, water quality following coagulation/flocculation/clarification can be substantially different in alkalinity, DOC, and pH; thus, treatment requirements for toxin destruction in clarified water may be different than those for untreated water. To further understand the effect of ozone on toxins in clarified water, experiments were conducted during this thesis research to determine the applied ozone concentrations required to suitably reduce toxin concentrations in settled water in the presence of moderately high and high pH and DOC.

Toxin destruction during drinking water treatment has been investigated using both extracted toxins and whole cells from both cultured and naturally occurring blooms (Al Momani et al., 2008; Svrcek and Smith, 2004). The use of extracted toxins is easier and more common in the reported literature. The benefit of studying ozonation of extracted toxin is that there is greater control in experimental design; specifically an exact amount of toxin can be added and thus, the proportion destroyed can be more accurately measured. This permits a more controlled evaluation of the impact of each investigated parameter on the elimination of toxin. For these reasons, in this thesis research, the quantitative relationship between microcystin, ozone, pH, and DOC was first established using extracted toxin.

Despite the accuracy of performance measurement that can be obtained using extracted toxin, if toxin is present in a real source water, it will likely also be contained in the cyanobacteria cells —and it is these cells within a drinking water treatment process that need to be removed. As discussed in Section 2.2, cells are lysed in ozonated water by the disintegration of the cell membrane by ozone; the cell cytoplasm, which contains DNA, pigment, and toxin, is released into the water matrix thereafter (Pietsch et al., 2001). Ozone is required to lyse the cells, and then to destroy the toxin previously contained within the cells. The organic matter released from the cells also reacts with ozone and can thus impact the amount of ozone available for toxin destruction (Wert et al., 2014).

A few studies have described the release of intercellular cyanobacterial material and its contribution to DOC concentrations in ozonated water (Korak et al., 2015; Coral et al., 2013). The impact of DOC in the water matrix on the proportion of intercellular and extracellular toxin remaining following ozonation was investigated in this thesis research.

2.4 Dissolved Organic Carbon

Source waters supplying drinking water treatment plants (WTPs) contain a heterogeneous mixture of sizes, charges, hydrophobicities, and chemical compositions of organic matter. Its origins can be terrestrial, anthropogenic, or microbial (Marhaba et al., 2000; Shutova et al.,

2014). The quantity and character of organic matter contributes substantially to the efficacy of various drinking water treatment approaches. For instance, coagulation effectively removes aromatic, hydrophobic organics of a higher molecular weight (Shutovva et al., 2014). In a study of four WTPs, organic matter removal by coagulation/flocculation/sedimentation ranged from 31 to 57% of the organics present at the intake. However, the more aromatic organics that are typically believed to be terrestrial in origin were the greater proportion of what was removed from the matrix, whereas more of the microbially-derived organics remained (Shutova et al., 2014). Waters with this type of character of organic matter are what would be expected in the influent of post-sedimentation ozonation processes.

As previously mentioned, the water matrix in a post-sedimentation treatment process is different than from both source water and pre-coagulation water matrices. There are several aspects of water quality that are altered throughout the treatment process; these include pH, alkalinity, organic matter concentration and character, and the presence of coagulant and flocculent. While many changes in water quality can have an impact on treatment efficacy, changes in pH and DOC affect the destruction of microcystin by ozonation (He et al., 2012). In particular, water with a pH above 7.5 in the presence of DOC results in incomplete oxidation of microcystin (Al Momani et al., 2008). It has also been reported that increases in DOC concentrations correspond to increased ozone requirements for reducing concentrations of cyanobacterial toxin to below regulatory levels (Hoeger et al., 2002; Miao et al., 2009; Schmidt et al., 2008). While this relationship is understood in qualitative terms, the limits of ozonation for toxin destruction have not been quantitatively established. Describing the quantitative relationship between pH, DOC, ozone concentrations, and toxin destruction is one objective of this thesis research.

The microbially-derived forms of organic matter that are likely to be present in clarified water include the organic material contained within cyanobacterial cells. These cells release their organic matter when they are lysed during ozonation; some of this material dissolves in the water matrix (Wert et al., 2013). Although other forms of organic matter such as larger hydrophobic organic molecules are still present in clarified water and in cellular material, hydrophilic organic matter predominates (Swietlik et al., 2004). It has been shown that the hydrophobic portion of dissolved organics is what is predominately removed (i.e. broken down) by ozonation (Swietlik et al., 2004; Marhaba et al., 2000). This reactivity of ozone with DOC can be non-ideal from a treatment perspective because the amount of ozone available for toxin destruction decreases when ozone reacts with hydrophobic dissolved organics. Moreover, cell

lysis releases additional carbon that is relatively hydrophilic and therefore less likely to be reduced by ozonation.

It should be noted that although this thesis research was not specifically designed to assess how various dissolved organics are affected by ozonation, the proportion of intercellular and extracellular toxin present following ozonation at various DOC concentrations provides an indication of the efficacy of ozone in the presence of cellular organic matter and aqueous organic matter (i.e. DOC) in the water matrix.

Chapter 3
Methodology

3.1 General Research Approach

Microcystin and *Microcystis aeruginosa* destruction by ozone at various water quality and operational conditions were investigated. Experiments were conducted using a pilot-scale ozone generator and a bench scale reactor. The procedure for ozonation and reaction of the ozonated water with the toxin or cells was consistent throughout all experiments.

The first experiment involved the use of extracted toxin to provide the greatest control of the amount of toxin initially present in the water matrix. The use of extracted toxin also allowed for the assessment of only one mechanism of destruction by ozone: direct attack of the toxin molecules by ozone or hydroxyl radicals (Shawwa and Smith, 2001). The effect of water matrix pH and DOC concentration on toxin destruction were quantified and directly correlated.

The second experiment utilized laboratory cultured *Microcystis aeruginosa* cells to perform the same experiment as with the extracted toxin; thus, the same operational conditions (various controlled levels of DOC concentration and pH) were applied. The efficacy of cell and subsequently released toxin destruction by ozonation was investigated. Both intercellular and extracellular toxins were measured after ozonation to provide an indication of the concentration and proportion of live cells and toxin remaining in the water matrix after treatment (Pietsch et al., 2002). Water matrix pH and DOC concentration were also confirmed.

The third experiment involved the use of laboratory cultured *Microcystis aeruginosa* cells to repeat the first two experiments in the absence of DOC and at neutral pH. The auto-fluorescence of *Microcystis* sp. was utilized to evaluate the proportion of cells destroyed by ozone (Nancharaiah et al., 2007). The physical effect of ozone on the cells was observed and the extent of the damage to cell morphology was evaluated (Korak, et al., 2015). The general experimental research approach is summarized in Figure 3.1.

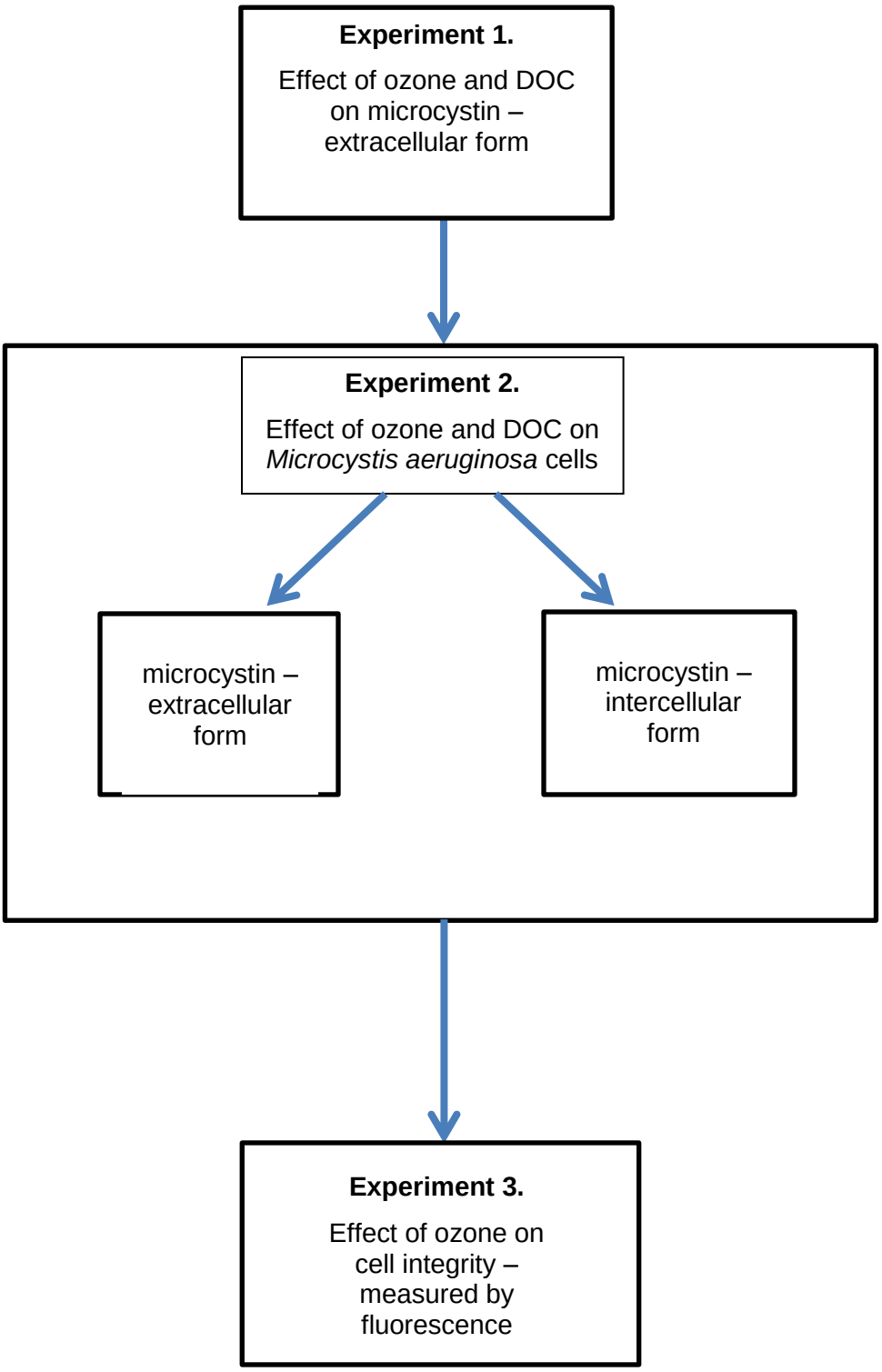

Figure 3.1: General Research Approach

3.2 Source Water

The partially treated (clarified) source water used during experiments 1 and 2 was obtained from the Mannheim Water Treatment Plant (MWTP) in Kitchener, Ontario (Figure 3.2). The MWTP treats surface water pumped from the Grand River via the Hidden Valley High Lift Pumping Station. The Pumping Station with its four reservoirs and the adjacent Grand River are shown in Figure 3.3.

Figure 3.2: Location of Mannheim Water Treatment Plant, Kitchener, Ontario

Figure 3.3: Hidden Valley High Lift Pumping Station and Grand River, Kitchener, Ontario

To evaluate toxin destruction by ozonation, water from the MWTP was obtained from a sample port downstream of the sedimentation tank and upstream of the ozone contact chamber, as shown in the process flow diagram provided in Figure 3.4.

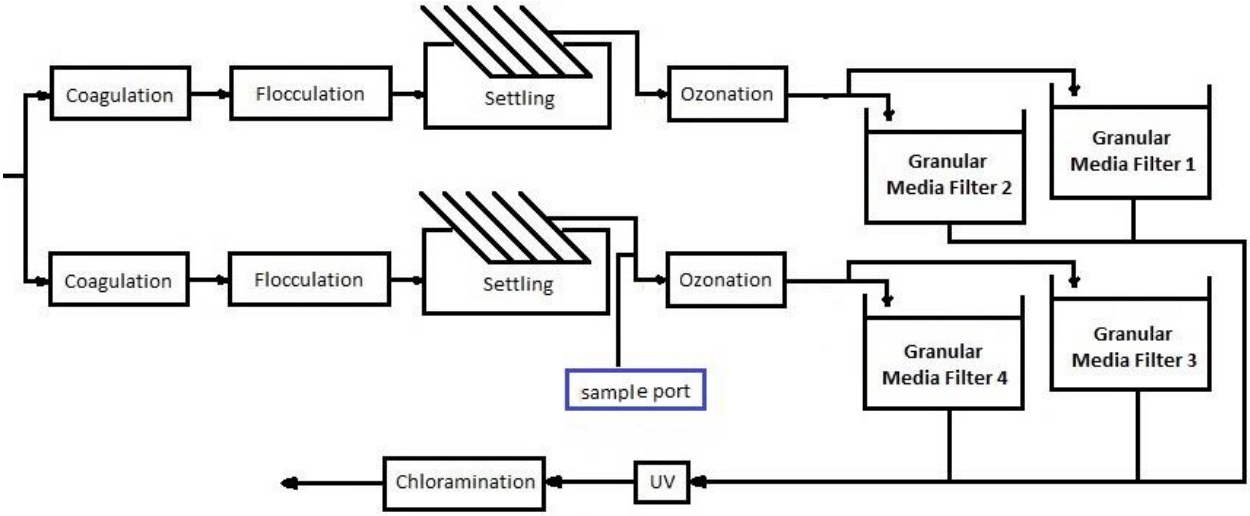

Figure 3.4: MWTP Process Flow Diagram

The nominal water quality characteristics of the influent to the MWTP and after clarification (i.e. collected at the sample port) are listed in Table 3.1 below.

Table 3.1: Nominal MWTP Water Quality Parameters

Parameter	Influent to MWTP	Prior to Ozonation
pH	8.0	--
DOC (mg/L)	5.7	4.2
TOC (mg/L)	5.8	4.5
Alkalinity as $CaCO_3$ (mg/L)	220	--
TDS (mg/L)	385	--
Hardness (mg/L)	268	--
Turbidity (mg/L)	3.4	1.4

3.3 Experiment Using Extracellular Toxin

3.3.1 Toxin Source and Analysis

500 micrograms (µg) of extracted microcystin-LR of ≥95% purity obtained by high performance liquid chromatography (HPLC) (Enzo Life Sciences, United Kingdom) were stored at -10°C. The microcystin-LR was extracted from *Microcystis aeruginosa*. The Certificate of Analysis is contained in Appendix 1. A 1000 microgram per litre (µg/L) solution of microcystin-LR dissolved in methanol was prepared using this extract.

Toxin analysis was performed by the enzyme-linked immunosorbent assay (ELISA) method (Engvall and Perlmann, 1971) using the Abraxis™ microcystins/nodularins-DM ELISA kit at the Central Ontario Analytical Laboratory (COAL) in Orillia, Ontario. The ELISA kit detailed procedure, the United States Environmental Protection Agency (EPA) Verification Program results, the COAL Standard Operating Procedure, and the COAL Determination of Detection Limits of Licensed Parameters are contained in Appendix 1. In brief, this method consists of a chlorometric analysis using enzyme conjugate and antibody solution to bind the toxins to the enzymes. The concentration of toxin is measured relative to the intensity of the dyed enzyme compound. The lab analysis reports are contained in Appendix 2. The samples were shipped by overnight courier in coolers with ice packs.

The reported method detection limit was 0.1 µg/L. All but one blank control sample (no toxin added) were reported as non-detects (ND). The sample with a detected concentration of toxin contained 2.81 µg/L microcystin-LR. It is unlikely that the settled water from the MWTP contained toxin, particularly in January when the water was collected. COAL acknowledged this was likely due to a mislabeled sample, but could not confirm.

3.3.2 Dissolved Organic Carbon (DOC)

A potassium hydrogen phthalate (99.95% purity, Nacalai Tesque Inc. Japan) solution at 1000 milligram per litre as carbon (mg/L-C) was prepared and verified. The solution was used to add additional DOC up to the desired concentration for each experimental trial.

Potassium hydrogen phthalate is a hydrophobic compound with a low molecular weight that is a commonly used laboratory carbon standard (Pradhan et al., 2015). Hydrophobic compounds constitute the majority of aqueous NOM, accounting for over half of the DOC in water (Matilainen et al., 2011). Particular to the research conducted, ozone more readily interacts with

hydrophobic compounds (Swietlik et al., 2004) and thus, the specific effect of the presence of DOC during ozonation of microcystin-containing water could be studied. As a result of the use of potassium hydrogen phthalate, the effect of DOC shown in this research could potentially be greater than would be present in water matrices where there is a greater presence of hydrophilic compounds.

The concentration of DOC in the solution and the water obtained from the MWTP was analysed using a Shimadzu VCPH Total Organic Carbon Analyser and reported to +/- 0.001 mg/L. The analysis was performed in accordance with Standard Method 5310B: High Temperature Combustion Method (AWWA et al., 2005). The method consists of homogenization and dilution of the sample as necessary. The sample is then acidifed to a pH less than 2 and sparged with ultrapure oxygen to drive off inorganic carbon from the sample. A small portion is then injected into a heated reaction chamber packed with a platinum catalyst. The water is vaporized and the organic carbon and inorganic carbon is oxidized to CO_2 and H_2O. The CO_2 is transported in the carrier-gas stream and is measured by a nondispersive infrared analyzer. Prior to analysis the water sample was vacuum filtered with a 0.45 micrometre (µm) Pall® Nylaflo hydrophilic nylon membrane disc bottle-top filter to obtain only the dissolved organic carbon. The sample was analysed immediately following filtration. All glassware used for DOC analysis, including sample filtrate bottles and the filter funnel apparatus were cleaned and acid washed for a minimum of twelve (12) hours in a 10% hydrochloric acid solution to ensure carbon did not leach into the samples (Khan and Pillai, 2007).

3.3.3 pH

An Orion 720A meter was used to measure pH. A three point calibration was performed with every 4 hours of use. Standard pH 4, 7, and 10 solutions were used for calibration.

3.3.4 Ozone

The ozone residuals utilized in this research were selected based on those utilized at the MWTP. 0.3 mg O_3/L is used in the winter season (November-March) and 0.6 mg O_3/L is used in the spring/summer season (April-October) (personal correspondence, Peter Clarke). The 0.1 mg O_3/L was chosen to assess if reduction of microcystin-LR by a lower ozone residual to below regulatory limits was possible under any of the conditions studied.

Ozone residual was measured using HACH™ Indigo Method for mid-range concentrations (0–0.75 milligrams of ozone per litre (mg O_3/L)). The HACH™ Method 8311 protocol is presented

in Appendix 1. Ozonated water was reacted with HACH™ AccuVac© ozone reagent ampoules and the ozone residual was measured with a HACH™ DR/2010 portable data-logging spectrophotometer (HACH Canada, Mississauga, Canada) set at the low range for trials conducted at 0.1 mg O_3/L, and medium range for trials conducted at 0.3 and 0.6 mg O_3/L.

The ozone residual was measured at the beginning of each trial to obtain the desired concentration. During the preliminary trials conducted to establish the required ozone generator set points, the ozone residual at the end of the reaction time was measured. No ozone residual was present, regardless of the initial residual concentration; thus, all of the ozone was consumed during the reaction period.

Table 3.2 shows the target ozone residuals and the range of actual ozone residuals used in the experiments conducted in accordance with Section 3.3.6.

Table 3.2: Target and Actual Ozone Residuals, Extracted Toxin

Target Ozone Residuals (mg O_3/L)	Actual Ozone Residuals (mg O_3/L)
0.1	0.06 – 0.18 (mean: 0.11)
0.3	0.22 – 0.45 (mean: 0.34)
0.6	0.51 – 0.70 (mean: 0.59)

3.3.5 Experimental Conditions

Table 3.3 is a summary of the different conditions investigated during this experiment. Each combination of experimental conditions was evaluated in triplicate. Thus, a total of 144 trials were conducted (48 factor combinations (4 pHs*4 DOCs*3 ozone residuals) * 3 replicates).

Table 3.3: Experimental Conditions using extracellular microcystin-LR

pH	DOC (mg/L)	Ozone residual (mg/L)
7.0	Source water concentration	0.1
7.5	5	0.3
8.0	10	0.6
8.5	15	

The complete listing of combinations of investigated variables (factorial design) is provided in Appendix 3.1.

3.3.6 Experimental Procedure

1. Water was obtained from a sample port downstream of the sedimentation tank, prior to ozonation at the Mannheim WTP in Kitchener, ON.
2. Water quality (pH and DOC) were characterized as described above.
3. Water was ozonated using a Pacific Ozone Model IC5005-C11 ozone generator with a 2900 watt (W) output to obtain desired ozone residual. Typical settings were 20 standard cubic feet per hour (scfh), 4.5 volts direct current (VDC), and a pressure of 6 pounds per square inch (psi). Figure 3.5 is a photograph of the ozone generator.
4. An approximately 50 millilitre (mL) sample of water was collected for ozone residual measurement.
5. Once the desired ozone residual was obtained, 1L of ozonated water was added to a 3L double-walled glass reactor (Figure 3.6). The ports at the top of the reactor were sealed with clamped butyl rubber during the experiment, not parafilm, as shown in Figure 3.6.
6. pH was measured and adjusted with 1.0 normal (N) sodium hydroxide (NaOH) or 1.0 N hydrochloric acid (HCl_{aq}) to obtain the desired pH.
7. A pre-determined volume of the DOC solution described above was added to the ozonated water to obtain the desired concentration of DOC in the solution.
8. 10 mL of toxin solution were added to reactor -- a concentration of 10 µg/L microcystin-LR in the ozonated water.
9. The reactor was sealed and treatment by ozonation occurred for 15 minutes. The time frame is comparable to reaction times at the MWTP (accounting for the volume of water and continuous flow conditions).
10. After 15 minutes, any remaining ozone residual was quenched with an excess of calcium thiosulphate (3 mL of a 1245 mg/L solution).
11. One (1) 500 mL sample of treated water was collected for microcystin-LR analysis. For every eight (8) samples collected a negative control sample (MWTP water only) was analysed.
12. The procedure was repeated for eight (8) sets of experimental conditions using the same batch of water obtained from the Mannheim WTP.

All sample results and data analysis are presented in Appendix 3.1.

Figure 3.5: Pilot-scale Ozone Generator

Figure 3.6: Bench-scale Ozone Reactor

3.4 *Microcystis aeruginosa* Cell Culture

A 30 mL culture of *Microcystis aeruginosa* was obtained from the Canadian Phycological Culture Centre (CPCC), University of Waterloo, Waterloo, Ontario.

3.4.1 Growth Medium

A BG-11 growth medium (Rippka et al., 1979) was used to culture the *Microcystis aeruginosa* cells. The individual solutions of the compounds comprising the growth medium were stored in glass 500 mL bottles, covered with aluminum foil, and refrigerated. The prepared BG-11 medium was also stored in 1L glass bottles, autoclaved, cooled, and then refrigerated. The vitamin solutions were stored in individual 1 mL cryovials and stored in the freezer (-10°C). The contents and methodology of preparation for the solutions and medium are provided in Appendix 1.

3.4.2 Culture Transfer

A 250 mL Erlenmeyer flask with a silicone plug was covered with Bioshield (a fabric used to cover instruments for sterilization), secured with twine, and autoclaved to sterilize the contents. The flasks were cooled to room temperature. Using a biological safety cabinet, a cryovial containing the vitamin solution was thawed and the solution was added to the sterilized growth medium. The growth medium and cell culture were added to the sterilized flasks at a volume ratio of 1:3 culture: medium.

3.4.3 Cell Culture Growth

The culture-containing flasks were stored in a Percival growth cabinet (John's Scientific Inc., Canada) with environmental conditions maintained as per the advice of Heather Rochon, Biologist at the CPCC. Specifically, the temperature in the culture chamber was maintained between 19 and 22°C, at a setpoint of 21°C on a 12 hour light/dark cycle. Three (3) full spectrum white light fluorescent lamps were used as the light source. The flasks were placed at the lower level of the cabinet where the light was measured to be approximately 1776 lux. Figure 3.7 shows a representative photograph of the cell cultures.

3.4.4 Cell Counts

Using a biological safety cabinet, the cell cultures were obtained with a sterilized Pasteur pipette and put into a 20 mL glass vial with the sterilized growth medium to obtain a desired dilution, usually of 50 times.

Using a Pasteur pipette the cell solution was placed on a Hausser Scientific Bright-Line Hemacytometer (VWR International, Mississauga, Canada) and covered with the cover slip. The counting chamber was 0.1 mm deep and contained exactly 10 µL. The entire middle grid of the chamber was used to count the cells.

Cells were enumerated at 200x and 400x magnification under white light using a Zeiss Axioskop 2 microscope (Carl Zeiss Canada, Toronto, Canada). The cell counts were used to determine if the cell culture should be transferred into fresh growth medium. Cultures were typically transferred after 1-2 weeks and/or cell counts in excess of $5.0x10^7$ cells/mL.

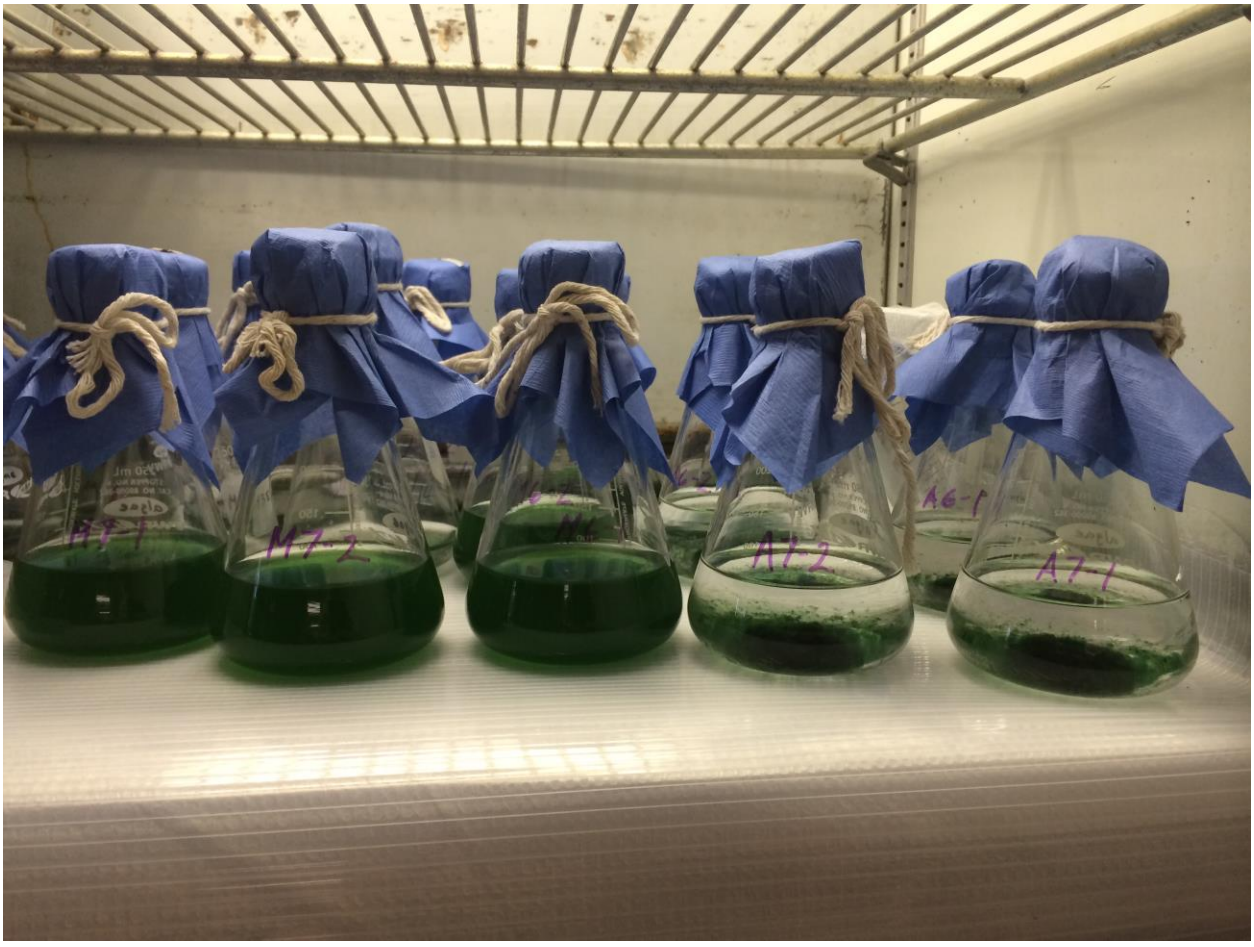

Figure 3.7: Representative *Microcystis aeruginosa* cell cultures in growth cabinet

3.5 Experiment using *Microcystis aeruginosa* cells to measure intercellular and extracellular toxin concentrations

3.5.1 Cell Culture

Microcystis aeruginosa cells cultured as described in Section 3.4 were used in this experiment. Cultures with a cell concentration between $2.5x10^7$ and $4.0x10^7$ cells/mL were used. Toxin concentrations in the cultured cells ranged from approximately 800 to 1000 µg/L. All toxin was intercellular (contained within the cells). Each set of nine (9) trials was conducted using cells cultured from the same flask.

3.5.2 Experimental Conditions

Table 3.4 shows the different system conditions evaluated during this experiment. Each combination of experimental conditions was evaluated in triplicate. Thus, 36 trials were conducted (12 factor combinations (2 pHs*3 DOCs*2 ozone residuals) * 3 replicates).

Table 3.4: Experimental Conditions using *Microcystis* aeruginosa cells

pH	DOC (mg/L)	Ozone residual (mg/L)
8.0	5	0.3
8.5	10	0.6
	15	

The complete listing of combinations of investigated variables (factorial design) is provided in Appendix 3.2.

3.5.3 pH, DOC, and Ozone

The pH, DOC, and ozone levels chosen for this experiment were based on the results obtained from the experiment in Section 3.3. The pH, DOC, and ozone were characterized and measured following the same procedure as in Section 3.3.

Table 3.5 shows the target ozone residuals and the range of actual ozone residuals used in the experiments conducted in accordance with Section 3.5.4.

Table 3.5: Target and Actual Ozone Residuals, *Microcystis* cells

Target Ozone Residuals (mg O_3/L)	Actual Ozone Residuals (mg O_3/L)
0.3	0.23 – 0.42 (mean: 0.31)
0.6	0.53 – 0.72 (mean: 0.60)

3.5.4 Experimental Procedure

The same procedure as described in Section 3.3.6 was followed for this experiment, with the exception of the following:

8. 10 mL of cell culture was added to the reactor. Based on initial toxin analysis of the cell cultures, the total toxin concentration was approximately 1000 µg/L. 10 mL of cell culture was added to the water in each trial to achieve an initial toxin concentration of approximately 10 µg/L in the ozonated water.
11. One (1) 20 mL sample of reacted water was collected for sample preparation for laboratory analysis of microcystin-LR. For every nine (9) samples collected a control sample (MWTP water only) and a sample of the cell culture was submitted for laboratory analysis of microcystin-LR. All samples were collected in 40 mL glass vials with screw caps.
12. The procedure was repeated for nine (9) sets of experimental conditions using the same batch of water obtained from the MWTP.

3.5.5 Toxin Analysis and Sample Preparation

Microcystin-LR in the cell cultures was analysed using liquid chromatography-mass spectroscopy-mass spectroscopy (LC-MS-MS) (Oehrle et al., 2010). The laboratory and instrument procedures and lab analysis reports are provided in Appendices 1 and 2, respectively. Samples were sent for analysis by overnight courier in sealed containers with ice packs.

The samples and cell cultures were prepared for total toxin analysis by the microwave method in accordance with the procedure outlined below (Metcalf and Codd, 2000). This method results in cell lysis and thus the release of intercellular toxin into the water matrix. Therefore, by performing this method, intercellular toxin becomes extracellular and the total toxin

concentration (extracellular toxin in the water matrix and the released toxin from the cells) in the sample can be measured.

To test the procedure, samples of the microwaved cells were viewed under UV light according to the method described in Section 3.6.4. No cells in the samples fluoresced, indicating there were no live intact cells remaining and thus the cells had lysed.

To measure for extracellular toxin concentration:

1. A portion of the sample was filtered with a 0.45 µm glass fibre membrane filter to collect only the microcystin-LR in solution and filter out all intact cyanobacterial cells. The filter was attached to a PVC disposable syringe and the sample was released into 2 mL amber vials with screw top caps, suitable for autosampler sample analysis.

To measure for total toxin concentration:

1. The screw top cap was loosely attached to the 40 mL glass vial containing the sample, as described in step 11 of the Experimental Procedure below.
2. The sample was placed in a Goldstar MS-71GMU 700W microwave on 'high' setting for 60 seconds in 10-15 second intervals to ensure the vial did not overheat.
3. Cells following microwaving were a pale green colour and the cell matter appeared "stringy."
4. The sample was allowed to cool to room temperature.
5. The sample was filtered with a 0.45 µm glass fibre membrane filter and attached to a PVC disposable syringe into 2 mL amber vials with screw top caps, suitable for autosampler sample analysis. A 0.45 µm glass fibre membrane filter allows microcystin-LR (between 1.2 and 2.6 nm in size) and matter dissolved in the water matrix (such as DOC) to pass through the membrane but would filter out any intact cyanobacteria, which are greater than 2 µm. Membrane filters ranging from 0.45 to 0.7 µm have previously been used to isolate microcystin-LR (Korak et al., 2015; Wert et al., 2014; Zamyadi et al., 2015) from cells.

All sample results and data analysis are presented in Appendix 3.2.

3.6 Cell Viability Experiment

3.6.1 Saline Solution

A saline solution was used as the water medium for this experiment in order to provide a water medium without carbon and one that would be conducive to cell viability, but would not encourage growth, such as the nutrient-rich growth medium described in 3.4.1.

A 10% saline solution was prepared using ultrapure (Type 1) MilliQ™ water, 14.4 grams (g) sodium phosphate dibasic heptahydrate, 80g sodium chloride, 2.0g potassium chloride, and 2.4g potassium dihydrogen orthophosphate to make a 1L solution. The pH of all solutions prepared for the trials were between 7.86 and 8.02. The solution was then autoclaved. A 0.1% saline solution was prepared by dilution of the 10% saline solution.

3.6.2 Cell Cultures, Toxin Analysis and Sample Preparation, and Ozone

The cell cultures, methods for sample preparation, and toxin and ozone residual analyses were the same as those described in Section 3.5.

Table 3.6 shows the target ozone residuals and the range of actual ozone residuals used in the experiments conducted in accordance with Section 3.6.3.

Table 3.6: Target and Actual Ozone Residuals, Cell Counts

Target Ozone Residuals (mg O_3/L)	Actual Ozone Residuals (mg O_3/L)
0.3	0.23 – 0.45 (mean: 0.31)
0.6	0.45 – 0.73 (mean: 0.57)

3.6.3 Experimental Procedure

1. The concentration of cells (cells/mL) of the cell culture was counted in accordance with the procedure described in Section 3.4.4.
2. A saline solution was prepared as described above and the pH was measured.
3. Water was ozonated in the ozone generator described in Section 3.3.6 to obtain the desired ozone residual.
4. An approximately 50 mL sample of water was taken to measure the ozone residual.

5. Once the desired ozone residual was obtained, 1L of ozonated water added to a 3L glass reactor.
6. The required volume of cell culture to achieve the desired cell concentration was added to reactor. For a cell culture concentration of $2.0x10^7$ cells/mL the following volumes were added:

Desired Concentration (cells/mL)	Volume added (mL)
100	0.005
2000	0.1
10 000	0.5
100 000	5

   The volume added was adjusted according to the concentration of the cell culture
7. The reactor was sealed and reacted for 15 minutes.
8. Any remaining ozone residual was quenched with 3 mL of calcium thiosulphate.
9. One (1) 20 mL sample of reacted water was collected for sample preparation for laboratory analysis of microcystin-LR. For every six (6) samples collected a control sample (unozonated saline solution containing a known concentration of cells) and a sample of the cell culture were submitted for microcystin-LR analysis. All samples were collected in 40 mL glass vials with screw top caps.
10. One (1) 50 mL sample of reacted water was collected for cell counts, as described below.

The procedure was repeated in triplicate for each ozone residual concentration and cell concentration. Thus, 24 trials were conducted (8 factor combinations (4 cell concentrations*2 ozone residuals) * 3 replicates). The complete listing of combinations of investigated variables (factorial design) is provided in Appendix 3.3. All sample results and data analysis are presented in Appendix 3.3.

3.6.4 Cell Counts Using Fluorescence

A portion (calculated based on the initial cell concentration added) of the 50 mL sample (Step 10 of section 3.6.3) was vacuum filtered using a 25 mm diameter, 3 µm nominal porosity Whatman Nuclepore polycarbonate filter (VWR International, Mississauga, Canada). The filter was transferred to a slide and covered with a cover slip. The auto-fluorescence-based method of Nancharaiah et al. (2007) was used for cell enumeration. This involved the use of a Zeiss

Axioskop 2 microscope (Carl Zeiss Canada, Toronto, Canada), using a Zeiss FluorArc UV lamp at a 546 nm excitation wavelength and a 590 nm emission wavelength was used to count the *Microcystis* cells at 200x and 400x magnification.

To verify the accuracy of the method, a solution at each cell concentration (i.e. 100, 2000, 10 000, and 100 000 cells/mL) used in the trials was prepared and enumerated, prior to conducting the experiments. The cell counts consistently had 98% or greater accuracy. All fluorescing cells on the filter were counted and the number of intact and damaged and potentially viable (DAPV) cells/mL was recorded.

3.7 Statistical Analyses

An anova 2-factor with replication regression analysis was conducted using Microsoft Excel 2010 Data Analysis software to evaluate the significance of operational conditions on toxin destruction. The descriptive statistics of the data sets, such as mean and standard deviation (Appendices 3.1, and 3.2) were also obtained using Microsoft Excel 2010 Data Analysis software.

P-values were generated to compare the results obtained at separate operational conditions. Two-tailed tests were performed and the p value was evaluated at a significance level of 0.05 ($\alpha = 0.05$). The analytical results of the tests are presented in Appendices 3.1, 3.2, and 3.3.

Chapter 4
Results and Discussion

4.1 Experiment Using Extracellular Toxin

Extracellular microcystin-LR was destroyed by ozonation to below ODWQ standards at ozone residuals above 0.3 mg O_3/L when aqueous DOC concentrations did not exceed 5 mg/L. Notably, the pH of the clarified water did not have a significant effect on the destruction of microcystin-LR. The efficacy of ozone for toxin destruction has been reported previously (Jurczak T. et al, 2005; Rositano J. et al, 2001), however, the design of this experiment and the size of the data set enabled description of the quantitative relationship between DOC concentration in the water matrix and the amount of ozone residual required to achieve toxin destruction to levels below regulatory limits in the presence of extracellular toxin.

The average concentration of extracted microcystin-LR remaining following ozonation of water containing an initial toxin concentration of 10 µg/L (at each experimental condition) is shown in Figure 4.1. The pH/DOC concentration combination at each ozone residual concentration is presented relative to the microcystin-LR concentration remaining after ozonation. Sw (source water) in Figure 4.1 denotes the clarified water DOC concentration obtained from the MWTP. The concentration was typically 3.5 mg/L. The exact concentrations are provided in Appendix 3.1. The same results are presented relative to the DOC concentration in the water matrix for each ozone residual (0.1, 0.3, and 0.6 mg O_3/L) and at all pH levels in Figures 4.2, 4.3, and 4.4. Each set of system conditions was evaluated in triplicate and all of the raw data are presented.

Not surprisingly, the reduction in toxin concentration from the initial 10 µg/L of microcystin-LR was not consistent across all pH/DOC concentrations at an ozone residual of 0.1 mg/L. The remaining toxin concentrations ranged from ND (0.1 µg/L) to 7.45 µg/L. The majority of these ozonated water samples contained toxin at a concentration that exceeded the 1.5 µg/L ODWQS, as indicated in Figure 4.1. No trend in the microcystin-LR concentration was observed with increasing DOC concentrations (Figure 4.2). Microcystin-LR elimination in the system with 0.1 mg O_3/L was statistically different from that in the systems with 0.3 and 0.6 mg O_3/L ($p=0.000023$, $\alpha=0.05$). These results demonstrate that 0.1 mg O_3/L of ozone residual is inadequate for treating an extracellular microcystin-LR concentration of 10 µg/L when DOC is present at concentrations between ~5 and 15 mg/L; which can be reasonably expected in the

settled water of a conventional drinking water treatment process that treats a municipal agriculturally-impacted surface water such as the Grand River.

The toxin concentrations remaining following ozonation at 0.3 and 0.6 mg O_3/L were similar to one another. As shown in Figure 4.1, the average concentration of microcystin-LR following ozonation was consistently less than the ODWQS at a pH/DOC combination of less than 8 pH units/10 mg/L when the ozone residual was 0.3. mg O_3/L. Similarly, the average microcystin-LR concentration was consistently less than the ODWQS at a pH/DOC combination of less than 8.5 pH units/5 mg/L when the ozone residual was 0.6 mg O_3/L (Figure 4.1).

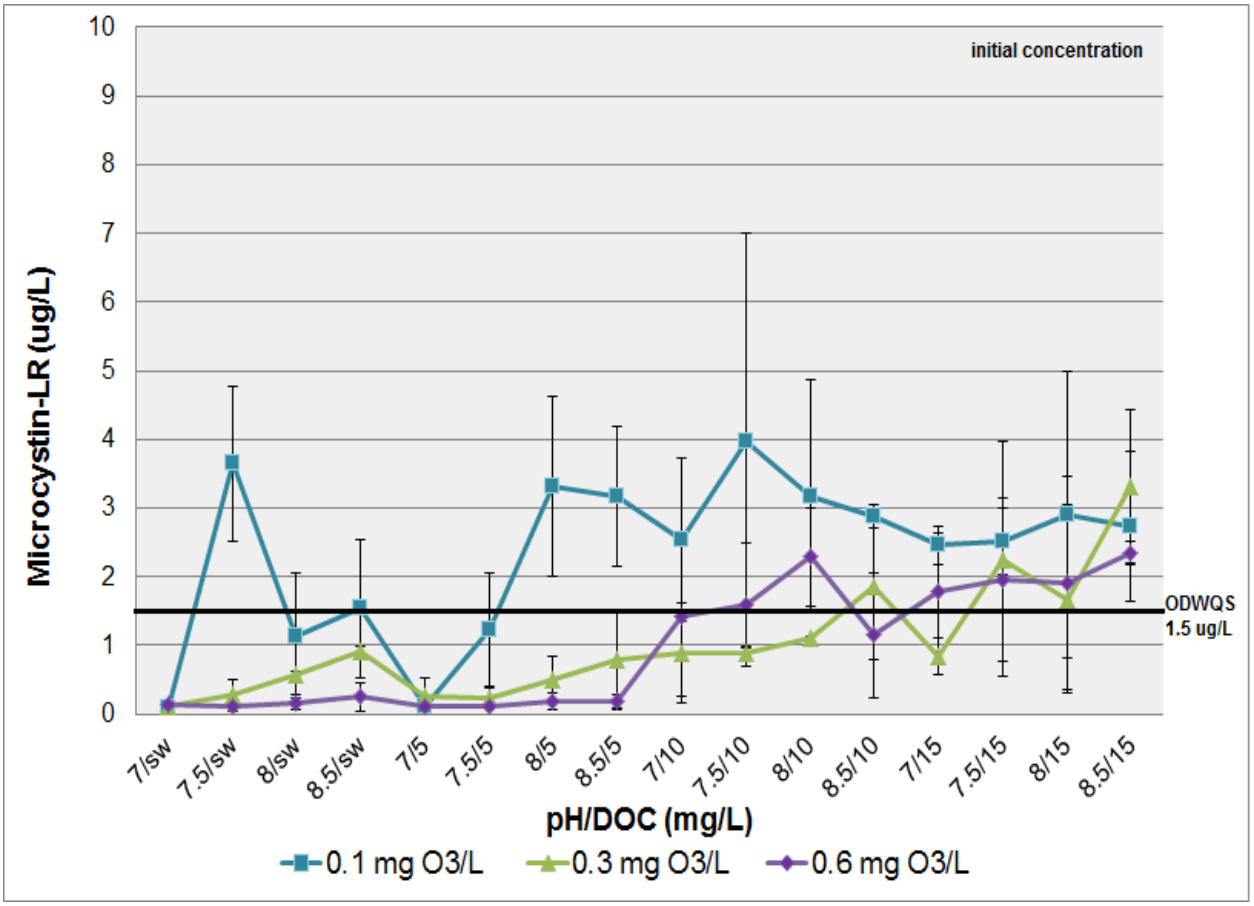

Figure 4.1: Extracellular toxin present (mean +/- standard deviation) at all pH/DOC combinations for all ozone residual concentrations (0.1, 0.3, and 0.6 mg/L) (n=144 samples)

Figures 4.3 and 4.4 illustrate that there was less than 1.5 µg/L of toxin remaining in most samples (all except for one sample at 0.6 mg O_3/L, and all except for two samples at 0.3 mg O_3/L) for ozonated water that contained 5 mg/L DOC. In contrast, a wider range of toxin concentrations (higher standard deviations) was observed at higher DOC concentrations of 10

and 15 mg/L. Specifically, the toxin concentrations remaining after ozonation ranged from 0.26 µg/L to 3.99 µg/L in samples containing 10 mg/L DOC, and ND (0.1 µg/L) to 3.01 µg/L in samples containing 15 mg/L DOC at both ozone residual concentrations. Both ozone residual concentrations adequately eliminated extracellular toxin to concentrations below the regulatory limits (mean: 0.35 µg/L) when clarified water contained 5 mg/L or less of DOC compared to toxin concentrations (mean: 1.76 µg/L) in water containing 10 and 15 mg/L of DOC ($p=0.00001$, $\alpha=0.05$). Although more toxin was destroyed when the ozone residuals were increased to 0.3 and 0.6 mg O_3/L (from 0.1 mg O_3/L), neither of these residual ozone concentrations was adequate for consistently eliminating toxin concentrations to below 1.5 µg/L when high DOC concentrations (10 and 15 mg/L) were present in the water matrix. These results underscore that sub-optimal coagulation situations that result in relatively elevated clarified water DOC concentrations (e.g., such as those that are frequently associated with cyanobacterial blooms) may significantly reduce the efficacy of ozonation as a barrier against toxin passage into treated drinking water supplies.

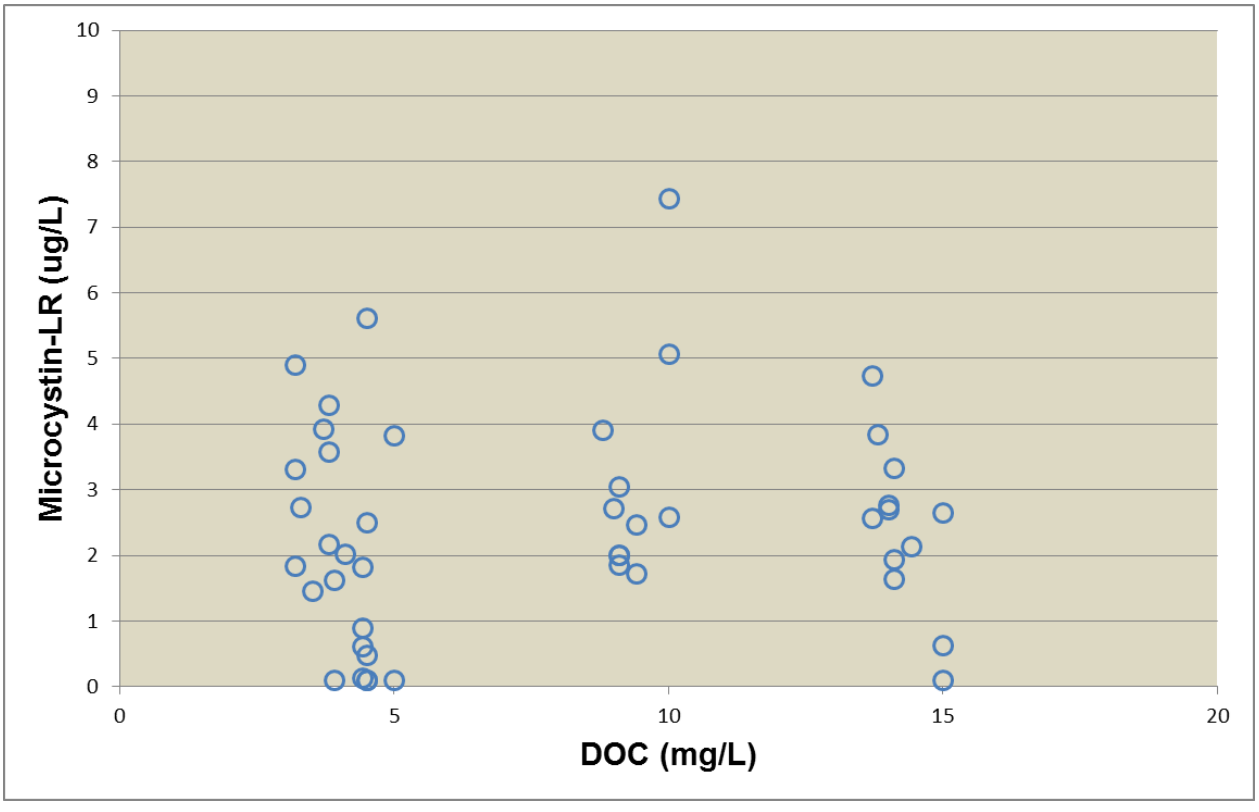

Figure 4.2: Extracellular toxin present following ozonation at 0.1 mg O_3/L (n=48)

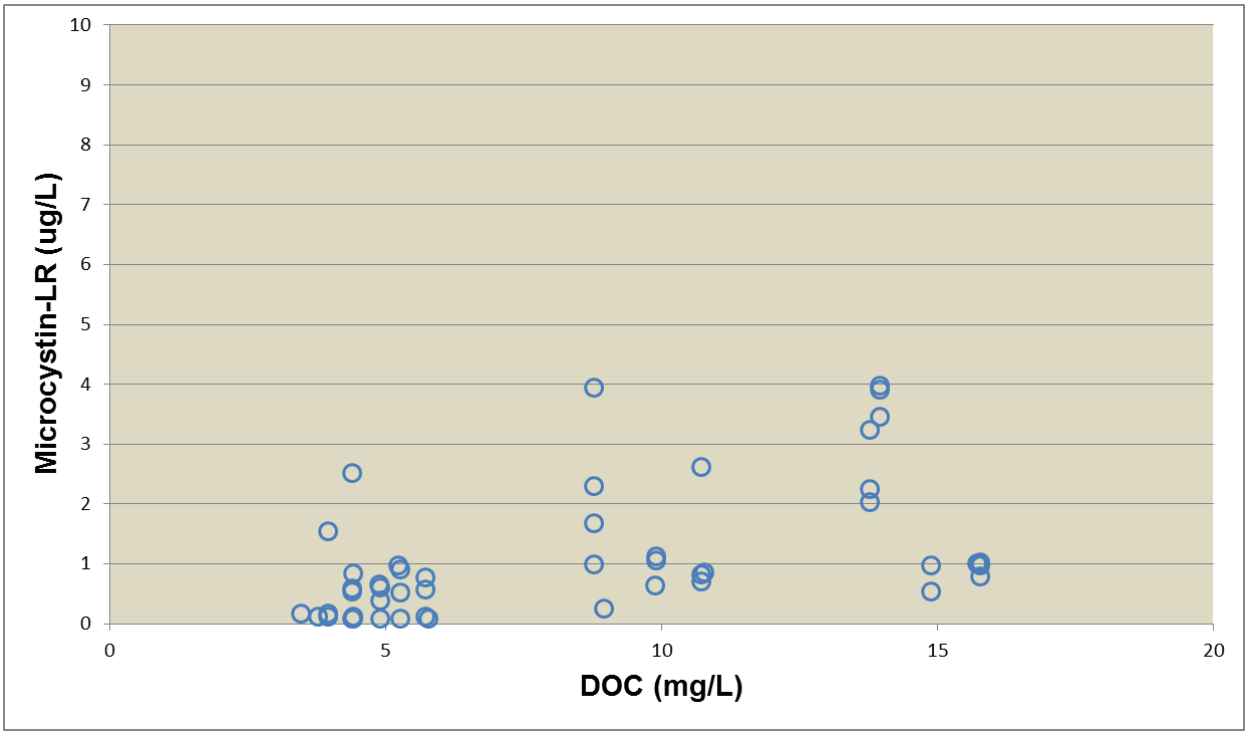

Figure 4.3: Extracellular toxin present following ozonation at 0.3 mg O_3/L (n=48)

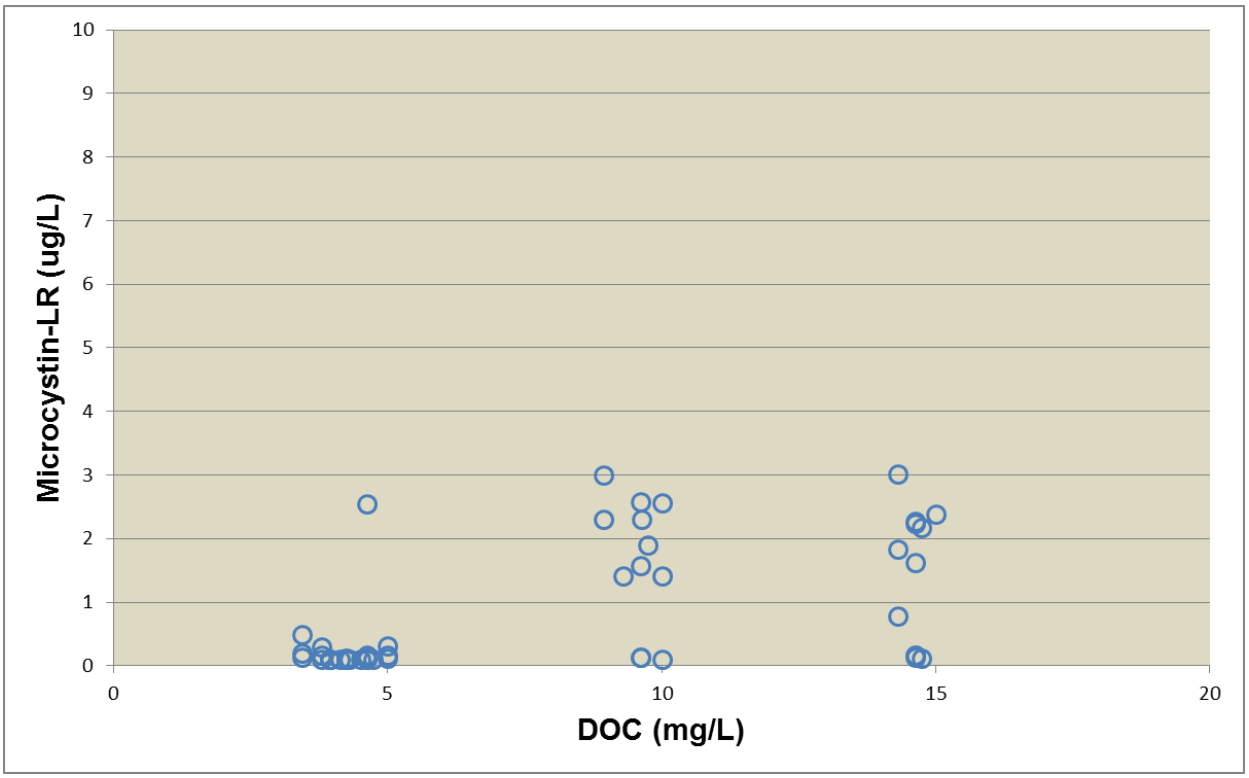

Figure 4.4: Extracellular toxin present following ozonation at 0.6 mg O_3/L (n=48)

pH was not a significant factor associated with elimination of extracellular toxin in the presence of DOC at concentrations between 5 and 15 mg/L, and ozone residuals of 0.1, 0.3, and 0.6 mg/L. This result appears contradictory to previous reports (Al Momani et al., 2007; Rositano et al., 1998) that demonstrated that lower pH resulted in greater toxin reduction. In those studies, the experiments were conducted at pH ranging from 2.0 to 9.9 (Rositano et al., 1998) and pH ranging from 2.0 to 11.0 (Al Momani et al., 2007). Notably, a narrower pH range of 7 to 8.5 was used in the present investigation to reflect the typical pH range in many treatment plants with surface water sources such as the Grand River. In contrast, the previously reported studies were conducted at much lower pH and at lower DOC concentrations. Thus, the presence of high DOC concentrations in clarified water at pHs consistent with typical surface waters appeared to monotonically, but non-linearly govern toxin elimination by ozonation, rendering pH effects on toxin reduction inconsequential. **This finding represents a novel contribution to the understanding of cyanotoxin elimination by ozonation at environmentally and operationally relevant conditions.**

Replicate water samples with ozone residual concentrations of 0.3 and 0.6 mg O_3/L that contained 5 mg/L DOC contained residual toxin concentrations with a standard deviation of ~0.25 µg microcystin-LR/L after treatment. By contrast, the standard deviations of the residual toxin concentrations measured in systems with higher DOC concentrations were 0.97 and 1.07 µg microcystin-LR/L in the systems with 10 and 15 mg/L DOC, respectively. This difference in standard deviation (which increased with increasing DOC concentration) suggests that there is an interaction effect between the DOC and ozone that affects cyanobacterial toxin elimination and that effect is not dependent solely upon DOC concentration. Alternatively, some reports have suggested that the ELISA method is susceptible to increased variability in determining toxin concentrations in the presence of natural organic matter (Amistadi et al., 1997); however, those reports are only speculative as they do not attribute a cause for such variability. Thus the present investigation demonstrates that DOC concentrations above 5 mg/L (specifically more hydrophobic DOC) somehow interfere with microcystin-LR treatment by ozonation or with quantitative analysis of microcystin-LR using the ELISA method.

Regardless of residual ozone concentration, differences in toxin concentration following ozonation were not statistically significant between DOC concentrations of 10 mg/L or 15 mg/L ($p=0.10$, $\alpha=0.05$). In contrast, the toxin concentrations remaining after ozonation in the water matrix that contained 5 mg/L DOC were significantly different from those observed in the other water matrices with higher DOC concentrations, as previously mentioned. This difference

appeared to be related to the limit of ozone availability. In the water matrix with 5 mg/L DOC, the concentration of toxin remaining after ozonation decreased when the ozone residual was increased from 0.3 mg O_3/L to 0.6 mg O_3/L; specifically, from less than 1.00 μg/L (22 of 24 samples) at 0.3 mg O_3/L to less than 0.5 μg/L (23 of 24 samples) at 0.6 mg O_3/L ($p=0.0022$, $\alpha=0.05$, including all samples). Thus, at proportionally higher ozone residuals, ozone achieved correspondingly greater toxin elimination; this result is consistent with the reported literature (Newcombe and Nicholson, 2004). Notably, however, this result was observed even in the presence of DOC. The threshold by which this relationship was maintained was at 5 mg/L DOC and ozone residuals of 0.3 mg O_3/L or greater, as shown in Figures 4.3 and 4.4. Beyond 5 mg/L DOC, ozone still eliminates toxin, but there is not a significant effect between ozone residual and toxin reduction, such that increasing ozone residual concentrations do not correspond to greater toxin elimination ($p=0.79$, $\alpha=0.05$). Thus, it appears that at higher system DOC concentrations, ozonation at the residual ozone concentrations typically applied in water treatment plants is insufficient for extracellular toxin elimination and much greater concentrations of ozone are required to achieve a reduction in toxin concentrations to below regulatory limits. **This result and the associated implication to practice have not been previously reported.**

4.2 Experiment using *Microcystis aeruginosa* cells to measure intercellular and extracellular toxin concentrations

4.2.1 Toxin destroyed using cell cultures compared to using extracellular toxin

Only ~43% of the microcystin-LR that was destroyed by ozone when in extracellular form was destroyed when *Microcystis aeruginosa* cells were present in the system at the same experimental conditions. **This is a significant new finding that emphasizes the need for caution when relying on ozonation for drinking water treatment during periods of elevated cyanobacterial risk to water supplies**. Specifically, the mean concentration of toxin remaining following ozonation of the *Microcystis* cells was 5.49 μg/L (+/- 1.28), whereas the mean concentration of toxin remaining following ozonation of the extracted toxin was 1.52 μg/L (+/- 1.22). This demonstrates that a significant oxidative capacity is required to lyse the cells before the ozone can destroy the intercellular toxin. Thus, the operational requirements of cyanobacterial toxin destruction processes such as ozonation are greater in the presence of cells, as compared to toxin in the water matrix. **While this result would logically be expected and has been indirectly suggested by previous investigations (Fan et al., 2014; Fan et al.,**

2013; Miao and Tao, 2009), the present research has directly demonstrated and quantified it.

Figure 4.5 illustrates the decrease in microcystin-LR destroyed in the water matrix following ozonation of cultured *Microcystis aeruginosa* cells as DOC concentrations in the system were increased. The microcystin-LR concentration is shown relative to DOC concentration in the water matrix at the residual ozone concentrations (0.3 and 0.6 mg O_3/L) and pHs (8.0, 8.5).

The concentration of microcystin-LR destroyed following ozonation of *Microcystis* cells as a percentage of destroyed toxin, as shown in Figure 4.5, ranges from less than 18% to 34%. Moreover, the remaining toxin concentrations exceeded 1.5 µg/L (ODWQS) in all cases. In contrast, greater than 60% and 70% of the initial extracellular toxin concentration at 0.3 mg O_3/L and 0.6 mg O_3/L, respectively was destroyed (Figures 4.3 and 4.4, Section 4.1).

It should be noted that the initial toxin concentration was slightly higher in the experiment described in Section 4.1 (10 µg/L extracellular toxin vs. 6.1 and 8.6 µg/L toxin contained in cells on average). It is important to note that the cells used in the experiments were healthy and abundant (2.0 - 4.3 x 10^7 cells/mL). It can be speculated that the cells in a drinking water treatment facility may not be as robust or numerous after chemical treatment with coagulation, flocculation, and sedimentation. However, cells entering treatment processes during bloom events have challenged and disrupted coagulation and filtration processes, thereby increasing treatment demands on downstream processes, such as ozonation (Zamyadi et al., 2012). Accordingly, the state and condition of the *Microcystis* cells utilized in the present investigation may reasonably be similar to cells that may pass into in a drinking water treatment facility's post-clarification ozone contact chamber.

The results presented herein are consistent with the general understanding of toxin destruction by ozone when toxin is entirely contained within cyanobacterial cells (Fan et al., 2013; Miao and Tao, 2009), as was the case for the cell cultures used in the present experiments. The oxidative energy required to cause sufficient physical damage to the cell membrane that would release toxin and cell contents into the water matrix, reduces the available ozone for interaction with (destruction of) the toxin itself (Coral et al., 2013).

The difference in ozone efficacy between the two sets of experiments, as measured by the toxin destroyed, is substantial and underlies the fact that toxin elimination in an ozone treatment process environment is a two-step process of cell lysis followed by toxin destruction.

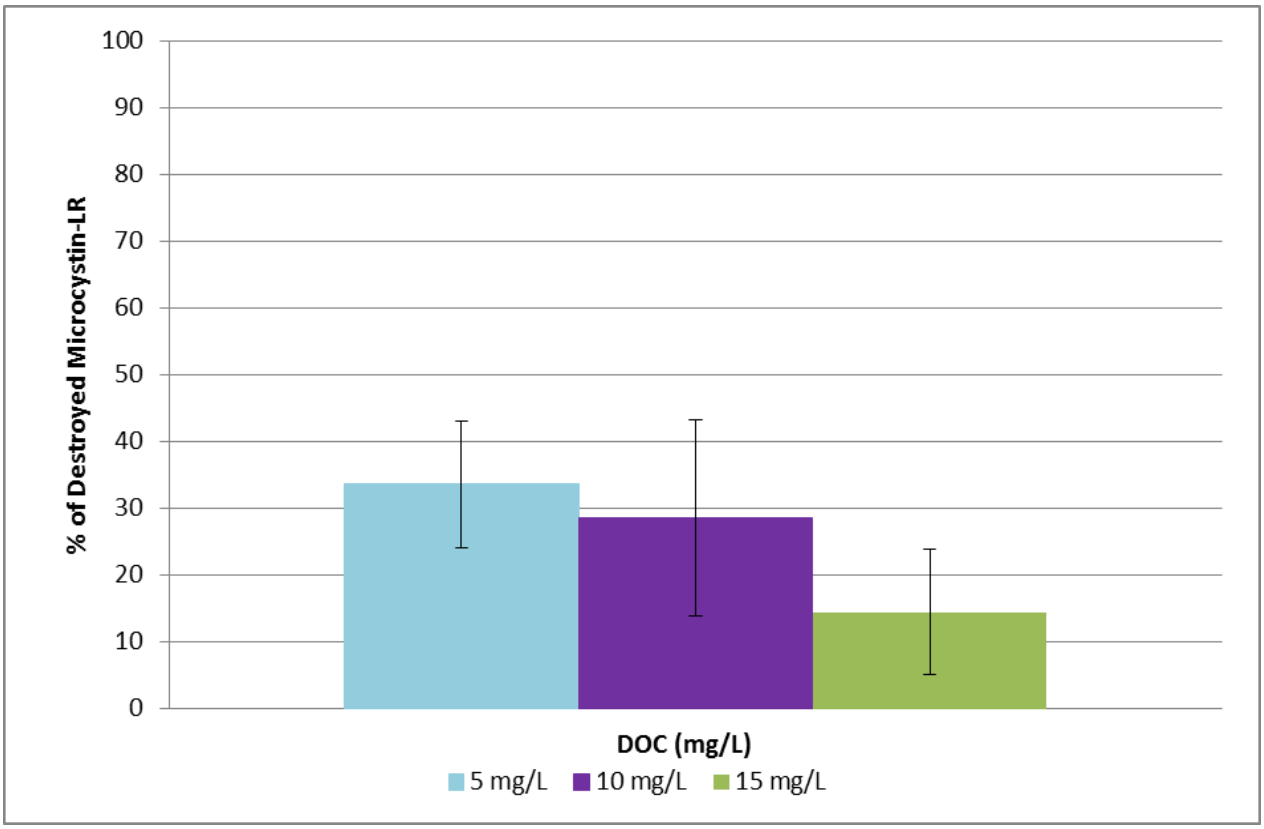

Figure 4.5: Microcystin-LR destruction (mean +/- standard deviation) following ozonation at various DOC concentrations in the water matrix (n=12 at 5 mg/L, n=11 at 10 mg/L, n=9 at 15 mg/L)

4.2.2 Extracellular vs. Intercellular Toxin Destruction

Distinguishing between the forms of remaining toxin in solution following ozonation provides insight into the proportion of intact cells. As the concentrations of DOC in the water matrix increased in the present investigation, the proportion live cells present (as measured by intercellular toxin concentrations) following ozonation also increased. Therefore, not only did DOC decrease the efficacy of ozone in destroying toxin, it also decreased the oxidative capacity of ozone in lysing cells; thus the rate of reaction was not directly proportional to the concentration of DOC.

The mean toxin concentration distribution between the extracellular (existing in the water matrix) and intercellular (contained within the cells) forms following ozonation of *Microcystis aeruginosa* cells is presented in Table 4.1. The microcystin-LR concentration shown relative to DOC concentration in the water matrix is at all the residual ozone concentrations (0.3, and 0.6 mg O_3/L) and pHs (8.0, 8.5) investigated.

The data demonstrate an increase in total toxin remaining at higher DOC concentrations, as illustrated in Figure 4.5. The increasing proportion of the average intercellular toxin and the corresponding decreasing proportion of the average extracellular toxin are demonstrated in Figures 4.6, 4.7, and 4.8. The concentration of intercellular toxin remaining after ozonation exceeded the concentration of extracellular toxin at a threshold DOC concentration of 9 mg/L DOC.

Table 4.1: Remaining microcystin concentration following ozonation of water containing *Microcystis aeruginosa* cells, expressed as a percentage of initial concentration (n=12 at 5 mg/L, n=11 at 10 mg/L, n=9 at 15 mg/L)

DOC (mg/L)	Mean total toxin (%)	Mean extracellular toxin (%)	Mean intercellular toxin (%)
5	66.4	51.4	15.0
10	71.4	31.8	39.6
15	85.5	17.8	67.7
p value (α=0.05)	0.00096 (between 5 and 15 mg/L)	0.00001 (between 5 and 15 mg/L)	Calculated value

Increased DOC concentrations in the water matrix both decreased the efficacy of toxin destruction by ozonation and shifted the form of toxin (i.e. extracellular vs. intercellular). In systems with 5 mg/L DOC, a third of the initial toxin was destroyed following ozonation and most of the remaining toxin was in the extracellular form. As all the initial toxin was contained within the cells, ozone lysed ~85% of the cells (15% of toxin remained within the cells) (Figure 4.6). With each 5 mg/L increment of additional DOC added to the water matrix, a non-linear proportional reduction in lysed cells was observed. The percentage of lysed cells decreased from 85% to 60% to 32% at 5, 10, and 15 mg/L DOC, respectively, as shown in Figures 4.6, 4.7 and 4.8. This result demonstrates that the effect of increased DOC concentrations decreased ozone efficacy in destroying toxin, and non-linearly increased the oxidative requirements necessary for achieving cell lysis relative to the DOC concentration present in the system. **Thus, this result provides important new insight into the design and application of ozone systems for mitigating cyanobacterial toxin passage into treated drinking water.**

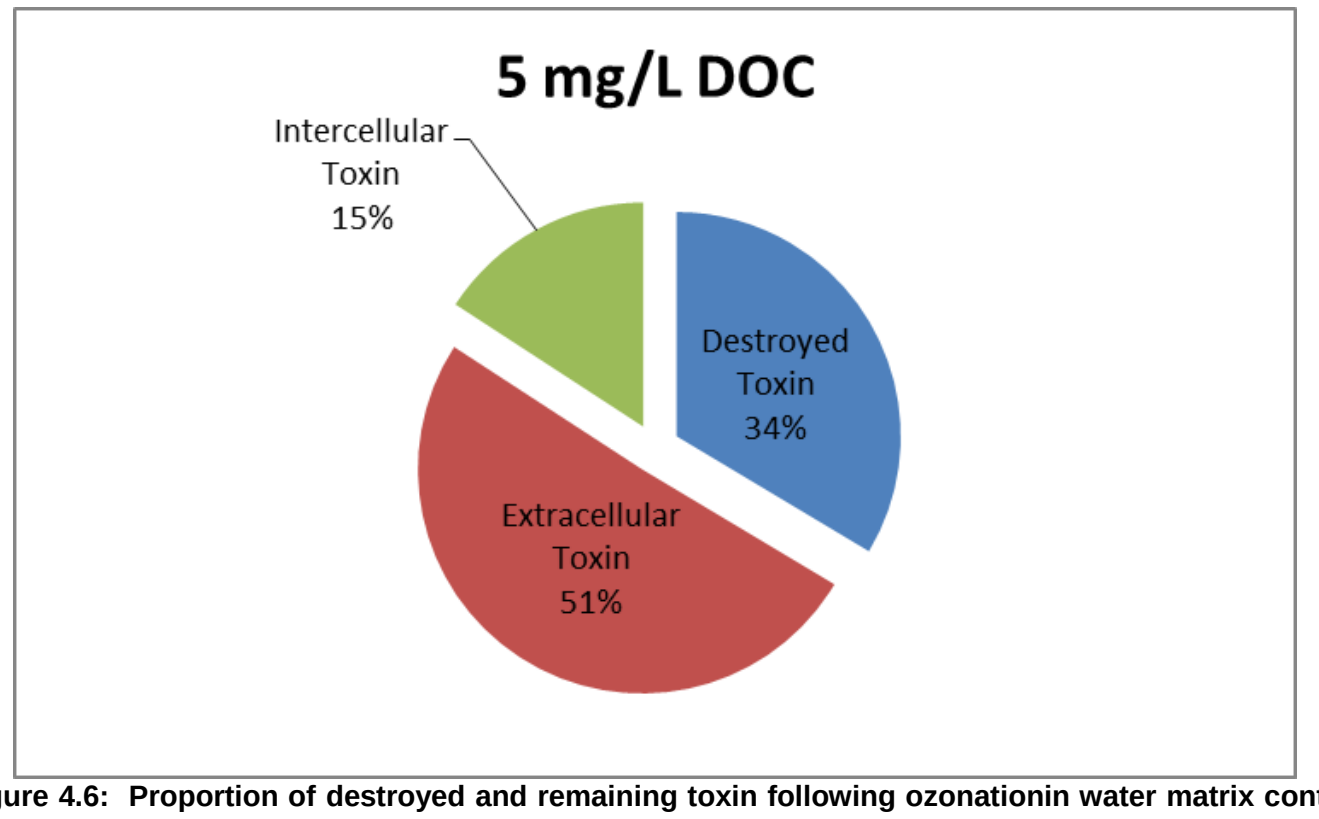

Figure 4.6: Proportion of destroyed and remaining toxin following ozonationin water matrix containing 5 mg/L DOC

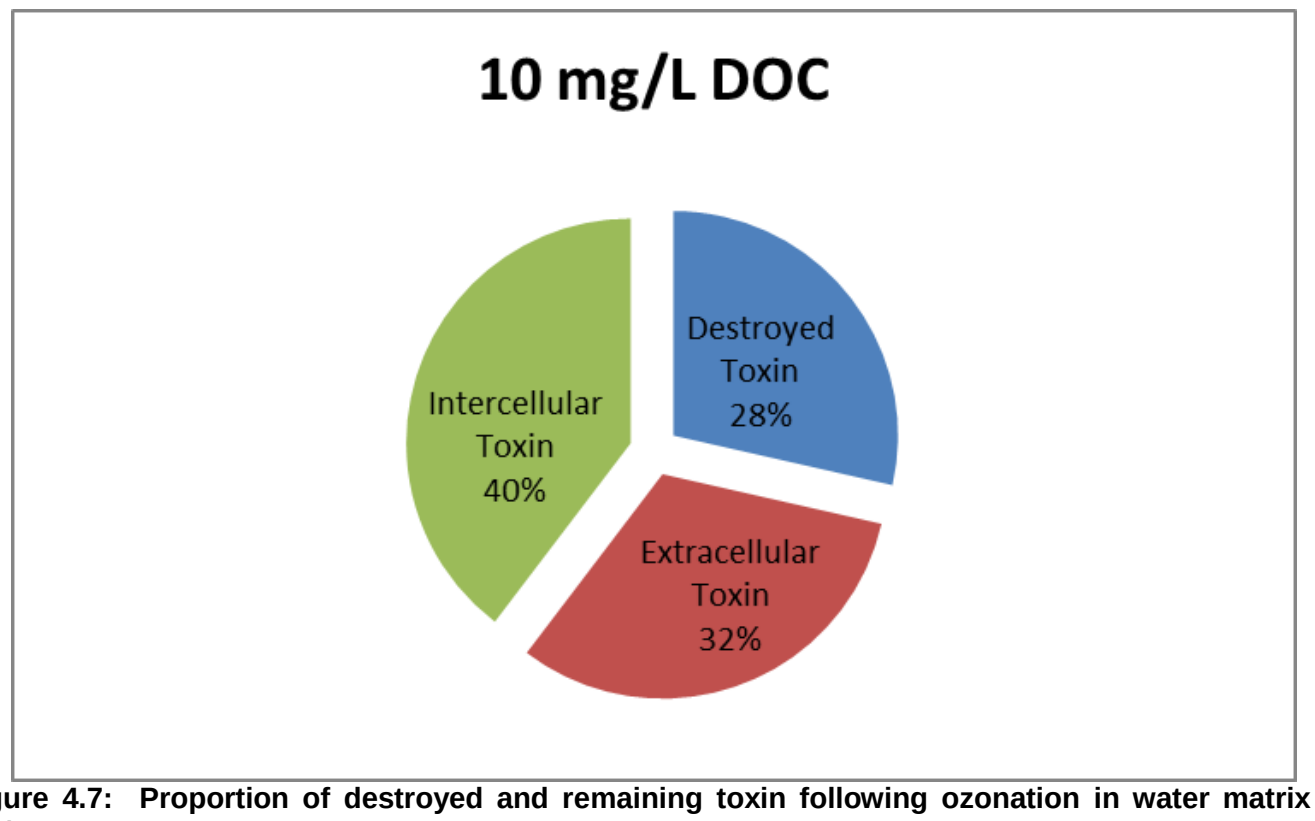

Figure 4.7: Proportion of destroyed and remaining toxin following ozonation in water matrix containing 10 mg/L DOC

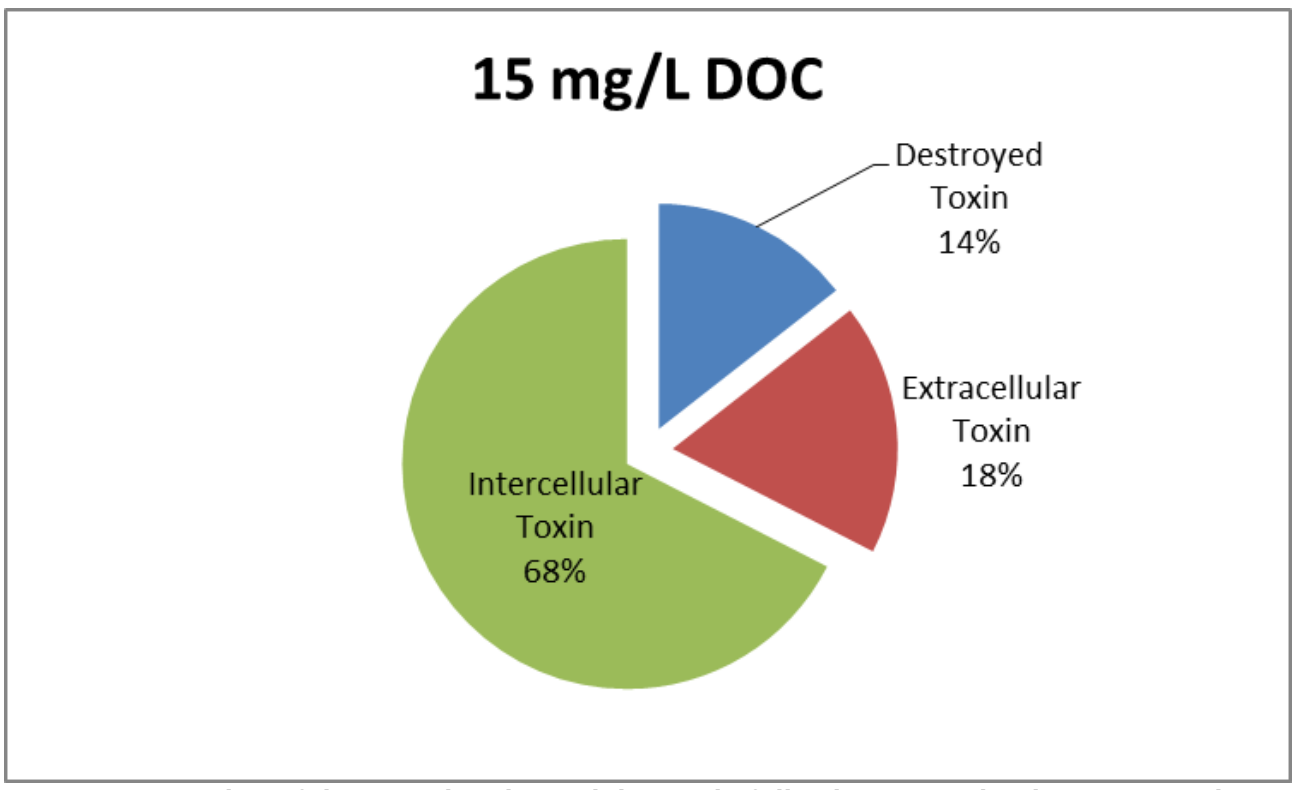

Figure 4.8: Proportion of destroyed and remaining toxin following ozonation in water matrix containing 15 mg/L DOC

Figure 4.9 illustrates the relationship between the total toxin remaining following ozonation at the three DOC concentrations investigated and the portion of that toxin in the extracellular form. Each set of system conditions was evaluated in triplicate and all of the raw data are presented. Figure 4.10 illustrates the relationship between total toxin and intercellular toxin for the same set of system conditions.

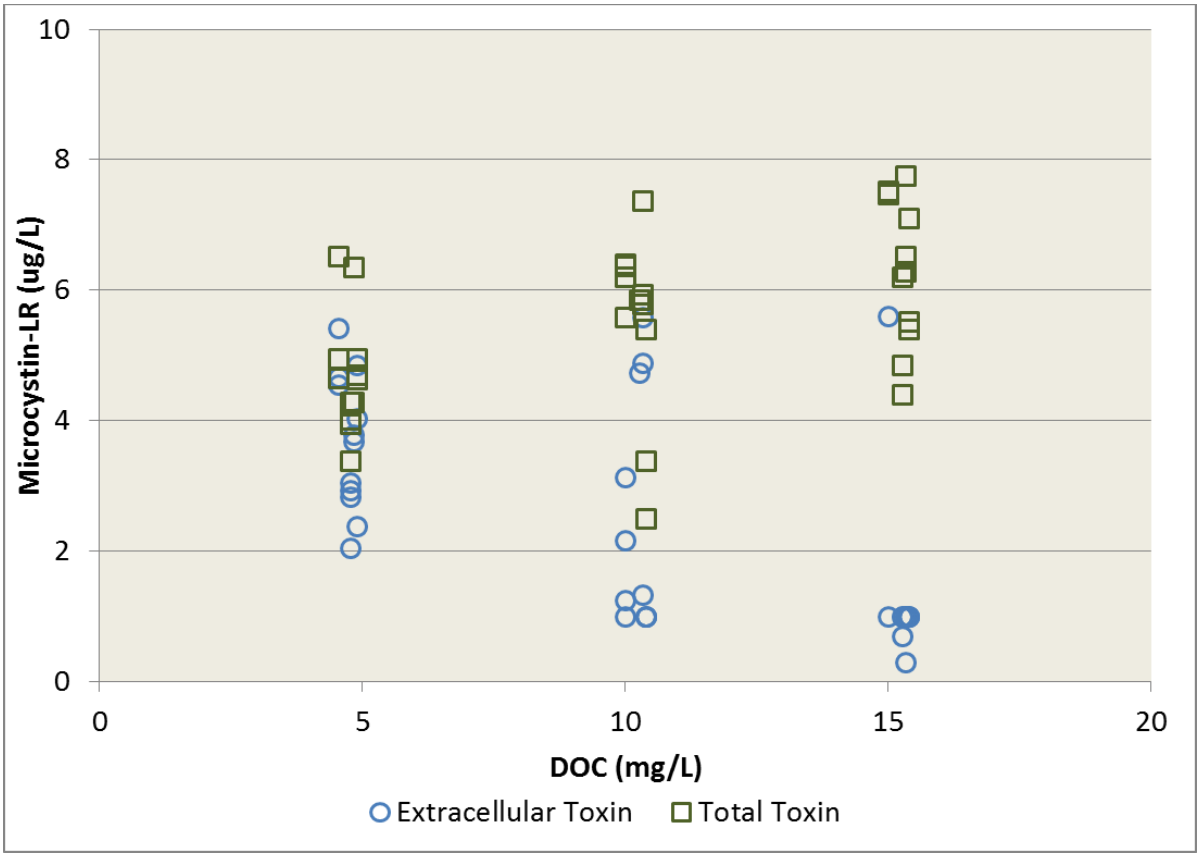

Figure 4.9: Total and extracellular toxin present following ozonation for all ozone residual concentrations as a function of DOC concentration

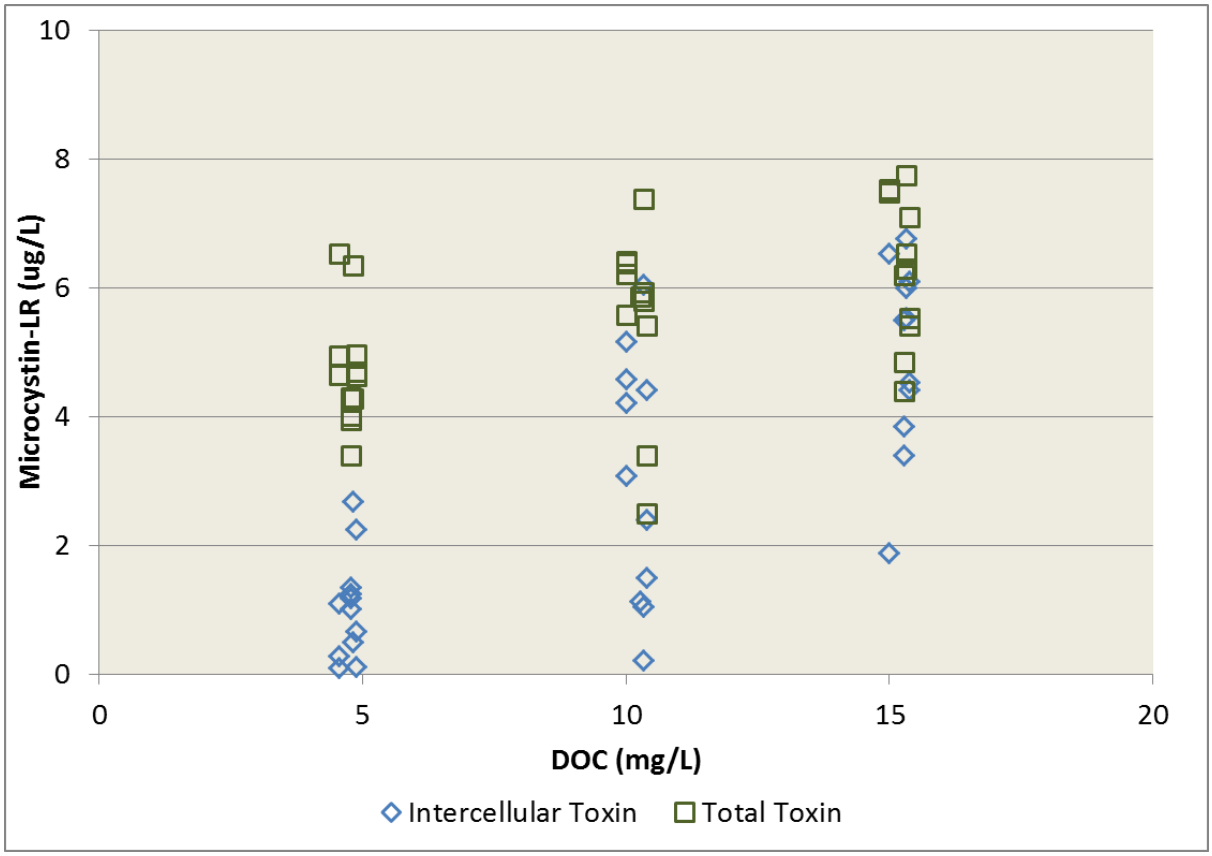

Figure 4.10: Total and intercellular toxin present following ozonation for all ozone residual concentrations as a function of DOC concentration

The variability in the data in Figures 4.9 and 4.10 can be attributed to a number of factors. The consistency in initial toxin concentration in the cell cultures cannot be easily controlled; here, it was either 6.1 or 8.6 µg/L. Thus, the absolute value of the remaining toxin concentrations may differ even if the proportion destroyed was similar. Also, the toxin concentration in the cells was assumed to be uniformly distributed. While this is a reasonable assumption for the purposes of assessing the concentrations of toxin at various experimental conditions, it is likely that there is natural variability in toxin concentration across the individual cells within a population. Further, the data presented here are aggregated across all experimental conditions (i.e., ozone residual concentrations and pHs) for each DOC concentration to demonstrate the consistency of the observed trends. Ozone concentration was not a critical factor in the proportion of total, extracellular, and intercellular toxin destroyed at the DOC concentrations investigated. This presentation of the data demonstrates that the form of toxin (intercellular vs. extracellular) present after treatment

appeared to be governed by the initial DOC concentration in the system, likely due to the significant oxidant/ozone demand of DOC when present at high aqueous (~10 mg/L) concentrations. Similar to the result from the experiments using extracellular toxin only (Section 4.1), pH did not a significantly affect toxin destruction by ozonation at the conditions investigated. Notably, despite the variability in the data, there was a significant increase in the total toxin concentration remaining following treatment by ozonation between water matrices with 5 mg/L DOC and those with 15 mg/L DOC. There was also a significant difference in the form of the remaining toxin (extracellular vs. intercellular) between water matrices with 5 mg/L DOC and those with 15 mg/L DOC.

4.3 Cell Viability Experiment

In the absence of DOC, a mean of 93% of cells were lysed at cell concentrations between 100 and 100 000 cells/mL, which when considered in conjunction with the results from Section 4.2 provides a baseline for the cell lysis rate attributable to ozonation in the presence of DOC. Fluorescence analysis enabled the observation that some cells were not entirely intact after ozonation; thus they were described as Damaged and Potentially Viable (DAPV) cells. These cells were present at initial ozone residuals less than 0.45 mg O_3/L, suggesting that incomplete oxidation occurs at lower ozone residual concentrations. These cells may have the potential to reproduce; accordingly, this possibility warrants further investigation and represents an important follow up to the present investigation.

The fraction of *Microcystis aeruginosa* cells remaining in the water matrix following ozonation is shown in Figure 4.11. The initial cell concentrations were based on Water Quality Research Australia (WQRA) Alert levels. Level One Alert is between 2000 and 6500 cells/mL, Level Two Alert is greater than 6500 cells/mL and Level Three Alert at greater than 65 000 cells/mL (Newcombe et al., 2009). Although these levels may seem low as compared to traditional bloom event concentrations, cyanobacteria are generally present in surface water and thus in treatment processes. Their behaviour at these concentrations is important to understanding drinking water treatment process efficacy. Thus, the efficacy of ozone in destroying microcystin-LR and lysing cells at 100 to 100 000 cells/mL in the absence of DOC provided a baseline for describing the relationship between ozone, DOC, and toxin-containing *Microcystis* cells.

With the exception of three trials, the cells remaining following ozonation at both 0.3 and 0.6 mg O_3/L represented less than 10% of the initial cell concentration. The mean lysis rate was 93% for all trials. There was no significant correlation between the initial cell concentration and the lysis rate ($p=0.15$, $\alpha=0.05$). Of the three trials in which the cell concentration remaining following ozonation was above 10%, the cell concentrations were 14.6%, 37.5%, and 57.4%. Factors that could contribute to these higher remaining cell concentrations after treatment include a higher initial cell concentration of the cell culture than was calculated, obtaining the cell volume from a high density portion of the cell culture resulting in an initial cell concentration that was higher than calculated, and the ozone not interacting with the cells to the same extent as in other trials.

The calculated total microcystin-LR concentration of the cells added to the ozonated water ranged between <0.01 µg/L (100 cells/mL) to >5.00 µg/L (100 000 cells/mL). The microcystin-LR concentration was calculated based on the measured toxin and cell concentration of the cell culture.

All toxin concentrations following ozonation were non-detect (ND at 1.0 µg/L) or below the detection limit (BDL) with the exception of one (1) sample that contained microcystin-LR at 1.94 µg/L. The initial cell concentration of the sample that contained microcystin-LR was 100 000 cells/mL and the cell concentration following ozonation was 7800 cells/mL. Although there was no correlation between the cell concentration following ozonation and the toxin concentration measured, the sample containing toxin was at a concentration above the ODWQS and at a cell concentration above Alert Level 2. Thus, even cell concentrations at much lower concentrations than bloom events can contain high levels of toxin that are not eliminated by ozone to below regulatory levels.

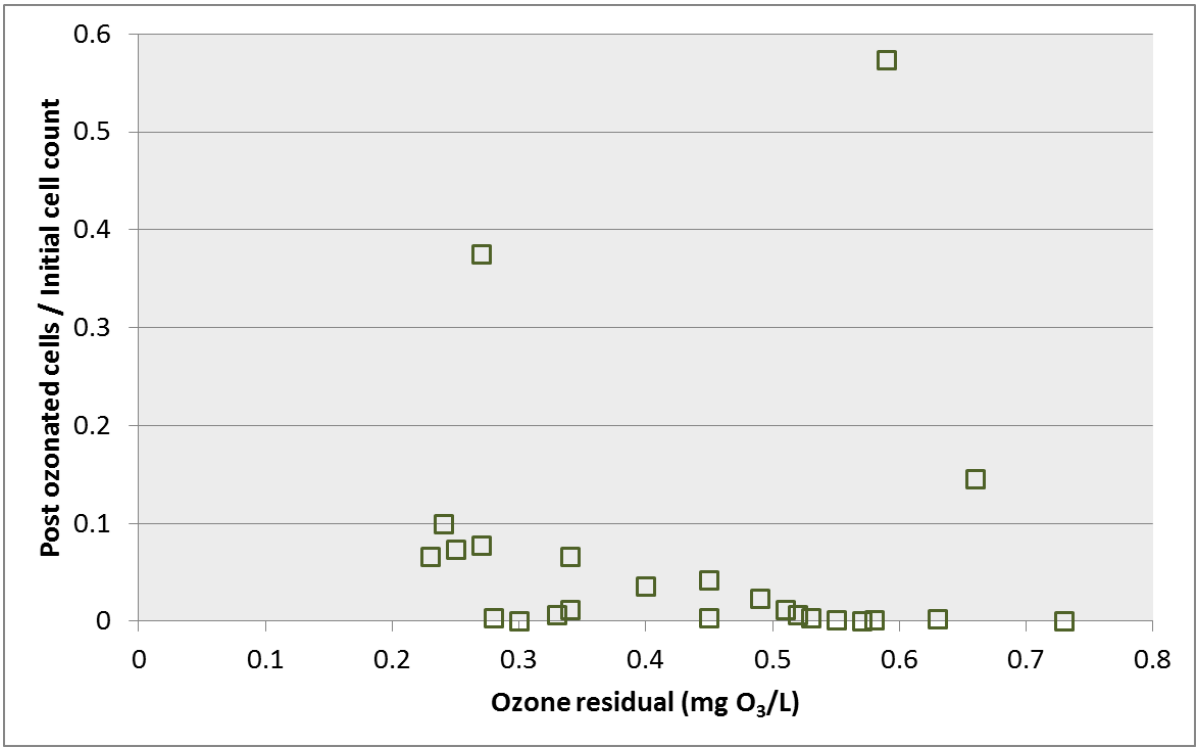

Figure 4.11: Cell lysis by ozone according to ozone residual concentration (n = 24)

These experiments conducted in the absence of DOC can be considered in conjunction with the results from Section 4.2. A relationship between cell lysis by ozone and DOC concentration in the water matrix also can be determined based on this work. This relationship is shown in Figure 4.12. There is a slight increase in the mean remaining cells following ozonation from 7% of cells remaining with no DOC to 15% of cells remaining with DOC at a concentration of 5 mg/L. The slope of the curve increases as DOC increases to 10 mg/L and the percentage of cells remaining increases to 40%. The slope of the curve is similar from 10 to 15 mg/L DOC as the percentage of cells remaining increases to 68%.

This demonstrates the oxidative capacity of ozone relative to the scavenging capability of DOC and the associated effect on cell lysis. As noted in Section 4.2, the determination of the proportion of toxin remaining in the cell following ozonation relative to DOC concentration provides an understanding of the limitations of ozone to affect the destruction of toxin. It also demonstrates that the concentration of ozone necessary to achieve toxin elimination is dependent upon 'overcoming' the DOC concentration present in the water matrix.

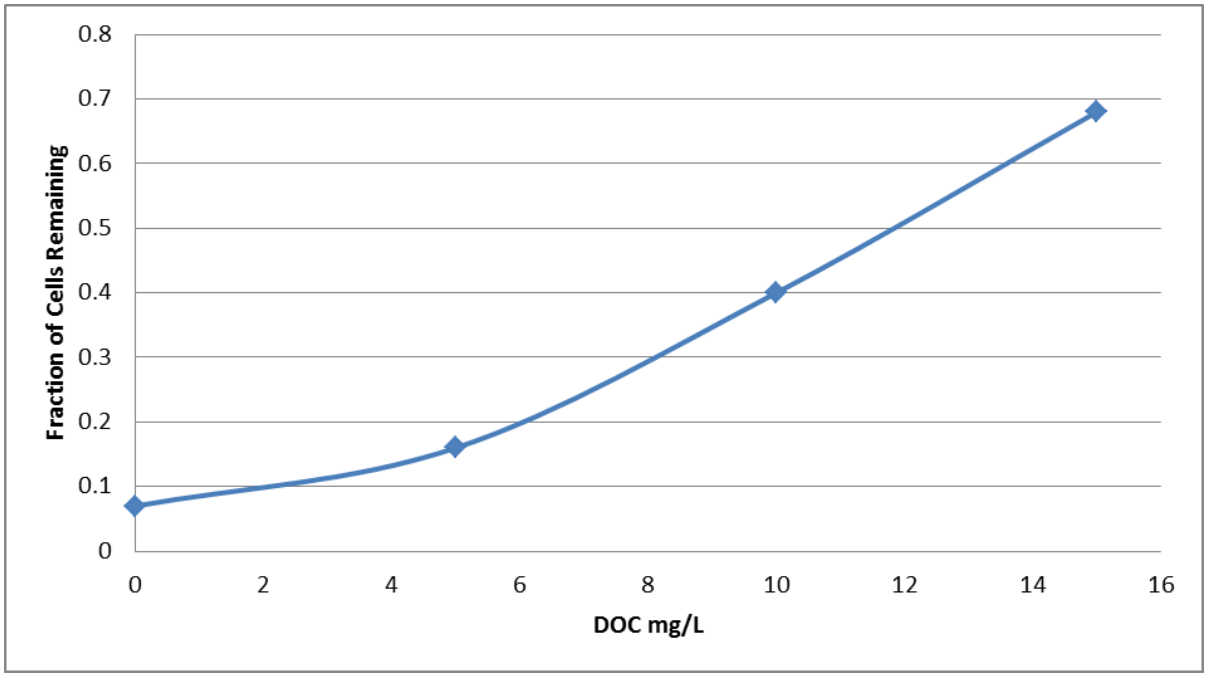

Figure 4.12: Cell lysis by ozone according to DOC present in water matrix at all ozone residuals investigated
(points are connected only to improve visualization of results)

The fluorescence method used to detect and count live *Microcystis* cells pre- and post-ozonation also enabled visualization of cells that were not similar to untreated *Microcystis* sp. Prior to ozonation, cells appeared to be smooth-surfaced, and spherical with a distinctive cell wall. Pictures of these cells are shown in Figures 4.13 and 4.15. Following ozonation, the cells were dimpled and appeared shrunken. Similar alteration in cell structure following ozonation was observed by Coral et al. (2013) and Miao and Tao (2009), including the release of cytoplasm from the cells. However, in this investigation, the pigment within the cells did fluoresce in the same manner as the pre-ozonated cells and the cell walls were visible. These cells did not appear to have a defined cell wall and the pigment did not always appear to be contained by a cell. The cells also appeared flattened or non-spherical. Often they were grouped together in clusters such that differentiating individual cells was difficult. Nonetheless, these cells fluoresced, which indicates the presence of pigmented cell matter, common to intact, live cyanobacterial cells. Pictures of these cells are shown on Figures 4.14 and 4.16.

These misshapen, DAPV cells clearly had suffered some damage due to ozonation, but it remains unclear whether they were damaged beyond an ability to reproduce or if their fluorescence indicated that their cell contents were intact and therefore the cells remained viable following ozonation.

The DAPV cells were enumerated in addition to the intact cells post-ozonation. The fractions of DAPV cells are shown as a function of ozone residual in Figure 4.17. Similar to the fraction of intact cells following ozonation, the fraction of DAPV cells was less than 10% of the initial cell count with the exception of two trials. In three trials the fraction of DAPV cells was greater than the fraction of intact cells remaining following ozonation. Thus, for some trials, the number of fluorescing cells approached 20% of the initial cell count. The DAPV cells were present at ozone residuals of 0.45 mg O_3/L and less, which reasonably suggests that incomplete cell lysis occurred at conditions with less oxidation.

The presence of DAPV cells adds to the complexity of cell lysis and toxin destruction by ozone as there is the potential for greater numbers of viable cells than originally estimated by solely accounting for traditionally intact cells. It is also unclear as to whether the toxin is contained within the cell or due to its damage, some or all of the toxin was released. One of the most important considerations is the reproducibility of these cells. Should these cells be able to reproduce, then the means by which we detect live cells needs to include the ability to determine potential viability. The fluorescence method does achieve this via the detection of pigmentation, however more work on the relationship between pigment and toxin release would be required to determine the significance of these cells to ozone treatment efficacy.

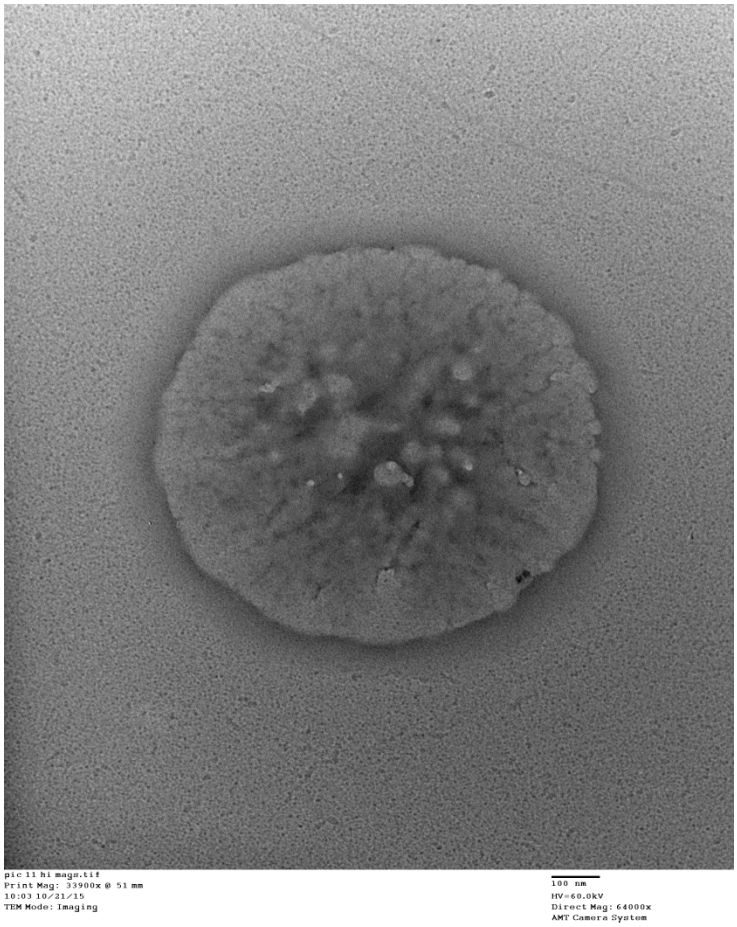

Figure 4.13: *Microcystis aeruginosa* cell

(Philips CM10 Transmission Electron Microscope (TEM) at 64 000x magnification)

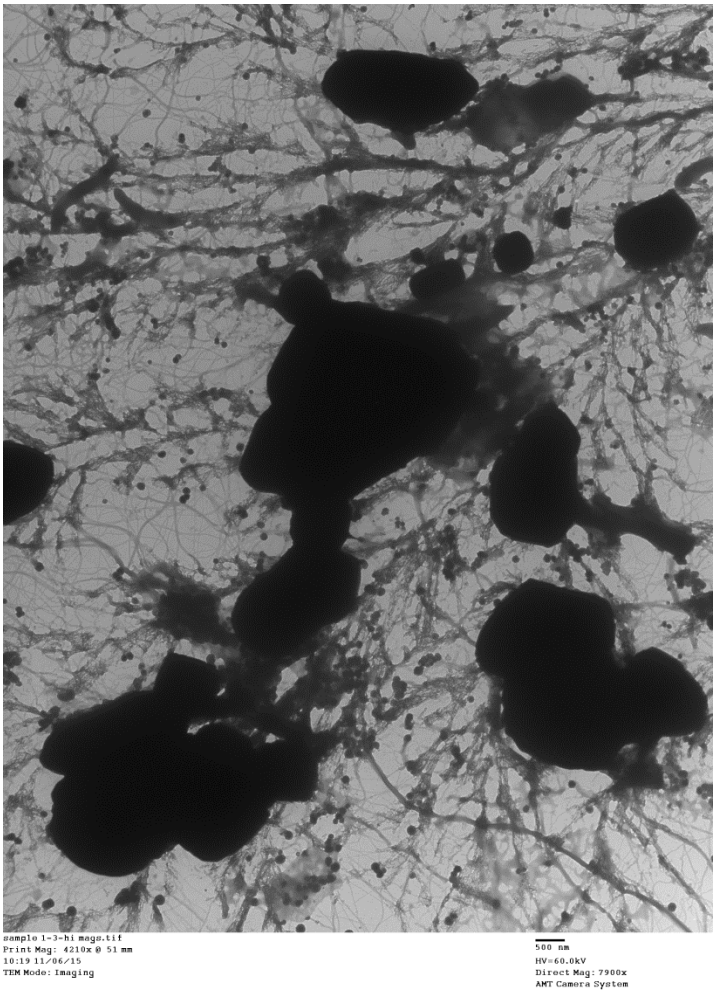

Figure 4.14: Ozonated *Microcystis aeruginosa* cells (Philips CM10 TEM at 7900 x magnification)

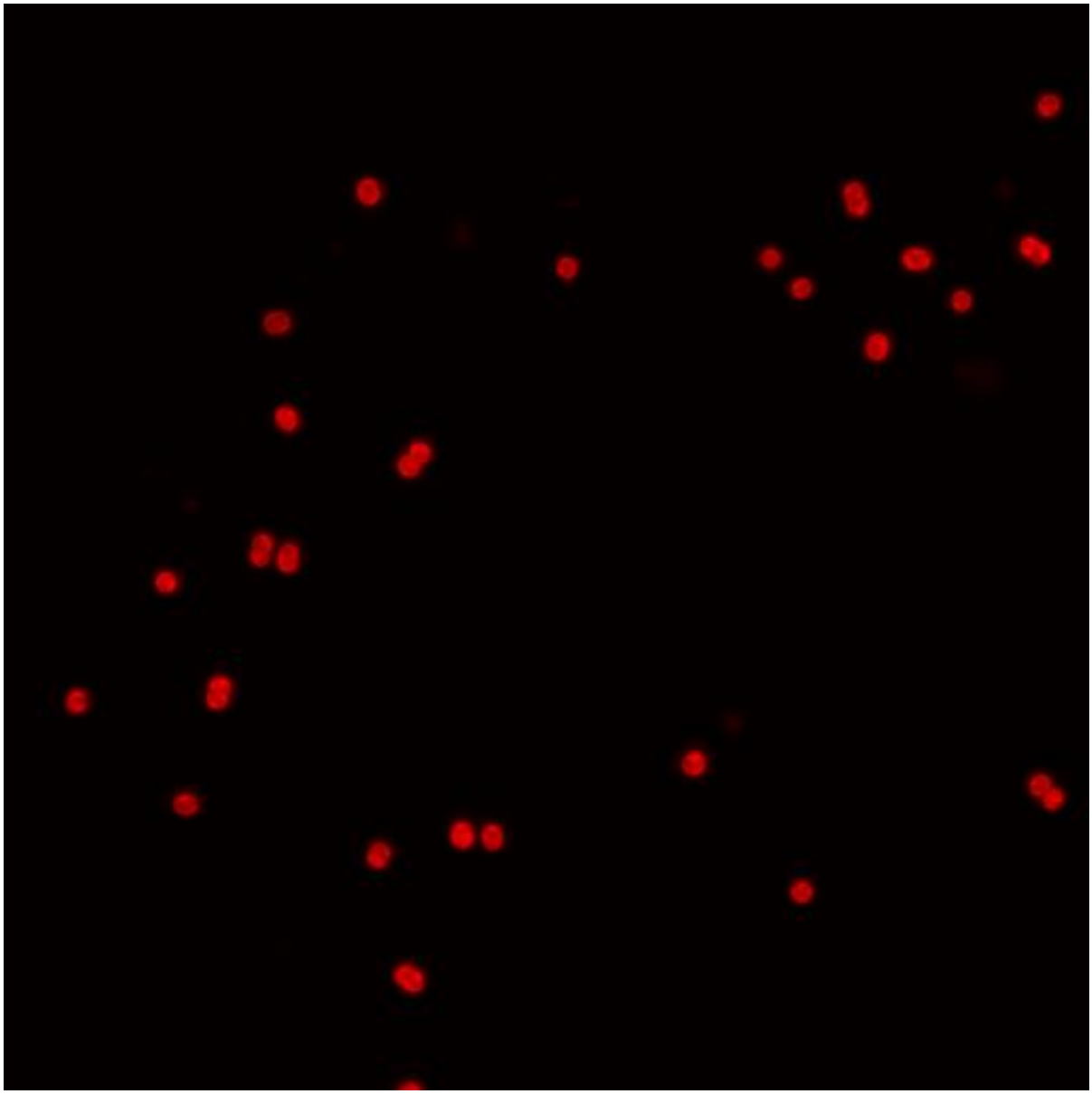

Figure 4.15: *Microcystis aeruginosa* cells

(Zeiss LSM 510 Meta Confocal Microscope and FluoArx UV lamp at 63x magnification, at 546/590 nm excitation/emission wavelength)

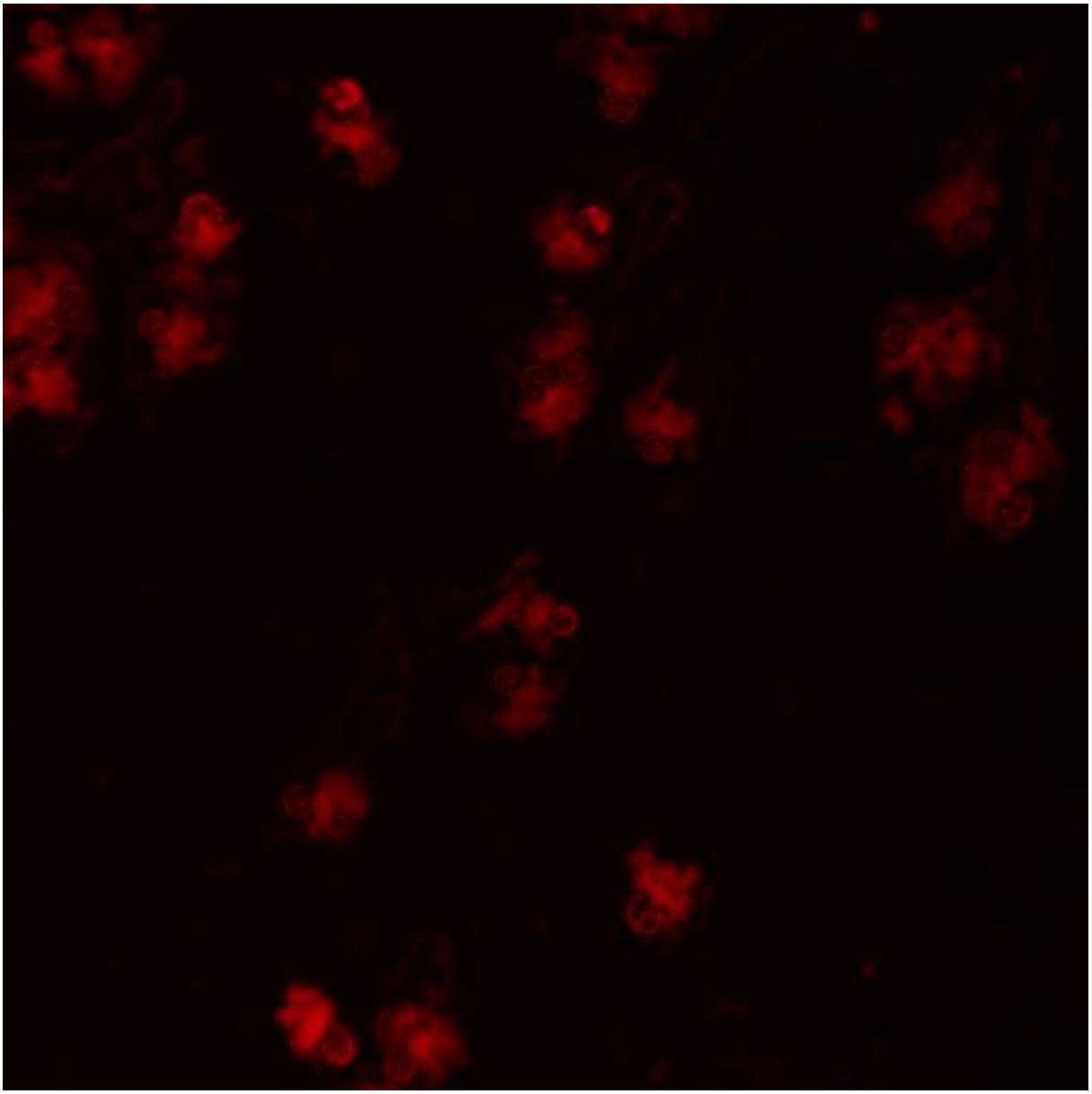

Figure 4.16: Ozonated *Microcystis aeruginosa* cells

(Zeiss LSM 510 Meta Confocal Microscope and FluoArx UV lamp at 63x magnification, at 546/590 nm excitation/emission wavelength)

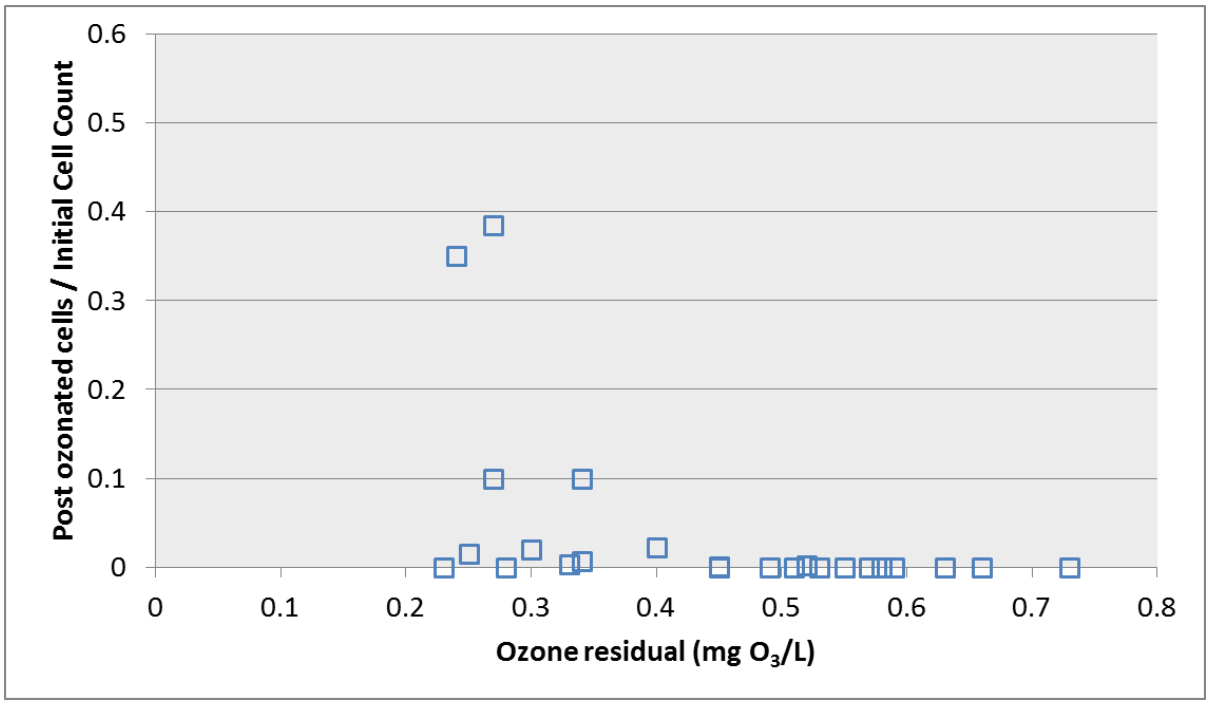

Figure 4.17: Presence of DAPV *Microcystis aeruginosa* cells relative to ozone residual

Chapter 5
Conclusions and Implications

The overall conclusions and implications from this work are as follows:

1. Ozonation can effectively destroy microcystin-LR, regardless of whether it is in extracted (i.e. extracellular) or intercellular forms.

   This was demonstrated using extracellular toxin at DOC concentrations of 5 mg/L and below, and ozone residual concentrations at 0.3 mg/L or higher. It was also demonstrated by the lack of toxin detection (at a detection limit of 1.0 µg/L) for all but one sample in the experiment on cell viability at cell concentrations between 100 and 100 000 cells/mL. These findings are consistent with the reported literature (Al Momani et al., 2008; Hoeger et al., 2002; Rositano et al, 1998; Rositano et al., 2001).

2. Proportionally, ~43% less microcystin-LR is destroyed by ozonation when present in *Microcystis* sp. cells than when present in extracted form. The novel contribution of this work is that this relationship was demonstrated through replication of identical experiments using toxin in extracted and cellular forms. The relationship was also maintained when all other relevant factors including initial ozone residual, DOC concentration, and water matrix, were the same.

   This result is consistent with the reported literature on the mechanics of oxidative cell disruption, which demonstrates that ozone lyses cyanobacterial cells and then destroys toxins (Coral et al., 2013; Korak et al., 2015). The controlled nature of the present investigation clearly underscores that effective treatment requires consideration of cellular matter when designing and evaluating processes such as ozonation for the purpose of cyanobacterial destruction. Notably, the evaluation of many treatment processes' toxin removal performance has historically relied on assessment of extracted toxin elimination (Wert et al., 2014; Zamyadi et al., 2012) and does not take into consideration the mechanics of cell disruption by oxidation.

3. a) This work suggests that a key DOC threshold concentration exists (~5 mg/L), above which DOC significantly precludes adequate microcystin-LR destruction by ozonation for meeting regulatory targets for treated drinking water.

   This threshold was observed at ozone residuals 0.3 mg O_3/L and 0.6 mg O_3/L during the experiment using extracellular toxin. The experiments conducted with *Microcystis aeruginosa* also demonstrate a significant difference between increasing concentrations of DOC and the efficacy of microcystin-LR destruction by ozonation. As the proportion of DOC increased, so did the concentration of toxin remaining following ozonation. This result is consistent with the reported literature, in which the presence of organic matter has been reported to act as a scavenger of ozone (Shawwa et Smith, 2001; von Gunten et al., 2003). Thus, DOC decreases the available oxidative capacity of ozone in destroying cyanotoxins.

   This work is the first to demonstrate the quantitative relationship between toxin concentration and DOC concentration at controlled ozone residual concentrations. Its critical implications are two-fold. First, it demonstrates that even at moderate DOC concentrations (>5 mg/L), sufficient toxin destruction by ozonation cannot be assured. Thus, any increase in DOC concentration in the influent water—as might occur during high precipitation events and often accompanies cyanobacterial bloom events—may require modifications to the method and/or concentration of ozone application. Alternatively, if such modifications are not possible, ozonation may be an inadequate barrier for the treatment of cyanotoxin. Second, this threshold illustrates the importance of the management of organic matter in source water (i.e. through active source water protection strategies), reservoirs, and upstream treatment processes to ozonation. For example, utilities that experience severe landscape disturbance by wildfire or hurricanes in source watersheds can expect significant increases in nutrients such as phosphorus and DOC (Emelko et al., 2016; Silins et al., 2014), which can concurrently promote cyanobacterial blooms and challenge treatment because of significantly elevated DOC concentrations, even in the highest quality source watersheds (Emelko et al., 2011). Accordingly, these concurrent climate associated changes in source water quality and challenges for conventional water treatment technologies, such as ozone, underscore

the need for drinking water utilities to increasingly weigh and balance the benefits of investment in both source water protection strategies and resilient treatment technologies.

The relationship established in this investigation provides an indication of the decreased efficacy of cyanobacteria destruction that can be expected in treatment processes when changes in influent DOC concentrations and/or toxin concentrations are experienced.

3.b) The experiments conducted with *Microcystis aeruginosa* cells demonstrated that the form of toxin, intercellular or extracellular, present in a treatment plant is important. Specifically, the presence of cells during ozonation requires a different operational strategy than ozone application for the treatment of extracellular toxin. This investigation demonstrated that the relative proportion of intercellular microcystin-LR remaining in the *Microcystis aeruginosa* cells following ozonation is greater with increasing DOC concentrations. Intercellular toxin concentrations increased from 7% to 15% to 40% to 68%, on average, with increases in DOC concentrations from 0 to 5 to 10 to 15 mg/L, respectively. The proportion of intercellular toxin remaining following ozonation exceeded that of extracellular toxin at system DOC concentrations of 9 mg/L.

The minimal effect of increases in ozone residual concentration on the efficacy of treatment was also demonstrated in these experiments. At the ozone concentrations investigated, any impact ozone may have had on the reduction of toxin was overshadowed by the DOC concentration present. As such, while increases in ozone concentrations would provide more oxidative capacity, it appears that increased ozone residual would not necessarily result in a reduction in toxin proportional to the increased DOC concentration. In other words, the relationship between DOC concentration and ozone efficacy in eliminating toxin is likely monotonic, but definitely not linear.

Higher proportions of intercellular toxin with increasing DOC may mean the potential for a higher proportion of live cells (which may possibly replicate and produce more toxin) after treatment. Thus, it is possible that ozonation may reach a limit of efficacy in treating cyanobacterial toxins.

4. The destruction of cyanobacterial cells by ozone is not just a matter of viable or inactivated, but also a matter of accounting for damaged and potentially viable (DAPV) cells. This work is the first to identify this possibility.

Although cell deformation following ozonation has previously been shown using scanning electron microscopy (SEM) (Coral et al., 2013), the use of fluorescence as a means of establishing the impact of treatment, particularly ozonation, on cyanobacteria in the present investigation has provided greater insight into the viability of ozonated cells. Here, 93% of the initial cyanobacterial cells were destroyed by ozonation (at both residual concentrations of 0.3 mg O_3/L and 0.6 mg O_3/L). More complete destruction of *Microcystis* sp. cells was achieved at higher ozone residuals, such that there were fewer damaged and potentially viable (DAPV) cells than at lower residual concentrations. Very few of these cells were observed above 0.45 mg O_3/L. This may have been expected, as ozone lyses cells through disruption of the cell wall, yet the use of fluorescence to measure the effects of ozone enabled observation and quantification of the presence of fluorescing (thus presumably viable) though clearly damaged cells. Thus, the presence of *Microcystis* sp. cells in the treatment system at the concentrations studied necessitates the application of an ozone residual of greater than 0.45 mg O_3/L to ensure a complete destruction of cells. These observations underscore the importance of identifying and assessing the significance of DAPV cells in future work. Critically, it is important to evaluate;

1) whether DAPV cells can reproduce (and thus produce more toxin) and,
2) if toxin is still present within the walls of DAPV cells.

Appendix 1

Standard Operating Procedures and Certificates of Analysis

Certificate of Analysis

Microcystin-LR

Product Number:	ALX-350-012
Lot Number:	L30057
CAS Number:	101043-37-2
Molecular Weight:	995.2
Purity:	≥95% (HPLC)
Physical Form:	Film adhered to inside of the vial.
Long Term Storage:	-20°C
Amount:	500µg
Summation Formula:	C49H74N10O12
Appearance:	Whitish.
Handling:	For maximum product recovery after thawing, centrifuge the vial before opening the cap.
Solubility:	Soluble in 100% ethanol, methanol or DMSO.
Hazard:	HIGHLY IRRITANT. MAY BE CARCINOGENIC. VERY TOXIC.
Identity determined by (NMR, IR, MS etc.):	Identity determined by MS.
Expiration Date:	1 Year Upon Receipt
Country of Origin:	UK
By:	R. Steinauer
Name:	René Steinauer
Date:	6/7/2013

6/7/2013

General Product Storage and Handling Information

Specific storage and handling information for each product is indicated on the product datasheet. **Reagents have a warranty of one year from the date of receipt, except for reagents with an expiration date indicated on the label or other supporting document, and conjugates and proteins which are warranted for six months.** Most Enzo Life Sciences products, stored under the recommended conditions, are stable for at least one year. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality of the product, but save you shipping charges by using the most economical storage conditions for an overnight shipment. Upon receipt of the product, follow the storage recommendations on the product data sheet.

Solubilization of Small Molecules
Solubility information can be found on the product datasheet. Concentrations listed are concentrations at which products have been tested for solubility by Enzo Life Sciences. If a solvent, but no concentration, is listed, the product should be soluble at typical stock concentrations. Make sure any organic solvents used are anhydrous. Unless stated otherwise, solutions can be warmed in a water bath to improve solubility. For very hydrophobic products, the addition of a carrier protein, such as BSA, to the aqueous media may also be helpful.

Using water or other aqueous media to make serial dilutions of compounds dissolved in organic solvents is not recommended. Rather, make a concentrated stock solution in an organic solvent and dilute directly to your working concentration in the appropriate aqueous medium. Some products may precipitate upon initial dilution, but warming the solution will often redissolve the precipitate. Some hydrophobic compounds can be effective in tissue culture experiments when used in suspension rather than in solution.

Solubilization, Handling and Storage of Peptides
For maximum stability, store lyophilized peptides desiccated at -20°C. If the peptide is to be weighed out, equilibrate to room temperature in a desiccator prior to opening. Adsorption of water reduces stability and affects sample weight. Changes in temperature during the defrost cycle in a frost-free freezer may reduce peptide stability. Reconstitute peptides in sterile, distilled water. A few drops of ammonium hydroxide for acidic peptides or 10% acetic acid for basic peptides will facilitate dissolution. If necessary the solution can be sonicated briefly. With extremely hydrophobic or neutral peptides, solubilize using a minimal amount of dimethylsulfoxide (DMSO) or dimethylformamide (DMF) then add water or buffer. In order to avoid serious solubility problems, fully dissolve peptides before adding buffer or saline. Use oxygen-free solutions and reducing agents with peptides containing Trp, Met or free Cys to avoid oxidation.

After reconstituting, aliquot the solution into individual tubes and freeze at -20 or -70°C. Avoid repeated freeze/defrost cycles. In solution, store peptides at pH 5-7 for maximal stability. Peptides in solution have limited stability, especially peptides containing Cys, Met, Trp, Asn and Gln. For maximum stability re-lyophilize reconstituted peptides.

Enzymes
Enzymes must be handled particularly carefully In order to retain maximal enzymatic activity. Defrost enzymes quickly in a room temperature water bath or by rubbing between fingers, then immediately store on an ice bath. Unused enzymes should be quickly refrozen by placing at -70°C. To minimize the number of freeze/thaw cycles, aliquot enzymes into separate tubes and store at -70°C.

Lipids
Lipids can be supplied either dissolved in solvent or neat (not dissolved in solvent). Lipids supplied neat are sold by weight and appear as oily liquids or waxy solids. To use, add a known volume of appropriate solvent, divide the weight of the lipid supplied by the volume of the solvent added to obtain the concentration. Aliquot as needed and store the remaining product as recommended on the datasheet.

Antibodies
Antibodies have a compact and stable secondary structure making them relatively stable proteins. Specific storage recommendations are listed on the datasheet. In general, antibodies can be stored at -20°C or -70°C. Avoid repeated freeze/defrost cycles. Aliquot undiluted antibody into smaller volumes (not less than 10 µL) prior to freezing if appropriate. Store diluted antibody at 2-4°C (do not freeze) and use within 1 month.

Species cross-reactivity and tested applications for each antibody are listed on the datasheet.

Technical Service
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techserv-int@enzolifesciences.com (outside of North & South America)

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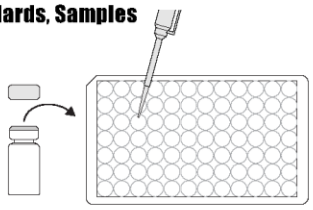
For Local Distributors please visit our Website.

Microcystin-DM ELISA Kit, Detailed Procedure

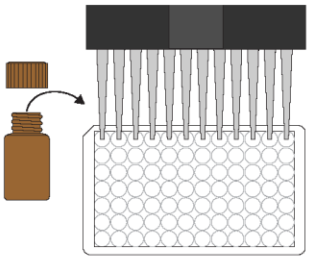
1. Addition of Standards, Samples

Add 100 µL of the standard solutions, control or samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.



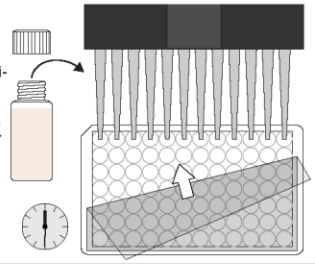
2. Addition of Enyzme Conjugate

Add 50 µL of the enzyme conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette.



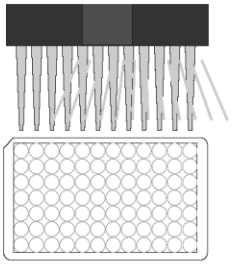
3. Addition of Antibody Solution

Add 50 µL of the Microcystin Monoclonal antibody solution to the individual wells successively using a multi-channel pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 90 min. at room temperature.



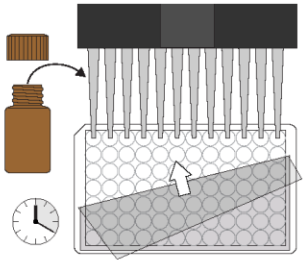
4. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips three times with a multi-channel pipette or using the diluted 1X washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



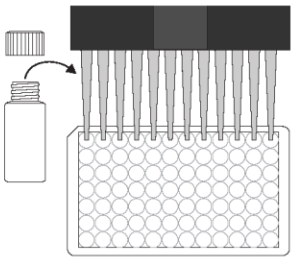
5. Addition of Substrate/Color Solution

Add 150 µL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 20 min. at room temperature.



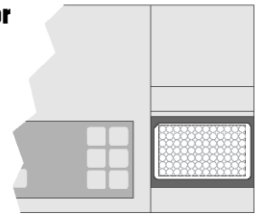
6. Addition of Stopping Solution

Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



7. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.



For Ordering or Technical Assistance Contact:
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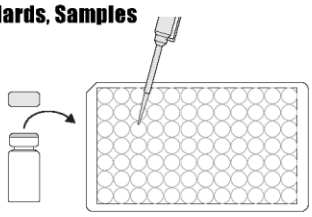
124 Railroad Drive, Warminster, PA 18974
Phone: 215-357-3911 Fax: 215-357-5232
www.abraxiskits.com

Microcystin-DM ELISA Kit, Concise Procedure

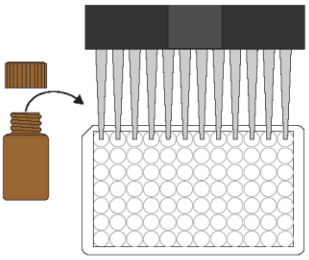
1. Addition of Standards, Samples

Add 100 µL of standard solutions, control or samples.



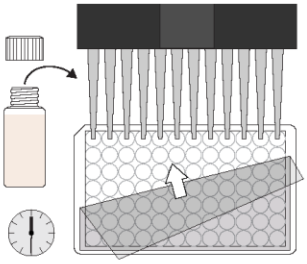
2. Addition of Enyzme Conjugate

Add 50 µL of enzyme conjugate.



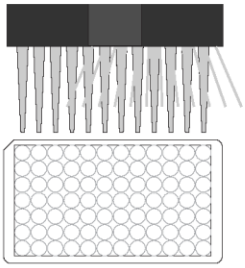
3. Addition of Antibody Solution

Add 50 µL of the antibody solution. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 90 minutes at room temperature.



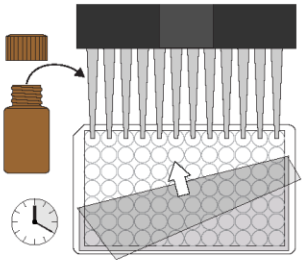
4. Washing of Plates

Wash the plates three times with 250 µL of diluted 1X washing buffer.



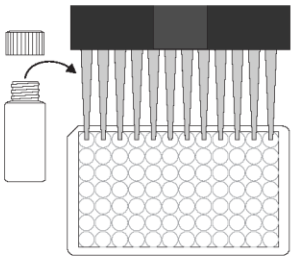
5. Addition of Substrate/Color Solution

Add 150 µL of substrate/color solution. Incubate 20 minutes at room temperature and away from direct sunlight.



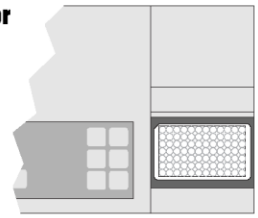
6. Addition of Stopping Solution

Add 100 µL of stop solution.



7. Measurement of Color

Measure color at 450 nm. Calculate results.



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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

TECHNOLOGY TYPE:	**MICROCYSTIN TEST KIT**
APPLICATION:	**RECREATIONAL WATER MICROCYSTIN DETECTION**
TECHNOLOGY NAME:	**Microcystin DM ELISA Test Kit**
COMPANY:	**Abraxis**
ADDRESS:	**54 Steamwhistle Drive Warminster, PA 18974** **PHONE: 215-357-3911**
WEB SITE:	**http://www.abraxiskits.com/**

ETV Joint Verification Statement

The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of microcystin test kits for water monitoring. This verification statement provides a summary of the test results for the Abraxis Microcystin DM enzyme-linked immunosorbent assay (ELISA) Test Kit.

VERIFICATION TEST DESCRIPTION

This verification test of the Abraxis Microcystin DM ELISA Test Kit was conducted from July 26 through August 12, 2010 at Battelle laboratories in Columbus, OH. Reference analyses by liquid chromatography tandem

mass spectrometry (LC-MS/MS) were performed the week of August 16, 2010 by the University of Nebraska Water Sciences Laboratory.

The objective of this verification test was to evaluate the microcystin test kit performance in analyzing known concentrations of microcystin in ASTM International Type II deionized (DI) water and in natural recreational water (RW) samples. The technology was used to analyze a variety of water samples for the variants microcystin-LR, microcystin-LA, and microcystin-RR. Because the technology cannot specify between the more than 80 microcystin variants, the samples prepared for this test were spiked with three individual variants. The Microcystin DM ELISA Test Kit provided a quantitative determination of microcystins and was evaluated in terms of:

- Accuracy - comparison of test kit results (samples prepared in DI water) to results from a reference method;
- Precision - repeatability of test kit results from three sample replicates analyzed in DI water, matrix interference, and RW samples;
- Linearity - determination of whether or not the test kit response increases in direct proportion to the known concentration of microcystin;
- Method detection limit - the lowest quantity of toxin that can be distinguished from the absence of that toxin (a blank value) at a 95% confidence level;
- Inter-kit lot reproducibility - determination of whether or not the test kit response is significantly different between two different lots of calibration standards within the kits;
- Matrix Interference - evaluation of the effect of natural recreational water matrices and chlorophyll-*a* on the results of the test kits; and
- Operational and sustainability factors - general operation, data acquisition, setup, consumables, etc.

Each microcystin test kit was operated according to the vendor's instructions by a vendor-trained Battelle technician. Samples and calibration standards were analyzed in duplicate and positive and negative controls were analyzed at the vendor-specified frequency.

The ability of the Abraxis Microcystin DM ELISA Test Kit to determine the concentration of microcystin was challenged using quality control (QC) samples, performance test (PT) samples and RW samples. QC, PT, and RW samples were prepared by Battelle technical staff the day before testing began. The test samples were prepared in glass volumetric flasks and stored in amber glass vials at 4 °C ± 3 °C until use. The reference samples that were prepared from the test solutions were stored in amber glass bottles at < -10°C. Replicate samples for the test kits were taken from the same sample bottle. The QC, PT, and RW samples were prepared blindly for the operator by coding the sample labels to ensure the results were not influenced by the operator's knowledge of the sample concentration and variant.

Unlike many contaminants, certified microcystin standards are not commercially available. In planning this verification test, multiple sources of standards were investigated. With agreement from the stakeholders, all vendors and the EPA project officer, the standards used for this verification were purchased from the most reputable sources (LR and RR from Canadian National Research Council and LA from Abraxis), based on a Performance Evaluation Audit, and used for both the testing solutions and the reference method calibration.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted technical systems audits of the both the laboratory and field testing, and Battelle QA staff conducted a data quality audit of at least 10% of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

Following is a description of the Microcystin DM ELISA Test Kit, based on information provided by the vendor. The information provided below was not verified in this test.

The Microcystin DM ELISA Test Kit is an ELISA for the determination of microcystins and nodularins in water samples. The assay utilizes monoclonal antibodies that have been raised against the ADDA moiety of the molecule, allowing for the detection of numerous microcystin and nodularin variants in drinking, surface, and groundwater at levels below World Health Organization (WHO) guidelines.

The test is a direct competitive ELISA and is based on the recognition of microcystins, nodularins and their variants by a monoclonal antibody. Microcystins and nodularins, when present in a sample, and a microcystins-HRP analog compete for the binding sites of anti-microcystin antibodies in solution. The microcystin antibodies are then bound by a second antibody (goat anti-mouse) immobilized on the plate. After a washing step and addition of a substrate/chromogen solution, a color signal is generated. The intensity of the color is inversely proportional to the concentration of the microcystins/nodularins present in the sample. The color reaction is stopped after a specified time and analyzed using a plate photometer to obtain the optical density (OD) at a wavelength of 450 nanometers (nm).

The Microcystin DM ELISA Test Kit is not able to distinguish the difference between two microcystin variants. Results from the Microcystin DM ELISA Test Kit are calibrated with respect to the microcystin-LR variant. However, other microcystin variants are known (based on information provided by Abraxis) to react to different extents with the antibodies used for detection; this is referred to as the cross reactivity (CR) of the variant. For this verification test, Microcystin DM ELISA Test Kit results for LR were reported from the calibration curve, results for the LA variant were reported as 48% of the kit results (based on the CR value of 125%) and the RR variant was reported as 53% of the test kit results.

VERIFICATION RESULTS

The verification of the Abraxis Microcystin DM ELISA Test Kit is summarized by the parameters described in Table 1.

Table1. Abraxis Microcystin DM ELISA Test Kit Performance Summary

Verification Parameters	LR	LA	RR
Accuracy (range of %D)			
0.10 ppb	50% to 94%		
0.50 ppb	47% to 94%	For LA, 147% to 229%, and for RR, 81% to 260%. For both variants, the data suggested that the uncorrected results in LR equivalents would provide concentrations that were more similar to the reference method concentrations.	
1.0 ppb	61% to 71%		
2.0 ppb	28% to 45%		
4.0 ppb	24% to 29%		
Precision (range of %RSD)	2% to 11%	1% to 9%	1% to12 %
Precision (RW samples)	2% to 9%		
Linearity (y=)	1.23x + 0.187 $r^2 = 0.993$	2.57x + 0.135 $r^2 = 0.997$	2.16x + 0.207 $r^2 = 0.985$
Method Detection Limit (ppb)	0.105	0.078	0.099

Inter-kit lot reproducibility. Calibration standards from two different lots were measured and the relative percent difference (RPD) of the resulting ODs ranged from 1% to 10%, with seven of the 12 being less than 5%.

Matrix Interference. Matrix interference effects were assessed by using a t-test to compare results from samples made by spiking undiluted and diluted interference matrices with the PT sample results at 2.0 ppb spiked concentration. Both the RW matrix results for LA and the 10x RW sample were significantly different from the DI water results and the diluted and undiluted RW were also significantly different from one another. The chlorophyll-*a* results for LR, LA, and RR were all statistically different when compared to the DI results except the 1 mg/L chlorophyll-*a* solutions spiked with RR ($p = 0.169$). There was no difference determined when comparing the two levels of chlorophyll-*a* solution results for all three variants. All of the undiluted and diluted RW samples were significantly different from one another for all three variants. The spiked undiluted RW samples each exhibited a higher microcystin concentration than did the diluted RW sample even after the samples

were corrected for any background microcystin present. Given that the molecular basis on which the test kits operate is well-characterized and understood from the literature, these results were unexpected. Two variants (LR and LA) demonstrated an interference effect but the third variant (RR) did not. This could have been caused by a number of factors, such as chlorophyll-*a* source and stability. However, due to the limited number of replicates and low power of this study, additional testing would be required to provide a better understanding as to whether there is a matrix interference due to chlorophyll-*a*, or another variable not investigated in this verification testing.

Recreational Water (RW). Because the reference method did not measure all possible microcystin variants, no quantitative comparison was made between the Microcystin DM ELISA Test Kit and the reference method results. The reference data were converted into LR-equivalents according to the Microcystin DM ELISA Test Kit cross reactivity for the variants. In general, the samples that were determined to have higher total concentrations by the Microcystin DM ELISA Test Kit had higher total concentrations as determined by the reference method. All of the Microcystin DM ELISA Test Kit total microcystin results were greater than the reference method results, which was consistent with the likelihood that all of the microcystins were not being measured by the reference method.

Operational Factors. The test kit operator reported that the Microcystin DM ELISA Test Kit was easy to use. Solution or sample preparation is minimal, mostly involving diluting samples that were above the quantification range. The procedure included two incubation periods that totaled 2 hours. The solutions in the kit produced a color change in the wells, confirming that those wells contain the solution. This feature was extremely helpful as technicians can become confused about what wells have had the solution added and which ones have not when analyzing 96-well plates. Previous knowledge or training on the use of micro-pipettes and or multi-channel pipettes with 96-well plates is recommended for consistent readings. A spectrophotometer plate reader is necessary for obtaining the spectrophotometric readings that are then analyzed using any commercial ELISA evaluation program (four-parameter is recommended by the vendor). Once the analysis was complete, the remaining solutions were disposed in the trash in accordance with local regulations.

The listed price for the Microcystin DM ELISA Test Kit at the time of the verification test was $400. The kit has a 12-month shelf life when received, and should be stored at 4 to 8 °C. Of the 96 wells on one plate, 16 wells were needed for calibrators and controls. The remaining 80 wells are for sample analyses that are performed in duplicate. Other consumables not included in the kit are pipettes, pipette tips, and distilled or DI water that can be supplied by the vendor.

Tracy Stenner 9/23/10
Date

Tracy Stenner
Manager
Environmental Product Line
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Battelle

Sally C Gutierrez 10/25/10
Date

Sally Gutierrez
Director
National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency

ADDITIONAL INFORMATION AND COMMENTS

Accuracy. Unlike many contaminants, certified reference standards are not commercially available. Several sources of standards were investigated and in our experience they can vary greatly in concentration and purity. The ETV study was conducted using a Microcystin-LR source from Canada, Abraxis standards are prepared using Microcystin-LR obtained from Dr. Carmichael. Our comparison of the 2 toxin sources indicate that both standard sources are within 20% of each other.

Another factor that needs to be considered when comparing results is that the reference method used in this study (LC-MS-MS) is not nearly as sensitive as the ELISA, therefore a SPE concentration step had to be performed with every sample. SPE extraction tended to give lower recoveries.

The combination of the standard source and lower SPE recoveries with the reference method lead to differences in accuracy between the instrumental (reference) and ELISA methods.

Matrix Interference. Matrix interference effects were assessed by using a t-test to compare results from samples made by spiking undiluted and diluted interference matrices with the PT sample results at 2.0 ppb spiked concentration. Both the RW matrix result for LA and the 10X RW sample were significantly different from the DI water results and the diluted and undiluted RW were also significantly different from one another. The chlorophyll-a results for LR, LA, and RR were all statistically different when compared to DI results except the 1 mg/mL chlorophyll-a solutions spiked with RR ($p = 0.169$). There was no difference determined when comparing the two levels of chlorophyll-a solution results for all the variants. All of the undiluted and diluted RW samples were significantly different from one another for all the variants. The spiked undiluted RW samples each exhibited a higher concentration than did the diluted RW sample even after the samples were corrected for any background microcystins present. *[Given that the molecular basis on which the test kit operates is well characterized and understood from the literature, these results were unexpected. Two variants (LR and LA) demonstrated interference effect but the third variant (RR) did not. This could have been caused by a number of factors, such as chlorophyll-a, stability, etc. In addition the chlorophyll-a used in the ETV study is insoluble and precipitated into a glob at the bottom of the vial when diluted in water. However, due to the limited number of replicates and low power of this study, additional testing would be required to provide a better understanding as to whether there is a matrix interference due to chlorophyll-a, or another variable not investigated in this verification testing].*

Based on the additional studies listed in Table 27A, **no interference of chlorophyll-a** can be found.

Table 27A. Chlorophyll-a Interferent Sample Results for the Abraxis DM Test Kit

Variant	Sample Description	Mean Kit Results: LR Equivalents (ppb)	Corrected Conc. by Variant (ppb)
LR	2.0ppb LR in DI	1.702	1.702
	2.0ppb LR in 1mg/mL Chlorophyll a in DI	1.720	1.720
	2.0ppb LR in 10mg/mL Chlorophyll a in DI	1.742	1.742
	2.0ppb LR in 100mg/mL Chlorophyll a in DI	1.695	1.695
RR	2.0ppb RR in DI	1.344	2.536
	2.0ppb RR in 1mg/mL Chlorophyll a in DI	1.343	2.534
	2.0ppb RR in 10mg/mL Chlorophyll a in DI	1.305	2.462
	2.0ppb RR in 100mg/mL Chlorophyll a in DI	1.305	2.462
LA	2.0ppb LA in DI	1.346	2.804
	2.0ppb LA in 1mg/mL Chlorophyll a in DI	1.285	2.677
	2.0ppb LA in 10mg/mL Chlorophyll a in DI	1.330	2.771
	2.0ppb LA in 100mg/mL Chlorophyll a in DI	1.410	2.938

Additional Factors. The Battelle **operator conducting the verification study** has 10 years of laboratory experience, but **was not experienced with ELISA analysis**. Our experience indicates that familiarity with ELISA and additional ELISA assay experience greatly increases performance, this will manifest in better precision and accuracy of results.

Fernando Rubio 11/24/2010

Fernando Rubio Date
President
Abraxis LLC

COAL TOX: The Screening and Semi-Quantitative Analysis of Water Samples for Microcystins by Enzyme-Linked Immunosorbent Assay (ELISA)

1.0 SCOPE

This method is used for the screening of microcystins and nodularins in water samples. The Abraxis Microcystins-DM (direct monoclonal) ELISA microtiter plate is an immunoassay for the quantitative and sensitive detection of microcystins and nodularins. The Abraxis Microcystin-ADDA ELISA microtiter plate is an immunoassay for the quantitative and sensitive congener-independent detection of microcystins and nodularins. Both microtiter plates (DM & ADDA) allows for the detection of microcystin and nodularins; the DM microtiter plate is more sensitive to microcystin-LR. The applicable range is 0.10 to 5.0 ppb.

2.0 SUMMARY

ELISA is the most prevalent immunoassay technique utilized for environmental analyses. The immunoassay test products available from manufacturers are devised for specific analytes but follow the same principles. The fundamental concept governing all immunoassays is the lock and key fit between the analyte molecule and the binding sites of the antibody. Immunossays can be performed in either liquid-phase or solid-phase. An immunosorbent is created when a known amount of antibody is immobilized on a solid-phase support (such as a disposable plastic tube or microtiter plate). The type of immunoassay employed in this method is known as competitive ELISA. The Microcystin DM kits are a direct competitive ELISA analysis and the Microcystin ADDA kits are an indirect competitive ELISA analysis.

For Microcystin DM kits, the direct competitive ELISA analysis is based on the recognition of microcystins, nodularins and their congeners by a monoclonal antibody. Microcystins, nodularins and their congeners when present in a sample, and a microcystins-HRP analogue compete for the binding sites of anti-microcystins antibodies in solution. The microcystins antibodies are then bound by a second antibody (goat anti-mouse) immobilized in the plate.

For Microcystin ADDA kits, the indirect competitive ELISA analysis is based on the recognition of microcystins, nodularins and their congeners by specific antibodies. Microcystins, nodularins and their congeners when present in a sample and a microcystins-protein analogue immobilized on the plate compete for the binding sites of antibodies in solution. After a washing step, a second antibody-HRP label is added.

For both kits, after a washing step and addition of the substrate solution, a colour signal is generated. The intensity of the blue colour is inversely proportional to the concentration of the microcystins present in the sample. The colour reaction is stopped after a specified time and the colour is evaluated using an ELISA reader. In the absence of microcystin in the sample, maximum binding of the conjugate to the antibodies occurs and would be retained after the washing step. Subsequent addition of substrate and chromogen would yield a maximum colour signal (absorbance). The maximum absorbance value produced by the negative control is regarded as the zero baseline.

The general analytical protocol used in screening of the microcystin samples involves the following steps:

i) Cell wall rupturing by microwave
ii) ELISA
iii) Spectrophotometer measurement
iv) Data processing and reporting

3.0 DEFINITIONS

Microcystins/Nodularins: are cyclic toxin peptides released from toxic cyanobacteria (blue-green algae) blooms. Microcystins (several structural variants or congeners are found) have been found in fresh water throughout the world. Nodularins are found in marine and brackish water. To date, approximately 80 variants of microcystins have been isolated; the most common variant is microcystin-LR.

ELISA (Enzyme-Linked Immunosorbent Assay): is a rapid immunochemical test that involves an enzyme used for measuring a wide variety of tests. An ELISA test detects substances that have antigenic properties, primarily proteins rather than small molecules and ions. Some of these substances include hormones, bacterial antigens, and antibodies. ELISA tests are generally highly sensitive and specific.

4.0 INTERFERENCES

Samples that are analyzed by ELISA must not contain mass-labeled internal standards or immunoassay compatible standards.

All labware that contact microcystin sample should have relatively inert surfaces; otherwise, compound losses may occur. Microcystin losses due to adsorption may occur in plastic wares.

This method cannot be used to quantify microcystin-LR to meet regulatory requirements under O.Reg. 169/03 but it can be used to satisfy this regulation when microcystin is below the concentration of 1.5 µg/L.

5.0 MATERIALS REQUIRED

5.1 Lab Ware

- VWR Multichannel 50-300 µL pipette
- Anachemia 50-200 µL
- Pipette tips
- Micro centrifuge tubes
- Distilled Water

5.2 Reagents

COAL uses Abraxis Microcystins Kits

DM Reagent Kit includes
- Microtiter plate coated with a second antibody (goat anti-mouse)
- Standards (6) and Control (1): 0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb. Control at 0.75 ppb
- Antibody solution (monoclonal anti-microcystins), 6 mL
- Microcystins-HRP Conjugate, 6mL
- Diluent/zero, 25 mL.
- Wash solution 5X Concentrate, 100 mL
- Color Solution (TMB, 16 mL)
- Stop Solution, 2X 6mL

ADDA Reagent Kit includes
- Microtiter plate coated with an analog of microcystins conjugated to a protein.
- Standards (6) and Control (1): 0, 0.15, 1.40, 1.0, 2.0, 5.0 ppb. Control 0.75 ppb.
- Antibody solution
- Anti-Sheep-HRP conjugate
- Wash Solution 5X Concentrate
- Color Solution (TMB)
- Stop Solution
- Diluent

5.3 Equipment
- Microplate Washer Stat Fax 2600
- Multi-purposed Photometer System Stat Fax 3200
- 600-watt microwave oven

6.0 SAMPLE REQUIREMENTS

6.1 Specifications

Samples must be collected in 500 mL or 1 L, amber glass bottles with Teflon-lined caps. About 1000 mL (2 x 500 mL bottles) must be submitted for testing. Microcystin stability is possibly matrix dependent. As per Abraxis instructions, no preservation is required, although preserved samples do not affect the results of the test.

In the laboratory, samples are stored at 4.0°C ± 3.0°C, preferably in the dark.

Samples must be analyzed within 7 days of the sampling date.

Note: If temperature conditions for sample storage are not met for whatever reason, samples in question will not be analyzed and a request for re-sampling is made for drinking water samples.

6.2 Contingencies

Microcystin stability is possibly matrix dependent. The presence of microbes, humic material and residual chlorine may contribute to compound losses. The requirement of holding time for samples can be circumvented by freezing a portion of the sample and storing it below -10ºC. In such cases a portion of sample must be frozen upon arrival in the bioassay laboratory. The frozen sample can be tested at any time after thawing.

Non-conforming samples are not analyzed (see WI-3).

6.3 Storage

Sample holding time for microcystins is 7 days. Samples are refrigerated at 4.0°C ± 3.0°C upon receipt at the laboratory. Drinking water samples that exceed 7 day holding time are not analyzed and are given the remark code NDHT (NO DATA: Hold Time Exceeded).

7.0 QUALITY CONTROL

7.1 Standards and Reference Materials

No other standards are required other than the ones provided in the kit.

7.2 Method Blank

Distilled or deionized water may be used as a method blank.

7.3 Spike Solution

Separate Spike solutions are not used. However, a set of purchased Microcystin LR Check Samples is used for positive control and tap water or diluent/zero (included in kits) blanks are run as negative control.

7.4 Positive Controls

The kit comes with a positive control that must be included as a QC sample in each run.

7.5 Calibration Procedure

The Multi-purpose photometer Stat Fax 3200 conducts a self-calibration as an automatic function during start up. The instrument does not require re-adjustment.

8.0 CALIBRATION AND STANDARDIZATION

8.1 Calibration Standard Solution

Each kit comes with a set of calibrator standards. The Abraxis DM and ADDA kit used in this method comes with 6 calibration standards at 0.0, 0.15, 0.40, 1.0, 2.0 and 5.0 ppb levels. These solutions are working calibration solutions.

9.0 PROCEDURE

9.1 Sample Preparation refer to WI 45 – ADDA Analysis and/or WI 46 – DM Analysis

Note: Any drinking water samples identified as regulatory submission are not filtered for analysis.

9.1.1 Samples are set up according to laboratory numbers.

9.2 Test Preparation

9.2.1 Micro-pipetting equipment and pipette tips for pipetting the standards and samples are necessary. Use a multi-channel pipette for adding the antibody, enzyme conjugate, substrate solution, and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Use only the reagents and standards from the same lot number in one test, as they have been adjusted in combination.

9.2.2 Ensure the microtiter plate and the reagents come to room temperature before use.

9.2.3 Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator at 4.0°C ± 3.0°C.

9.2.4 The standard, control, antibody solution, enzyme conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.

9.2.5 The wash solution is a 5X concentrated solution and needs to be diluted with deionized water.

9.2.6 The stop solution has to be handled with care as it contains diluted H_2SO_4.

9.3 Operations

9.3.1 Follow the assay procedure provided with the Abraxis ADDA or DM test kits (or see WI-45 and/or WI-46), which details the proper volume for the standards, controls, test sample, antibody, conjugate, colour, and stop solution. The procedure also defines the incubation periods.

10.0 DATA ANALYSIS AND CALCULTATION

10.1 Read the absorbance at 450 nm using the Multi-purposed Photometer System Stat Fax 3200. Turn on the Microplate reader power and wait 30 minutes to warm up.

10.2 The evaluation of the DM and ADDA microcystin is performed using a commercial evaluation program. The concentrations of the samples are determined using a standard curve. Competitive ELISA yields an inverse curve, where higher values of microcystin in the samples or standards yield a lower optical density.

10.3 Report results only if the R-value on the curve equals or is greater than 0.95. If the curve is below 0.95, re-read plate. If the curve is still below 0.95 repeat analysis.

10.4 Report results if the control is within range. The acceptable range it between 0.56 and 0.94 ppb or ± 25% of the control at 0.75 ppb. If the control is outside of range repeat analysis.

10.5 If the spike sample is negative or any blanks read positive, repeat analysis with fresh spike or blank, as required. If this does not solve the issue, use a new test kit lot.

10.6 If a replicate sample is performed report the higher result.

10.7 Any sample containing 0.14 ppb of microcystins or less are considered negative. Samples containing 0.15 to 1.49 ppb of microcystins are positive. Samples over 5.00 ppb are reported as greater than (>5.00 ppb).

10.8 Any drinking water samples reading $\geq$ 1.5 ppb is considered presumptive positive for microcystin-LR and triggers the provisional exceedance reporting (WI –13).

11.0 SUPPORTING DOCUMENTATION

2.1 List of Instruction Manuals

Refrigerator Use and Care Guide 3-24308-031 Rev. 1
Stat Fax 2600 Microplate Washer Owner's Manual Rev 1.2-9/99
Stat Fax 3200 Operator's Manual Rev E 07/2005

2.2 List of Work instructions

WI 3 – Receiving Waters
WI 7 – Pipettes
WI 11 – Fridge Temperatures
WI 13 – Adverse Reporting
WI 17 – Monitoring of Purchased Distilled and DI Water
WI 19 – Handling of Legal Submissions
WI 28 – Disposal Procedures
WI 45 – ADDA Analysis
WI 46 – DM Analysis
WI 47 – Microcystin Instrumentation Maintenance

12.0 REFERENCES

M. G. Weller, A. Zeck, A. Eikenberg, S. Nagata, Y. Ueno, and R. Niessner, Development of a Direct Competitive Microcystins Immunoassay of Broad Specificity. Analytical Sciences. 17, 2001, 1445-1448.

Microcystins-ADDA ELISA (Microtiter Plate) by Abraxis (www.abraxis.com)

Microcystins-DM ELISA (Microtiter Plate) by Abraxis (www.abraxis.com)

13.0 REVISION HISTORY

Date	Revision #	Section Name	Comments
*For revisions prior to 2012, please refer to Quality Documentation Master List			
March 2012	2.0	All Sections	Complete rewrite of Section 5 of SFA Chemistry Manual and SOP 69
May 2012	2.1	Header/Footer/Title	Removed "Methodology" from title/header, added "approved for use…" to footer
April 2013	2.2	4.0 – 7.0, 10.0	Editorial changes
July 2013	2.3	6.0	Removed duplicate info; added preservation details
		7.0	Editorial changes; clarified blanks
		9.0	Moved procedure details to WI 45 & 46
		10.0	Fixed control ranges; added rerun requirements for spike/blank fails; specified reporting for results over 5.00

DETERMINATION OF DETECTION LIMITS OF LICENSED PARAMETERS

CENTRAL ONTARIO ANALYTICAL LABORATORY
FEBRUARY 2011

Determination of Detection Limits of Licensed Parameters for Central Ontario Analytical Laboratory

Please note for all parameters, method detection limits (MDLs) and reliable detection limits (RDLs) were calculated based on the analysis of 8 low level (lowest standards provided, or blanks) replicate samples carried through all sample-processing steps. The standard deviation of the results was determined and used to find the MDL and RDL at 95% probability of detection (See SOP 24 – Test Methods and Method Validation). The method detection limit is defined as the measured response at which there is 95% probability that the analyte is present. The reliable detection limit is defined as the lowest analyte concentration that can be detected with 95% probability.

Where RDLs fall below the lowest standard provided for the parameter in question, it is the policy of Central Ontario Analytical Laboratory to use the lowest standard as the RDL.

From the data calculated in the following tables, it has been confirmed that the current MDLs and RDLs are acceptable and all methods for licensed parameters are fit for the purpose of testing drinking water.

Table 1. Method Detection Limits for Licensed C.O.A.L. Parameters

Parameter	Detection Limit
Microcystin DM	0.10
Microcystin ADDA	0.10
Nitrate+Nitrite	0.010
Nitrite	0.010
Ammonia	0.050
ortho-Phosphate	0.050
Total Coliform	0
E. coli	0
HPC	10
Fecal Strep	4

Table 2. MDL/RDL Calculations for Microcystin DM and ADDA

DM		ADDA	
Abs	*ppb*	*Abs*	*ppb*
1.336	0.00	0.801	0.01
1.357	-0.01	0.918	-0.02
1.394	-0.04	0.921	-0.02
1.404	-0.05	0.987	-0.04
1.421	-0.06	0.764	0.02
1.366	-0.02	0.886	-0.01
1.386	-0.04	0.847	0.00
1.298	0.03	1.101	-0.07
Mean	-0.02	Mean	-0.02
Std dev	0.03	Std dev	0.03
MDL	**0.05**	**MDL**	**0.05**
RDL	**0.10**	**RDL**	**0.10**

Table 3. MDL/RDL Calculations for Nitrate+Nitrite, Nitrite, Ammonia and ortho-Phosphate

	Nitrate + Nitrite	Nitrite	Ammonia	ortho-Phosphate
	Sample 0.01mg/L	*Sample 0.01mg/L*	*Sample 0.05mg/L*	*Sample 0.05mg/L*
	0.015	0.008	0.075	0.090
	0.014	0.008	0.071	0.080
	0.016	0.007	0.072	0.073
	0.012	0.007	0.068	0.069
	0.012	0.008	0.070	0.065
	0.012	0.007	0.066	0.059
	0.013	0.008	0.094	0.059
	0.014	0.007	0.084	0.057
Mean	0.014	0.008	0.075	0.069
Std dev	0.002	0.001	0.009	0.012
MDL	**0.002**	**0.001**	**0.015**	**0.019**
RDL	**0.005**	**0.002**	**0.031**	**0.038**

Table 4. MDL/RDL for Total Coliform, E. coli, Background, HPC and Fecal Strep

	Endo 100mL Blank	*DC 100mL Blank*	*HPC 0.1mL Blank*	*FS 25mL Blank*
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
	0	0	0	0
MDL	**0**	**0**	**10***	**4****
RDL	**0**	**0**	**10***	**4****

*based on reporting final value at CFU/1mL
**based on reporting final value at CFU/100mL

Ozone

DOC316.53.01106

Indigo Method **Method 8311**

0.01 to 0.25 mg/L O_3 (LR), 0.01 to 0.75 mg/L O_3 (MR), 0.01 to 1.50 mg/L O_3 (HR) **AccuVac® Ampuls**

Scope and application: For water.

Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter requirement for AccuVac Ampul tests.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for AccuVac Ampuls

Instrument	Adapter
DR 6000 DR 5000 DR 900	—
DR 3900	LZV846 (A)
DR 3800 DR 2800 DR 2700	LZV584 (C)
DR 1900	9609900 or 9609800 (C)

Before starting

Samples must be analyzed immediately after collection and cannot be preserved for later analysis.
Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.
Use tap water or deionized water for the blank (ozone-free water).
In this method, the instrument is intentionally zeroed on the sample, not the blank.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

AccuVac Ampuls

Description	Quantity
Ozone AccuVac® Ampuls, 0-0.25 mg/L	2
Ozone AccuVac® Ampuls, 0-0.75 mg/L	2
Ozone AccuVac® Ampuls, 0-1.5 mg/L	2
Beaker, 50 mL	1

AccuVac Ampuls (continued)

Description	Quantity
Stoppers, for 18-mm tubes and AccuVac Ampuls	2
Water, ozone-free	varies

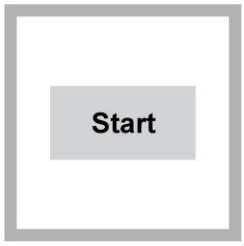
Refer to Consumables and replacement items on page 4 for order information.

Sample collection

- Samples must be analyzed immediately after collection and cannot be preserved for later analysis.
- The most important consideration during sample collection is to prevent the escape of ozone from the sample.
- Collect the sample gently and analyze immediately. Do not shake or stir the sample or allow the sample temperature to increase.
- Do not transfer the sample from one container to another unless absolutely necessary.

AccuVac Ampul procedure

Note: *For this procedure, the zero step is done on the prepared sample, and the read step on the blank.*



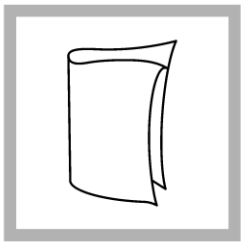
1. Start program **454 Ozone LR AV**, **455 Ozone MR AV** or **456 Ozone HR AV**. For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.

Note: *Although the program name can be different between instruments, the program number does not change.*

2. Prepare the blank: Pour at least 40 mL of ozone-free water in a 50-mL beaker. Fill an Indigo Ozone Reagent AccuVac Ampul with the ozone-free water. Keep the tip immersed while the Ampul fills fully.

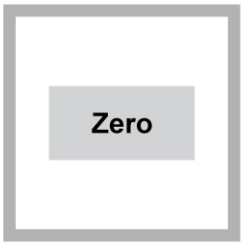
3. Prepare the sample: Pour at least 40 mL of sample in a 50-mL beaker. Fill an Indigo Ozone Reagent AccuVac Ampul with the sample. Keep the tip immersed while the Ampul fills fully.

4. Quickly invert the AccuVac Ampuls several times to mix.
Some of the blue color will be bleached if ozone is present.

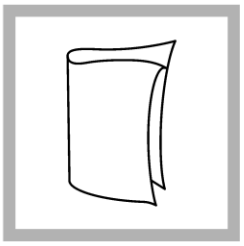


5. Clean the prepared sample AccuVac Ampul.

6. Insert the prepared sample AccuVac Ampul into the cell holder.



7. Push **ZERO**. The display shows 0.00 mg/L O_3.



8. Clean the blank AccuVac Ampul.

9. Insert the blank AccuVac Ampul into the cell holder.

10. Push **READ**. Results show in mg/L O_3.

Reagent stability

The indigo reagent is light-sensitive. Keep the unused AccuVac Ampuls in the dark. The indigo solution decomposes slowly under room light after the AccuVac Ampul is filled. The filled blank Ampul can be used for multiple measurements during the same day.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% Confidence Interval)	Sensitivity Concentration change per 0.010 Abs change
454	0.15 mg/L O_3	0.14–0.16 mg/L O_3	0.01 mg/L O_3
455	0.45 mg/L O_3	0.43–0.47 mg/L O_3	0.01 mg/L O_3
456	1.00 mg/L O_3	0.97–1.03 mg/L O_3	0.01 mg/L O_3

Summary of method

The reagent formulation adjusts the sample pH to 2.5 after the Ampule has filled. The indigo reagent reacts immediately and quantitatively with ozone. The blue color of indigo is bleached in proportion to the amount of ozone present in the sample. Other reagents in the formulation prevent chlorine interference. No transfer of sample is needed in the procedure, therefore ozone loss due to sampling is eliminated. The measurement wavelength is 600 nm for spectrophotometers or 610 nm for colorimeters.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
Ozone AccuVac® Ampuls, 0-0.25 mg/L	2	25/pkg	2516025
Ozone AccuVac® Ampuls, 0-0.75 mg/L	2	25/pkg	2517025
Ozone AccuVac® Ampuls, 0-1.5 mg/L	2	25/pkg	2518025

Required apparatus

Description	Quantity/test	Unit	Item no.
AccuVac Snapper	1	each	2405200
Beaker, 50-mL	1	each	50041H
Stoppers for 18-mm tubes and AccuVac Ampuls	2	6/pkg	173106
Beaker, polypropylene, 50-mL, low form	1	each	108041

Optional reagents and apparatus

Description	Unit	Item no.
Water, deionized	4 L	27256
SpecCheck™ Gel Secondary Standard Kit, Ozone, 0–0.75 mg/L set	each	2708000

BG-11 MEDIUM

Reference: Rippka, R., J. Deruelles, J. Waterbury, M. Herdman and R. Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111: 1-61.

This medium is used for successfully for most cyanobacteria. Vitamin B_{12} may be added for those species that require it. Use f/2 vitamin solution.

STOCK	STOCK SOLUTION		ml/Litre
	prepare ~~250mL~~ 500mL		
1. $NaNO_3$ (omitted for heterocystous species)	150 g/L	75 g	10 ml
2. $K_2HPO_4.3H_2O$ or $*K_2HPO_4$	40 g/L or *30 g/L	15 g	1 ml
3. $MgSO_4.7H_2O$	75 g/L	37.5 g	1 ml
4. $CaCl_2.2H_2O$	36 g/L	18 g	1 ml
5. Citric Acid combined with	6 g/L	3 g	1 ml
Ferric Ammonium Citrate	6 g/L	3 g	
6. Na_2EDTA	1 g/L	0,5 g	1 ml
7. Na_2CO_3	20 g/L	10 g	1 ml
8. Trace Metal solution	See below		1 ml
9. F/2 vitamins			1 mL

Adjusting the pH of the medium to approximately 7.5 will avoid heavy precipitation. (Initial pH is approximately 8.5.) When making solid media, you can add agar directly to medium or make double strength medium and double strength agar solution, then after autoclaving combine the two. Omit $NaNO_3$ for media used to culture cyanobacteria with heterocysts e.g. *Nostoc, Anabaena* in order to maintain their ability to produce heterocysts.

OPTION: 0.5 g/L of HEPES buffer can be added to the final medium as a buffer. $FeCl_3$ and EDTA added in a 1:1 ratio may be substituted.

Trace Metal Solution: prepare 500mL or 1L

Substance	g/Litre
1. H_3BO_3	2.86 g
2. $MnCl_2.4H_2O$	1.81 g
3. $ZnSO_4.7H_2O$	0.222 g
4. $Na_2MoO_4.2H_2O$	0.390 g
5. $CuSO_4.5H_2O$	0.079 g
6. $Co(NO_3)_2.6H_2O$	0.0494 g

Dissolve each of the above substances for the Trace Metal solution separately prior to adding the next on the list.

VITAMIN SOLUTIONS

f/2 VITAMIN SOLUTION

Reference: Guillard, R.R.L. and J.H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can. J. Microbiol. 8: 229-239.

Reference: Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates in "Culture of Marine Invertebrate Animals." (Eds: Smith W.L. and Chanley M.H.) Plenum Press, New York, USA. pp 26-60.

STOCK	STOCK SOLUTION
1. Vitamin B12 (Cyanocobalamin)	5mg/5ml distilled H_2O
2. Biotin	1 mg/10ml distilled H_2O

To make the working solution add the following amounts of the stock solutions to 100 ml of distilled water:

1. Vitamin B12	0.1 ml
2. Biotin	1.0 ml
3. Thiamine HCl	20 mg

Dispense working solution according to amounts required for media preparation. One ml aliquots are conveniently stored frozen, in cryovials for periods of 1-2 months. Store the remainder of the working solution frozen, in a polyethylene bottle of 100 ml. Wrap with Parafilm to avoid moisture loss. and store all solutions frozen.

Vitamin Mix S_3

(Ref: Watanabe, M. and N. Hisayoshi. 1997. National Institute for Environmental Studies (NIES) List of Strains. Fifth Edition. Microalgae and Protozoa. Japan

Thiamine HCl	5 mg
Nicotinic acid	1 mg
Calcium pantothenate	1 mg
P – aminobenzoic acid	0.1 mg
Biotin	0.01 mg
Inositol	50 mg
Folic acid	0.02 mg
Thymine	30 mg
Distilled water	100 ml

Filter-sterilize the solution and add aseptically to medium. Store frozen in vials.

UPLC-MS/MS DETECTION OF MICROCYSTIN LR

Johnna Birbeck, Nicole Lenca, Judy Westrick
Lumigen Instrument Center
Wayne State University

UPLC-MS/MS DETECTION OF MICROCYSTIN LR

INSTRUMENTATION

LC SYSTEM

Shimadzu Nexera X2
Controller: CBM-20A
Pumps: LC-30AD Binary Pump System
Column Oven: CTO-20AC
Autosampler: SIL-30ACMP

MS DETECTOR

Shimadzu LCMS-8040

COLUMN

Waters Acquity UPLC HSS T3 1.8 µm 2.1 mm x 30 mm Column

METHOD PARAMETERS

SOLVENTS

Solvent A: 0.1% Formic Acid in LC-MS Grade Water
Solvent B: 0.1% Formic Acid in LC-MS Grade Acetonitrile

INSTRUMENT PARAMETERS

*Settings are based on tabs available under the Advanced section of the Realtime Analysis Window

MS

Advanced Settings

Applied Voltage 4.5kV (positive mode)

MRM Events

Compound	*Precursor m/z*	*Product m/z*	*Dwell Time (msec)*	*Pause Time (msec)*	*Q1 Pre Bias (V)*	*CE*	*Q3 Pre Bias (V)*
Microcystin LR	498.10	134.95	10.0	3.0	-25.0	-16.0	-24.0
Microcystin LR	498.10	482.10	10.0	3.0	-25.0	-12.0	-23.0
Microcystin LR	498.10	861.25	10.0	3.0	-25.0	-17.0	-40.0

*The 134.95 product m/z transition was used as the quantifier for Microcystin-LR.

Interface

Electrospray Ionization (ESI)
Nebulizing Gas Flow: 3 L/min
DL Temperature: 250 °C
Heat Block Temperature: 400 °C
Drying Gas Flow: 15 L/min

Data Acquisition

LC Time Program

LC Stop Time: 8.00 min

LC Time Prog.

Time	Module	Command	Value
0.4	Pumps	Pump B Conc.	30
2.50	Pumps	Pump B Conc.	30
2.51	Pumps	Pump B Conc.	95
5.00	Pumps	Pump B Conc.	95
5.01	Pumps	Pump B Conc.	30
8.00	Controller	Stop	

Pump

Mode: Binary Gradient
Total Flow: 0.400 mL/min
Pump B Conc.: 30.0%
Pump B Curve: 0
Pressure Limits

Maximum: 15000 psi
Minimum: 0 psi

Column Oven
- Oven Temperature: 35 °C
- Temperature Limit (Maximum): 90 °C
- Ready Check: On

Controller
- External Output
  - Power on: box checkmarked
- Acquisition cycle time optimization
  - Autosampler pretrement beginning: Off
  - Pretreatment overlap time: 0.00 min

Autosampler
- Injection Settings
  - Sampling Speed: 1.0 uL/sec
  - Cooler Temperature: 10 °C
  - Temperature Control: Rack plate L, M, R and Ctrl Rack boxes are check marked
- Rinse Type: External Only
  - Rinse Settings
    - Rinse Mode: Before and after aspiration
    - Rinse Dip Time: 0 sec
  - Rinse Pump
    - Rinse Method: Rinse port only
    - Rinse Time: 2 sec
- Injection Volume
  - 5 uL

QA/QC

For all samples QA/QC during each run included a positive control, negative control, spiked sample and a sample replicate. The QA/QC's were run approximately every 3-10 samples depending on the amount of samples being run. Percent recoveries were calculated for the positive controls in which the values must be within ± 15% of the true concentration. For spiked samples, the spiked concentration was subtracted from the calculated concentration and the value was compared to the same sample that was not spiked. The concentrations for the spiked samples were evaluated to be ± 15% of the un-spiked sample using percent coefficient of variance (% CV). Replicate values were also compared to one another using % CV with the % CV values having to be ± 15%.

Appendix 2

Laboratory Analyses

CENTRAL ONTARIO ANALYTICAL LABORATORY INC.

4260 Burnside Line, R.R. #4
Orillia, ON L3V 6H4
Tel: (705) 326-8285 Fax: (705) 326-9316
www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2013-12-23 14:15
2013-12-23 13:30	9.3	JT	Date & Time ELISA Procedure:	2013-12-23 16:46

Date Sampled: 2013-12-16

Collected By: Gemma Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By:

Twp/Town	Waterloo
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	23
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave W. Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research. Purolator # NHX000005497 NDBT= No Data: Bottle Broken in Transit

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
11:00	1	R	R	5					MC-695	<	0.10	✔									
11:40	2	R	R	5					MC-696		2.50		✔								
12:20	3	R	R	5					MC-697		2.59		✔								
13:00	4	R	R	5					NDBT												
13:40	5	R	R	5					NDBT												
14:30	6	R	R	5					MC-698	<	0.10	✔									

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2013-12-24 09:30 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: AB Authorized & Rechecked By: TJ/JT Copy Released By: JT/AD/HM

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2013-12-23 14:15
2013-12-23 13:30	9.3	JT	Date & Time ELISA Procedure:	2013-12-23 16:46

Date Sampled: 2013-12-16 & 2013-12-17

Collected By: Gemma Charlebois

Name of Facility: UW

Drinking Water System (DWS) #: N/A

Submitted By:

Twp/Town	Waterloo
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	23
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave W. Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research. Purolator # NHX000005497 *Bottle says Sample 9 but sampled on December 17, 2013. Sample Identifier's 7,8,9 were sampled on December 16, 2013 & Sample Identifier's 10,11,12 were sampled on December 17, 2013.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
15:10	7	R	R	5					MC-699		3.83		✔								
15:50	8	R	R	5					MC-700		5.08		✔								
16:30	9	R	R	5					MC-701		2.81		✔								
11:00	10	R	R	5					MC-702	<	0.10	✔									
11:45	11*	R	R	5					MC-703		5.62		✔								
12:30	12	R	R	5					MC-704		0.48		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2013-12-24 09:30 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: AB Authorized & Rechecked By: TJ/JT Copy Released By: JT/AD/HM

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

Final Certificate Microcystin, PITA 111, 1311, Revision 1.4
Page 1 of 1

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www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2013-12-23 14:15
2013-12-23 13:30	9.3	JT	Date & Time ELISA Procedure:	2013-12-23 16:46

Date Sampled: 2013-12-17

Collected By: Gemma Charlebois

Name of Facility: UW

Drinking Water System (DWS) #: **N/A**

Submitted By:

Twp/Town	Waterloo
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	23
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave W. Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research. Purolator # NHX000005497

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
13:15	13	R	R	5					MC-705	<	0.10	✔									
13:50	14	R	R	5					MC-706		2.66		✔								
14:30	15	R	R	5					MC-707		0.11	✔									
15:15	16	R	R	5					MC-708		7.45		✔								
16:00	17	R	R	5					MC-709		0.63		✔								
16:30	18	R	R	5					MC-710	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2013-12-24 09:30 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: AB Authorized & Rechecked By: TJ/JT Copy Released By: JT/AD/HM

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2013-12-23 14:15
2013-12-23 13:30	9.3	JT	Date & Time ELISA Procedure:	2013-12-23 16:46

Date Sampled: 2013-12-18

Collected By: Gemma Charlebois

Name of Facility: UW

Drinking Water System (DWS) #: N/A

Submitted By:

Twp/Town	Waterloo
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	23
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave W. Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research. Purolator # NHX000005497

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
11:10	19	R	R	5					MC-711		0.14	✔									
11:50	20	R	R	5					MC-712	<	0.10	✔									
12:30	21	R	R	5					MC-713		8.24		✔								
13:10	22	R	R	5					MC-714		8.95		✔								
13:50	23	R	R	5					MC-715		9.01		✔								
14:30	24	R	R	5					MC-716		0.21		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2013-12-24 09:30 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: AB Authorized & Rechecked By: TJ/JT Copy Released By: JT/AD/HM

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2013-12-23 14:15
2013-12-23 13:30	9.3	JT	Date & Time ELISA Procedure:	2013-12-23 16:46

Date Sampled: 2013-12-18

Collected By: Gemma Charlebois

Name of Facility: UW

Drinking Water System (DWS) #: **N/A**

Submitted By:

Twp/Town	Waterloo
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	23
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave W. Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Purolator # NHX000005497 NDBT= No Data: Bottle Broken in Transit

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
15:10	25	R	R	5	—				NDBT												
15:50	26	R	R	5		—			MC-717		5.80		✔								
16:30	27	R	R	5	—				NDBT												

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2013-12-24 09:30 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: AB Authorized & Rechecked By: TJ/JT Copy Released By: JT/AD/HM

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

CENTRAL ONTARIO ANALYTICAL LABORATORY INC.

4260 Burnside Line, R.R. #4
Orillia, ON L3V 6H4
Tel: (705) 326-8285 Fax: (705) 326-9316
www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-13 10:30
2014-01-10 10:27	7.7	AD	Date & Time ELISA Procedure:	2014-01-13 13:11

Date Sampled: 2014-01-09
Collected By: G. Charlebois
Name of Facility: University of Waterloo
Drinking Water System (DWS) #: **N/A**
Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	23

Report To: **Gemma Charlebois**
Address: 200 University Ave., Waterloo, ON
Telephone: 519 503-7166 Ext: Fax:
Email Address: gcharlebois@uwaterloo.ca
"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005546
NDBT=No Data container broken in transit

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	28		R	5					MC-1		4.75		✔								
	29		R	5					NDBT		NDBT										
	30		R	5					NDBT		NDBT										
	31		R	5					MC-2		3.32		✔								
	32		R	5					MC-3		3.93		✔								
	33		R	5					MC-4		1.85		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-13 14:00 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: CAB Authorized & Rechecked By: TJ/JT Copy Released By: AD/AB

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-13 10:30
2014-01-10 10:27	7.7	AD	Date & Time ELISA Procedure:	2014-01-13 15:11

Date Sampled: 2014-01-09

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005546
NDBT=No Data container broken in transit

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	34		R	5					MC-5		2.57		✔								
	35		R	5					MC-6		4.91		✔								
	36		R	5					MC-7	<	0.10	✔									
	37		R	5					MC-8		4.30		✔								
	38		R	5					NDBT		NDBT										
	39		R	5					MC-9		2.73		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-13 14:00 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: CAB Authorized & Rechecked By: TJ/JT Copy Released By: AD/AB

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-13 10:30
2014-01-10 10:27	7.7	AD	Date & Time ELISA Procedure:	2014-01-13 13:11

Date Sampled: 2014-01-09

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: **N/A**

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	23

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005546
NDBT=No Data container broken in transit

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	40		R	5					MC-10		2.17		✔								
	41		R	5					MC-11		3.58		✔								
	42		R	5					MC-12		3.84		✔								
	43		R	5					NDBT		NDBT										
	44		R	5					MC-13		3.91		✔								
	45		R	5					MC-14	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-13 14:00 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: CAB Authorized & Rechecked By: TJ/JT Copy Released By: AD/AB

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-13 10:30
2014-01-10 10:27	7.7	AD	Date & Time ELISA Procedure:	2014-01-13 13:11

Date Sampled: 2014-01-09
Collected By: G. Charlebois
Name of Facility: University of Waterloo
Drinking Water System (DWS) #: **N/A**
Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	23

Report To: **Gemma Charlebois**
Address: 200 University Ave., Waterloo, ON
Telephone: 519 503-7166 Ext: Fax:
Email Address: gcharlebois@uwaterloo.ca
"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005546

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	46		R	5					MC-15		3.34		✔								
	47		R	5					MC-16		3.05		✔								
	48		R	5					MC-17		1.65		✔								
	49		R	5					MC-18		1.86		✔								
	50		R	5					MC-19		2.01		✔								
	51		R	5					MC-20		1.95		✔								
	52		R	5					MC-21		2.02		✔								
	53		R	5					MC-22		2.01		✔								
	54		R	5					MC-23	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-13 14:00 Analyzed By: AD Audited By: JT Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: CAB Authorized & Rechecked By: TJ/JT Copy Released By: AD/AB

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-16 10:00
2014-01-15 09:15	10.1	JT	Date & Time ELISA Procedure:	2014-01-16 13:05

Date Sampled: 2014-01-09

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	25

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research ONLY
Purolator #NHX000005580

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	55		R	5					MC-24		0.13	✔									
	56		R	5					MC-25		0.84		✔								
	57		R	5					MC-26		1.07		✔								
	58		R	5					MC-27	<	0.10	✔									
	59		R	5					MC-28		0.40		✔								
	60		R	5					MC-29	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-16 14:00 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-16 10:00
2014-01-15 09:15	10.1	JT	Date & Time ELISA Procedure:	2014-01-16 13:05

Date Sampled: 2014-01-09

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	25

Report To: **Gemma Charlebois**

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Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research ONLY
Purolator #NHX000005580

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	61		R	5					MC-30		0.62		✔								
	62		R	5					MC-31		1.13		✔								
	63		R	5					MC-32	<	0.10	✔									
	64		R	5					MC-33		1.04		✔								
	65		R	5					MC-34	<	0.10	✔									
	66		R	5					MC-35		0.92		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-16 14:00 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

CENTRAL ONTARIO ANALYTICAL LABORATORY INC.

4260 Burnside Line, R.R. #4
Orillia, ON L3V 6H4
Tel: (705) 326-8285 Fax: (705) 326-9316
www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-16 10:00
2014-01-15 09:15	10.1	JT	Date & Time ELISA Procedure:	2014-01-16 13:05

Date Sampled: 2014-01-09

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	25

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research ONLY
Purolator #NHX000005580 NDBT= No Data. Container Broken in Transit

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	67		R	5					NDBT		NDBT										
	68		R	5					MC-36		0.98		✔								
	69		R	5					MC-37		0.53		✔								
	70		R	5					MC-38		0.87		✔								
	71		R	5					MC-39		0.79		✔								
	72		R	5					MC-40	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-16 14:00 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-16 10:00
2014-01-15 09:15	10.1	JT	Date & Time ELISA Procedure:	2014-01-16 13:05

Date Sampled: 2014-01-09
Collected By: G. Charlebois
Name of Facility: University of Waterloo
Drinking Water System (DWS) #: **N/A**
Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	25

Report To: **Gemma Charlebois**
Address: 200 University Ave., Waterloo, ON
Telephone: 519 503-7166 Ext: Fax:
Email Address: gcharlebois@uwaterloo.ca
"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research ONLY
Purolator #NHX000005580

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	73		R	5					MC-41		0.78		✔								
	74		R	5					MC-42		0.58		✔								
	75		R	5					MC-43		0.72		✔								
	76		R	5					MC-44		1.01		✔								
	77		R	5					MC-45		0.83		✔								
	78		R	5					MC-46		0.12	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-16 14:00 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-16 10:00
2014-01-15 09:15	10.1	JT	Date & Time ELISA Procedure:	2014-01-16 13:05

Date Sampled: 2014-01-09

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	25

Report To: **Gemma Charlebois**

Address: 200 University Ave,, Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water. Research ONLY
Purolator #NHX000005580 NDBT= No Data. Container Broken in Transit.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	79		R	5					MC-47		0.99		✔								
	80		R	5					NDBT		NDBT										
	81		R	5					MC-48	<	0.10	✔									

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-16 14:00 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology: Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology: Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-23 10:30
2014-01-22 12:30	6.3	JT	Date & Time ELISA Procedure:	2014-01-23 13:22

Date Sampled: 2014-01-15

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	27

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005653
Dates taken from bottles.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	82		R	5					MC-49		0.98		✔								
	83		R	5					MC-50		0.60		✔								
	84		R	5					MC-51		0.64		✔								
	85		R	5					MC-52		0.54		✔								
	86		R	5					MC-53		0.67		✔								
	87		R	5					MC-54		2.53		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-23 14:00 Analyzed By: JT Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, JT

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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Tel: (705) 326-8285 Fax: (705) 326-9316
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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-23 10:30
2014-01-22 12:30	6.3	JT	Date & Time ELISA Procedure:	2014-01-23 13:22

Date Sampled: 2014-01-15

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: **N/A**

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	27

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005653
Dates taken from bottles. #88-90 Jan 15. #91-93 Jan 17.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	88		R	5					MC-55		0.54		✔								
	89		R	5					MC-56		0.10	✔									
	90		R	5					MC-57	<	0.10	✔									
	91		R	5					MC-58		0.13	✔									
	92		R	5					MC-59		3.99		✔								
	93		R	5					MC-60		0.18		✔								

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-23 14:00 Analyzed By: JT Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, JT

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-23 10:30
2014-01-22 12:30	6.3	JT	Date & Time ELISA Procedure:	2014-01-23 13:22

Date Sampled: 2014-01-18

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	27

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005653
Dates taken from bottles.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb					
	94		R	5					MC-61		0.18		✔								
	95		R	5					MC-62		1.56		✔								
	96		R	5					MC-63		3.92		✔								
	97		R	5					MC-64		3.47		✔								
	98		R	5					MC-65		0.26		✔								
	99		R	5					MC-66	<	0.10	✔									

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-23 14:00 Analyzed By: JT Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, JT

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-23 10:30
2014-01-22 12:30	6.3	JT	Date & Time ELISA Procedure:	2014-01-23 13:22

Date Sampled: 2014-01-18

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: **N/A**

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	27

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005653
Dates taken from bottles.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	100		R	5					MC-67		3.25		✔								
	101		R	5					MC-68		2.31		✔								
	102		R	5					MC-69		2.04		✔								
	103		R	5					MC-70		3.95		✔								
	104		R	5					MC-71		1.00		✔								
	105		R	5					MC-72		2.25		✔								

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-23 14:00 Analyzed By: JT Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, JT

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

CENTRAL ONTARIO ANALYTICAL LABORATORY INC.

4260 Burnside Line, R.R. #4
Orillia, ON L3V 6H4
Tel: (705) 326-8285 Fax: (705) 326-9316
www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-23 10:30
2014-01-22 12:30	6.3	JT	Date & Time ELISA Procedure:	2014-01-23 13:22

Date Sampled: 2014-01-18

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	5
# of samples sent	27
# of samples received	27

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Conditions/ Comments: Not Drinking Water Research ONLY
Purolator #NHX000005653
Dates taken from bottles.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb					
	106		R	5					MC-73		0.13	✔									
	107		R	5					MC-74		1.68		✔								
	108		R	5					MC-75	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-23 14:00 Analyzed By: JT Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, JT

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-29 11:00
2014-01-28 15:48	2.9	AD	Date & Time ELISA Procedure:	2014-01-29 13:44

Date Sampled: 2014-01-20

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 253 20 5573 1588
Dates as per bottles.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	109		R	5					MC-76		0.14	✔									
	110		R	5					MC-77		0.49		✔								
	111		R	5					MC-78		2.30		✔								
	112		R	5					MC-79		0.20		✔								
	113		R	5					MC-80	<	0.10	✔									
	114		R	5					MC-81	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-29 14:30 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB,AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-29 11:00
2014-01-28 15:48	2.9	AD	Date & Time ELISA Procedure:	2014-01-29 13:44

Date Sampled: 2014-01-20

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 1588
*Bottles #115-117 dated Jan 20/14; #118-120 dated Jan 22/14.

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	115		R	5					MC-82		0.10	✔									
	116		R	5					MC-83		3.00		✔								
	117		R	5					MC-84	<	0.10	✔									
	118		R	5					MC-85		1.83		✔								
	119		R	5					MC-86	<	0.10	✔									
	120		R	5					MC-87		0.16		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-29 14:30 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Orillia, ON L3V 6H4
Tel: (705) 326-8285 Fax: (705) 326-9316
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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-29 11:00
2014-01-28 15:48	2.9	AD	Date & Time ELISA Procedure:	2014-01-29 13:44

Date Sampled: 2014-01-22

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 1588

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	121		R	5					MC-88	<	0.10	✔									
	122		R	5					MC-89		0.78		✔								
	123		R	5					MC-90		0.29		✔								
	124		R	5					MC-91		1.41		✔								
	125		R	5					MC-92		3.01		✔								
	126		R	5					MC-93		0.18		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-29 14:30 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-29 11:00
2014-01-28 15:48	2.9	AD	Date & Time ELISA Procedure:	2014-01-29 13:44

Date Sampled: 2014-01-24

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

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Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 1588

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	127		R	5					MC-94		0.32		✔								
	128		R	5					MC-95		0.12	✔									
	129		R	5					MC-96	<	0.10	✔									
	130		R	5					MC-97		2.38		✔								
	131		R	5					MC-98		2.57		✔								
	132		R	5					MC-99		0.16		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-29 14:30 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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2014-01-28 15:48	2.9	AD	Date & Time ELISA Procedure:	2014-01-29 13:44

Date Sampled: 2014-01-24

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
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"Live Person" After Hours Contact Name and Tel. No:

Submitted By: G. Charlebois

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 1588
*Bottles #133-135 dated Jan 24/14; #136-138 dated Jan 25/14

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb					
	133		R	5					MC-100	<	0.10	✔									
	134		R	5					MC-101		1.41		✔								
	135		R	5					MC-102	<	0.10	✔									
	136		R	5					MC-103		0.12	✔									
	137		R	5					MC-104		0.12	✔									
	138		R	5					MC-105		1.90		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-29 14:30 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

Final Certificate Microcystin, PITA 111, 1311, Revision 1.4
Page 1 of 1

CENTRAL ONTARIO ANALYTICAL LABORATORY INC.

4260 Burnside Line, R.R. #4
Orillia, ON L3V 6H4
Tel: (705) 326-8285 Fax: (705) 326-9316
www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-01-29 11:00
2014-01-28 15:48	2.9	AD	Date & Time ELISA Procedure:	2014-01-29 13:44

Date Sampled: 2014-01-25
Collected By: G. Charlebois
Name of Facility: University of Waterloo
Drinking Water System (DWS) #: **N/A**
Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**
Address: 200 University Ave., Waterloo, ON
Telephone: 519 503-7166 Ext: Fax:
Email Address: gcharlebois@uwaterloo.ca
"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 1588

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	139		R	5					MC-106		0.12	✔									
	140		R	5					MC-107	<	0.10	✔									
	141		R	5					MC-108	<	0.10	✔									
	142		R	5					MC-109		2.17		✔								
	143		R	5					MC-110	<	0.10	✔									
	144		R	5					MC-111	<	0.10	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-01-29 14:30 Analyzed By: JT Audited By: AD Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AD

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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www.coalab.ca

Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-02-05 12:00
2014-02-04 15:25	4.3	AD	Date & Time ELISA Procedure:	2014-02-05 14:27

Date Sampled: 2014-02-03

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 253 20 5573 5486

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	149		R	5					MC-112		0.16		✔								
	150		R	5					MC-113		2.27		✔								
	151		R	5					MC-114		1.62		✔								
	152		R	5					MC-115		2.31		✔								
	153		R	5					MC-116		0.17		✔								
	154		R	5					MC-117		2.58		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-02-05 15:00 Analyzed By: AD Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB,AV

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-02-05 12:00
2014-02-04 15:25	4.3	AD	Date & Time ELISA Procedure:	2014-02-05 14:27

Date Sampled: 2014-02-03

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: N/A

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 5486

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Treatment Information: Other	Treatment Information: Total Cl_2 (mg/L)	Treatment Information: Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	Microcystin DM Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	Microcystin ADDA Total: ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	155		R	5					MC-118		0.14	✔									
	156		R	5					MC-119		2.55		✔								
	157		R	5					MC-120		1.57		✔								
	158		R	5					MC-121		0.79		✔								
	159		R	5					MC-122		2.42		✔								
	160		R	5					MC-123		0.28		✔								

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-02-05 15:00 Analyzed By: AD Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AV

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-02-05 12:00
2014-02-04 15:25	4.3	AD	Date & Time ELISA Procedure:	2014-02-05 14:27

Date Sampled: 2014-02-03

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: **N/A**

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 5486

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	161		R	5					MC-124		1.85		✔								
	162		R	5					MC-125		0.15		✔								
	163		R	5					MC-126	<	0.10	✔									
	164		R	5					MC-127		0.62		✔								
	165		R	5					MC-128		1.82		✔								
	166		R	5					MC-129		0.13	✔									

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Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-02-05 15:00 Analyzed By: AD Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AV

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-02-05 12:00
2014-02-04 15:25	4.3	AD	Date & Time ELISA Procedure:	2014-02-05 14:27

Date Sampled: 2014-02-03
Collected By: G. Charlebois
Name of Facility: University of Waterloo
Drinking Water System (DWS) #: **N/A**
Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**
Address: 200 University Ave., Waterloo, ON
Telephone: 519 503-7166 Ext: Fax:
Email Address: gcharlebois@uwaterloo.ca
"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 5486

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information: U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)	Laboratory Number	Microcystin DM Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total: >/<	ppb	Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
	167		R	5					MC-130		1.72		✔								
	168		R	5					MC-131		2.14		✔								
	169		R	5					MC-132		2.47		✔								
	170		R	5					MC-133		0.90		✔								
	171		R	5					MC-134	<	0.10	✔									
	172		R	5					MC-135		1.62		✔								

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-02-05 15:00 Analyzed By: AD Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AV

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-02-05 12:00
2014-02-04 15:25	4.3	AD	Date & Time ELISA Procedure:	2014-02-05 14:27

Date Sampled: 2014-02-03

Collected By: G. Charlebois

Name of Facility: University of Waterloo

Drinking Water System (DWS) #: **N/A**

Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**

Address: 200 University Ave., Waterloo, ON

Telephone: 519 503-7166 Ext: Fax:

Email Address: gcharlebois@uwaterloo.ca

"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 25320 5573 5486

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	173		R	5					MC-136		2.87		✔								
	174		R	5					MC-137		1.46		✔								
	175		R	5					MC-138		2.72		✔								
	176		R	5					MC-139		2.71		✔								
	177		R	5					MC-140		2.77		✔								
	178		R	5					MC-141	<	0.10	✔									

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-02-05 15:00 Analyzed By: AD Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB, AV

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

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Final Certificate Microcystin, PITA 111, 1311, Revision 1.4
Page 1 of 1

CENTRAL ONTARIO ANALYTICAL LABORATORY INC.

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Final Certficate of Microcystin Analysis of Water Only

Date & Time Received:	Temp. Rec'd. (°C):	Receiver:	Date & Time Heat Treatment:	2014-02-05 12:00
2014-02-04 15:25	4.3	AD	Date & Time ELISA Procedure:	2014-02-05 14:27

Date Sampled: 2014-02-03
Collected By: G. Charlebois
Name of Facility: University of Waterloo
Drinking Water System (DWS) #: **N/A**
Submitted By: G. Charlebois

Twp/Town	
Health Unit	
Regulation	N/A
Total # of pages sent	6
# of samples sent	36
# of samples received	36

Report To: **Gemma Charlebois**
Address: 200 University Ave., Waterloo, ON
Telephone: 519 503-7166 Ext: Fax:
Email Address: gcharlebois@uwaterloo.ca
"Live Person" After Hours Contact Name and Tel. No:

Conditions/ Comments: Not Drinking Water Research ONLY
UPS#1Z F85 253 20 5573 5486

Time Sampled	Sample Identifier	Identification of Collection Site	Water Type	Temp. Of Sample (°C)	Treatment Information				Laboratory Number	Microcystin DM Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results	Microcystin ADDA Total		Negative	Positive - Non Reportable	Provisional Positive Reportable	LaSB Results
					U.V.	Other	Total Cl_2 (mg/L)	Free Cl_2 (mg/L)		>/<	ppb					>/<	ppb				
	179		R	5					MC-142		2.62		✔								
	180		R	5					MC-143	<	0.10	✔									
	145		R	5					MC-144		0.13	✔									
	146		R	5					MC-145		2.24		✔								
	147		R	5					MC-146	<	0.10	✔									
	148		R	5					MC-147	<	0.10	✔									

All samples will be considered DISTRIBUTION samples unless otherwise indicated. All times are transcribed in the 24-hour clock for upload to the Ministry of the Environment (MOE) and/or Ministry of Health and Long Term Care (MoHLTC). Analysis performed by qualified analysts. Results relate only to the aliquot submitted. COAL is accredited by SCC and licenced by the MOE in these specific microcystin parameters. COAL accepts no responsibility for parameters selected, this is the responsibility of the submitting agency. For collection and handling procedures visit. www.coalab.ca/watersamples.html. Microcystin ADDA (COAL TOX) - Total (includes all Microcystin, Nodularins & Congeners); Microcystin DM (COAL TOX)- Total (includes all Microcystins). Microcystin LR Non Reportable limit is < 1.0 ppb or <1.0 µg/l in a raw water and a result ≥ 1.5 ppb or ≥1.5 µg/l in treated sample will cause the sampling agency to take appropriate action. (1 ppb = 1 µg/l) ELISA methodology is a screening for presence absence.

Raw = R
Distribution = D
Point of Entry = E
Recreational = L
Raw Water Consumed = RWC

Analysis Date & Time: 2014-02-05 15:00 Analyzed By: AD Audited By: AV Microcystin DM ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Analysis Date & Time: Analyzed By: Audited By: Microcystin ADDA ppb/ µg/l Abraxis ELISA Screening Methodology (COAL TOX)

Transcribed By: HM Authorized & Rechecked By: TJ/JT Copy Released By: AB,AV

Reported Provisional Adverse Submitting Agency @ Health Unit @ Spills Action Centre (SAC) @ AWQI # By: Date:

Final Results from LaSB Submitting Agency @ By: Date:

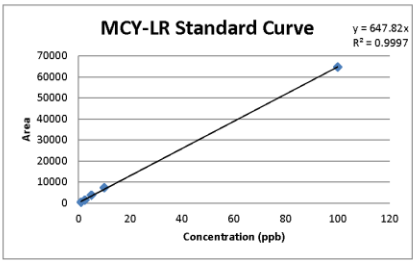
Test Methodology and Detection Limit

DM	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins DM using Abraxis ELISA Microtitre WO 01/18059
ADDA	COAL TOX	Detection Limit	0.10 ppb	Reportable Limit:	≥1.5 ppb	Methodology:	Determination of Microcystin-Nodularins ADDA using Abraxis ELISA Microtitre WO 01/18059 A2

Approved for use by TJ prior to use.

Microcystin-LR sample data for Gemma: 20141216

Standards (ppb)	Area	
1	286	
2.5	1283	
5	3632	
10	7182	
50	36676	excluded
100	64704	



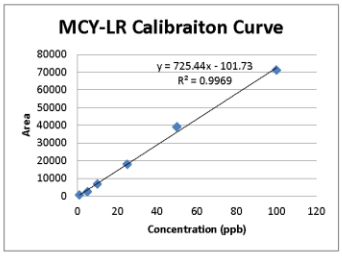
Sample	Concentration (ppb)	Expected (ppb)
1	ND	ND
2	ND	ND
3	4.94	< 2
4	5.06	< 5
5	6.37	< 2
6	2.16	< 5
7	5.58	< 2
8	ND	< 5
9	7.48	< 2
10	5.61	< 5
11	7.51	< 2
12	7.69	< 5
13	6.21	< 2
14	3.13	< 5
15	6.35	< 2
16	3.68	< 5
17	5.55	< 2
18	7.67	< 5
19	5.79	< 2
20	5.58	< 5
21	6.41	< 2
22	1.25	< 5
23	6.52	< 2
24	5.42	< 5
25	7.52	< 2
26	ND	< 5
27	7.38	< 2
28	1.33	< 5
29	4.28	< 2
30	3.79	< 5
31	7.75	< 2
32	0.99	< 5
33	6.29	< 2
34	BDL (0.26)	< 5
35	6.52	< 2
36	ND	< 5
37	ND	< 1
38	ND	< 1
39	BDL (0.15)	< 1
46	816.61	> 500
47	837.24	> 500
48	928.63	> 500

Postive Control	Concentration (ppb)
1	8.62
2	13.53
3	10.41
4	11.31
5	13.21
6	13.38
7	10.97
8	10.86
AVERAGE	11.54
Std Deviation	1.72
% CV	14.95
% Recovery	115.36

Negative Control	Concentration (ppb)
1	ND
2	ND
3	ND
4	ND
5	ND
6	ND
7	ND
8	ND

Microcystin-LR data for Gemma: 12-22-2014

Standards(ppb)	Area	Concentration (ppb)	Accuracy
1	649	1.04	103.50
5	2440	3.50	70.08
10	6755	9.45	94.52
25	17910	24.83	99.31
50	39085	54.02	108.03
100	71110	98.16	98.16



Sample	Concentration (ppb)	Expected (ppb)
49	ND	ND
50	ND	ND
51	7.10	<5
52	ND	<5
53	4.96	<5
54	4.85	<5
55	3.39	<5
56	ND	<5
57	2.49	<5
58	ND	<5
59	3.50	<5
60	6.53	<5
61	5.41	<5
62	ND	<5
63	5.41	<5
64	ND	<5
65	4.63	<5
66	2.38	<5
67	5.52	<5
68	ND	<5
69	3.95	<5
70	2.94	<5
71	3.95	<5
72	4.40	<5
73	4.85	<5
74	ND	<5
75	4.29	<5
76	3.05	<5
77	4.40	<5
78	ND	<5
79	5.86	<5
80	4.74	<5
81	3.39	<5
82	4.63	<5
83	3.39	<5
84	2.05	<5
85	6.20	<5
86	BDL (0.700)	<5
88	ND	ND
89	673.95	<500
90	ND	ND
91	556.98	<500
92	ND	ND

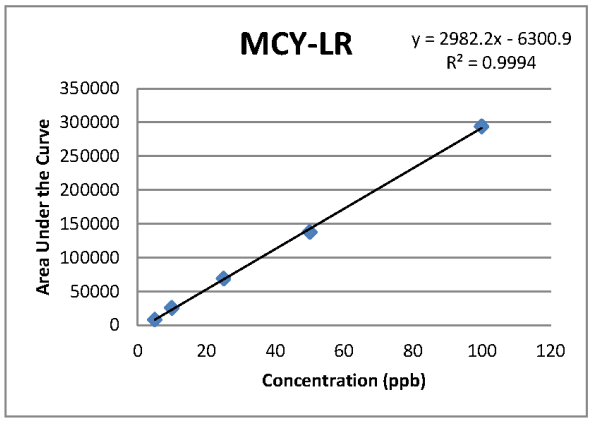
Postive Control	Concentration (ppb)
1-N	12.48
2-N	9.89
3-N	8.11
4-N	10.12
5-N	7.65
AVERAGE	9.65
Std Deviation	1.914901903
% CV	19.84230932
% Recovery	96.51

Negative Control	Concentration (ppb)
1	ND
2	ND
3	ND
4	ND
5	ND

Microcystin-LR Data for Gemma
Rerun of specified samples 20150206

(ppb)	MCY-LR		
Concentration	Area	Conc. (ppb)	Accuracy
1	1924		
5	8367	4.918482999	98.36966
10	25895	10.79602307	107.9602
25	69003	25.25112333	101.0045
50	137876	48.34581852	96.69164
100	293975	100.6893904	100.6894

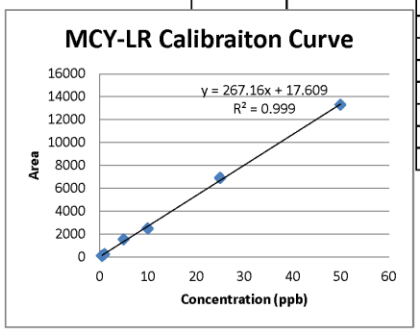
* Excluded



Sample	Area	Conc. (ppb)
3	7570	4.65
4	7256	4.55
11	8466	4.95
12	7615	4.67
17	11379	5.93
18	8260	4.88
59	7705	4.70
60	5733	4.04
71	8915	5.10
72	9183	5.19
81	5643	4.01
82	2149	2.83

Microcystin-LR data for Gemma: 03-13-2015

Standards(ppb)	Area	Conc. (ppb)	Accuracy
0.5	101	0.31	62.43
1	242	0.84	83.99
5	1520	5.62	112.47
10	2483	9.23	92.28
25	6910	25.80	103.19
50	13295	49.70	99.40



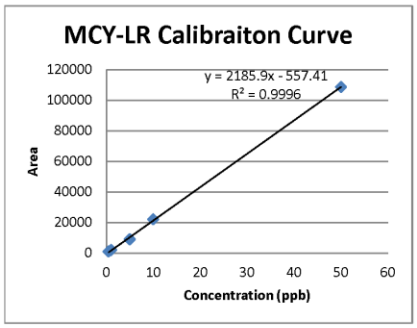
Sample	Conc. (ppb)	Expected (ppb)
1	13.42	8 ppb
2	BDL	< 1 ppb
3	15.24	8 ppb
4	BDL	< 1 ppb
5	14.67	8 ppb
6	BDL	< 1 ppb
7	16.06	8 ppb
8	BDL	< 1 ppb
9	14.87	8 ppb
10	BDL	< 1 ppb
11	15.06	8 ppb
12	BDL	< 1 ppb
13	1380.00	800 ppb
14	BDL	< 1 ppb

Postive Control	Conc. (ppb)
1	9.90
2	10.47
AVERAGE	10.19
Std Deviation	0.407600106
% CV	4.0014259
% Recovery	101.86

Negative Control	Conc. (ppb)
1	ND
2	ND

Microcystin-LR data for Gemma: 04-13-2015

Standards(ppb)	Area	Conc. (ppb)	Accuracy
0.5	1012	0.72	143.59
1	1863	1.11	110.73
5	8928	4.34	86.79
10	22050	10.34	103.42
25	47377	21.93	87.72
50	108725	49.99	99.99



Sample	Conc. (ppb)	Expected (ppb)
1	ND	< 2 ppb
2	ND	< 2 ppb
3	ND	< 2 ppb
4	ND	< 2 ppb
5	BDL	< 2 ppb
6	ND	< 2 ppb
7	ND	< 2 ppb
8	ND	< 2 ppb
9	BDL	< 2 ppb
10	BDL	< 2 ppb
11	1.94	< 2 ppb
12	BDL	< 2 ppb
13	4.35	< 5ppb
14	ND	< 5ppb
15	1096.55	5 ppb
16	0.74	5 ppb

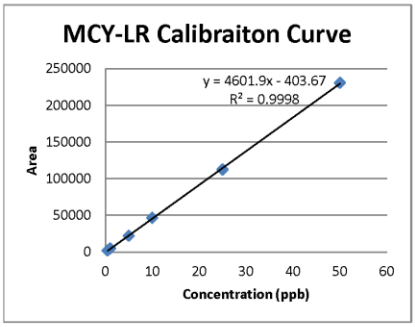
* ND = Not Detected
* BDL = Below Detection Limit

Postive Control	Conc. (ppb)
1	9.14
2	9.82
3	10.26
AVERAGE	9.48
Std Deviation	0.479728879
% CV	5.061755621
% Recovery	94.78

Negative Control	Conc. (ppb)
1	ND
2	ND
3	ND

Microcystin-LR data for Gemma: 05-06-2015

Standards(ppb)	Area	Conc. (ppb)	Accuracy
0.5	1968	0.46	92.98
1	4807	1.08	108.00
5	22164	4.84	96.81
10	46554	10.12	101.25
25	112603	24.43	97.74
50	230554	49.99	99.98



* ND = Not Detected

* BDL = Below Detection Limit

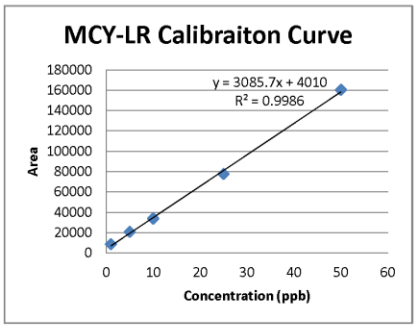
Sample	Conc. (ppb)	Expected (ppb)
17	ND	ND
18	ND	ND
19	ND	ND
20	ND	ND
21	BDL	< 2 ppb
22	ND	< 2 ppb
23	ND	ND
24	ND	ND
25	ND	ND
26	ND	ND
27	ND	ND
28	ND	ND
29	BDL	< 1 ppb
30	ND	< 1 ppb
31	674.80	~ 1000 ppb
32	BDL	~ 1000 ppb
33	ND	ND
34	ND	ND
35	ND	ND
36	ND	ND
37	ND	ND
38	ND	ND
39	ND	ND
40	ND	ND
41	ND	< 2 ppb
42	BDL	< 2 ppb
43	ND	< 2 ppb
44	ND	< 2 ppb
45	BDL	< 1 ppb
46	ND	< 1 ppb
47	1007	~ 1000 ppb
48	BDL	< 1 ppb

Postive Control	Conc. (ppb)
1	10.18
2	10.46
3	11.55
4	11.56
AVERAGE	10.94
Std Deviation	0.722619483
% CV	6.608166092
% Recovery	109.35

Negative Control	Conc. (ppb)
1	ND
2	ND
3	ND
4	ND

Microcystin-LR data for Gemma: 05-18-2015

Standards(ppb)	Area	Conc. (ppb)	Accuracy
0.5	8410	1.17	233.40
1	8653	1.50	150.47
5	20698	5.41	108.16
10	33766	9.64	96.43
25	77593	23.85	95.39
50	160136	50.60	101.19



* ND = Not Detected
* BDL = Below Detection Limit

Sample	Conc. (ppb)	Expected (ppb)
49	ND	ND
50	ND	ND
51	ND	ND
52	ND	ND
53	ND	ND
54	ND	ND
55	ND	ND
56	ND	ND
57	ND	ND
58	ND	ND
59	ND	ND
60	ND	ND
61	ND	ND
62	ND	< 2 ppb
63	945.81	~ 1000 ppb
64	1.10	ND
65	ND	ND
66	ND	ND
67	ND	ND
68	ND	ND
69	ND	< 2 ppb
70	ND	< 2 ppb
71	ND	ND
72	ND	ND
73	ND	ND
74	ND	ND
75	ND	ND
76	ND	ND
77	ND	ND
78	ND	ND
79	1040.41	~ 1000 ppb
80	ND	ND

Postive Control	Conc. (ppb)
1	10.93
2	11.42
3	11.36
4	12.63
5	12.82
6	11.74
AVERAGE	11.82
Std Deviation	0.752397981
% CV	6.365706422
% Recovery	118.20

Negative Control	Conc. (ppb)
1	ND
2	ND
3	ND
4	ND
5	ND
6	ND

Appendix 3

Data Analyses

Appendix 3.1

Experiment with Extracellular Toxin

experiments for all three residual values (0.6, 0.3, 0.1 mg/L)			Bottle #		0.10								
#	doc	pH		pH	O3 residual	DOC	MC-LR	bottle #	pH	O3 residual	DOC	MC-LR
1	0	7	1	7.10	0.11	4.50 <	0.10					
2	0	8.5	2	8.40	0.10	4.50	2.50					
3	10	8	3	7.98	0.08	10.00	2.59					
4	0	7	4	7.10	0.13	4.50		163	7.20	0.15	3.91 <	0.10
5	5	7.5	5	7.60	0.15	5.00		164	7.59	0.17	4.41	0.62
6	5	7	6	7.13	0.13	5.00 <	0.10					
7	5	8	7	8.08	0.06	5.00	3.83					
8	10	8	8	7.95	0.07	10.00	5.08					
blank			9			4.50 *	2.81					
9	15	7.5	10	7.57	0.11	15.00 <	0.10					
10	0	7	11	7.10	0.06	4.50	5.62					
11	0	8.5	12	8.44	0.08	4.50	0.48					
12	5	7	13	7.04	0.10	5.00 <	0.10					
13	15	7	14	6.97	0.08	15.00	2.66					
14	0	8	15	8.05	0.09	4.50	0.11					
15	10	7.5	16	7.49	0.11	10.00	7.45					
16	15	8	17	7.90	0.10	15.00	0.63					
blank			18			4.50 <	0.10					
17	5	8	19	7.99	0.15	5.00	0.14	165	7.97	0.14	4.41	1.82
18	5	7	20	7.15	0.09	5.00 <	0.10	166	7.14	0.09	4.41	0.13
19	10	7	21	7.25	0.12	10.00	8.24	167	7.03	0.10	9.41	1.72
20	15	7	22	7.10	0.08	15.00	8.95	168	7.10	0.15	14.41	2.14
21	10	7.5	23	7.70	0.06	10.00	9.01	169	7.64	0.08	9.41	2.47
22	5	7.5	24	7.56	0.18	5.00	0.21	170	7.55	0.14	4.41	0.90
23	0	8.5	25	8.48	0.10	4.50		172	8.55	0.15	3.91	1.62
24	10	8.5	26	8.41	0.14	10.00	5.80	173	8.61	0.12	9.41	2.87
blank			27			4.50		171	blank		3.91 <	0.10

experiments for all three residual values (0.6, 0.3, 0.1 mg/L)			Bottle #	0.10								
#	doc	pH		pH	O3 residual	DOC	MC-LR	bottle #	pH	O3 residual	DOC	MC-LR
25	15	8	28	7.64	0.12	13.70	4.75					
26	0	8	29	7.87	0.15	3.20		174	7.91	0.12		1.46
27	10	8.5	30	8.38	0.15	8.70		175	8.60	0.06		2.72
28	0	7.5	31	7.41	0.07	3.20	3.32					
29	5	8.5	32	8.51	0.08	3.70	3.93					
30	0	8	33	7.88	0.08	3.20	1.85					
31	15	7	34	7.00	0.15	13.70	2.57					
32	0	7.5	35	7.36	0.10	3.20	4.91					
blank			36			3.20 <	0.10					
33	5	8	37	8.05	0.14	3.80	4.30					
34	15	8.5	38	8.31	0.12	13.80		176	8.51	0.07		2.71
35	0	7.5	39	7.64	0.15	3.30	2.73					
36	5	7.5	40	7.56	0.07	3.80	2.17					
37	5	8.5	41	8.55	0.08	3.80	3.58					
38	15	8.5	42	8.45	0.10	13.80	3.84					
39	15	7.5	43	7.64	0.09	13.80		177	7.45	0.12		2.77
40	10	7	44	7.19	0.11	8.80	3.91					
blank			45			3.30 <	0.10					
41	15	8	46	7.86	0.11	14.10	3.34					
42	10	8.5	47	8.38	0.12	9.10	3.05					
43	15	8.5	48	8.45	0.15	14.10	1.65					
44	10	8	49	8.18	0.09	9.10	1.86					
45	10	7.5	50	7.51	0.12	9.10	2.01					
46	15	7.5	51	7.64	0.08	14.10	1.95					
47	5	8.5	52	8.45	0.07	4.10	2.02					
48	10	7	53	7.25	0.07	9.10	2.01					
blank			54			3.60 <	0.10					
		min			0.06							
		max			0.18							

experiments for all three residual values (0.6, 0.3, 0.1 mg/L)			bottle #	0.3									bottle #	0.6			
#	doc	pH		pH	O3 residual	DOC	MC-LR	bottle#	pH	O3 residual	DOC	MC-LR		pH	O3 residua	DOC	MC-LR
1	0	7	55	7.20	0.27	4.39	0.13						109	7.14	0.67	3.44	0.14
2	0	8.5	56	8.43	0.31	4.39	0.84						110	8.61	0.62	3.44	0.49
3	10	8	57	7.99	0.22	9.89	1.07						111	8.11	0.63	8.94	2.3
4	0	7	58	7.16	0.24	4.39 <	0.10						112	7.08	0.61	3.44	0.2
5	5	7.5	59	7.51	0.33	4.89	0.40						113	7.48	0.57	3.94 <	0.1
6	5	7	60	7.2	0.40	4.89 <	0.10						114	7.17	0.67	3.94 <	0.1
7	5	8	61	8.09	0.25	4.89	0.62						115	8.1	0.54	3.94	0.1
8	10	8	62	7.92	0.41	9.89	1.13						116	7.89	0.58	8.94	3
blank			63			4.39 <	0.10						117			3.44 <	0.1
9	15	7.5	64	7.51	0.30	15.76	1.04						118	7.51	0.63	14.30	1.83
10	0	7	65	7.21	0.28	5.26 <	0.10						119	7.11	0.56	3.80 <	0.1
11	0	8.5	66	8.53	0.25	5.26	0.92						120	8.37	0.59	3.80	0.16
12	5	7	67	7.15	0.34	5.76		178	7.12	0.25	<	0.1	121	7.05	0.67	4.30 <	0.1
13	15	7	68	7.13	0.43	15.76	0.98						122	7.06	0.56	14.30	0.78
14	0	8	69	8.01	0.41	5.26	0.53						123	8.1	0.63	3.80	0.29
15	10	7.5	70	7.6	0.32	10.76	0.87						124	7.59	0.53	9.30	1.41
16	15	8	71	7.96	0.38	15.76	0.79						125	8.05	0.53	14.30	3.01
blank			72			5.26 <	0.10						126			3.80	0.18
17	5	8	73	7.83	0.28	5.71	0.78						127	7.76	0.59	5.00	0.32
18	5	7	74	6.76	0.25	5.71	0.58						128	7.01	0.55	5.00	0.12
19	10	7	75	7.29	0.38	10.71	0.72						129	6.96	0.56	10.00 <	0.1
20	15	7	76	7.22	0.40	15.71	1.01						130	6.8	0.65	15.00	2.38
21	10	7.5	77	7.43	0.43	10.71	0.83						131	7.58	0.7	10.00	2.57
22	5	7.5	78	7.7	0.32	5.71	0.12						132	7.6	0.58	5.00	0.16
23	0	8.5	79	8.43	0.24	5.21	0.99						133	8.55	0.55	4.50 <	0.1
24	10	8.5	80	8.48	0.39	10.71		179	8.52	0.26		2.62	134	8.49	0.68	10.00	1.41
blank			81			5.21 <	0.10	180	blank		<	0.1	135			4.50 <	0.1

experiments for all three residual values (0.6, 0.3, 0.1 mg/L)			bottle #	0.3									bottle #	0.6			
#	doc	pH		pH	O3 residual	DOC	MC-LR	bottle#	pH	O3 residual	DOC	MC-LR		pH	O3 residua	DOC	MC-LR
25	15	8	82	7.97	0.45	14.88	0.98						136	8.08	0.55	14.74	0.12
26	0	8	83	8.41	0.38	4.38	0.60						137	8.09	0.65	4.24	0.12
27	10	8.5	84	8.29	0.23	9.88	0.64						138	8.5	0.65	9.74	1.9
28	0	7.5	85	7.49	0.45	4.38	0.54						139	7.53	0.7	4.24	0.12
29	5	8.5	86	8.61	0.39	4.88	0.67						140	8.47	0.53	4.74 <	0.1
30	0	8	87	8.17	0.31	4.38	2.53						141	8.2	0.63	4.24 <	0.1
31	15	7	88	7.32	0.34	14.88	0.54						142	7.2	0.52	14.74	2.17
32	0	7.5	89	7.61	0.40	4.38	0.10						143	7.62	0.63	4.24 <	0.1
blank			90			4.38 <	0.10						144			4.24 <	0.1
33	5	8	91	7.9	0.30	3.95	0.13						145	7.81	0.61	4.63	0.13
34	15	8.5	92	8.6	0.27	13.95	3.99						146	8.33	0.53	14.63	2.24
35	0	7.5	93	7.55	0.29	3.45	0.18						147	7.6	0.61	4.13 <	0.1
36	5	7.5	94	7.5	0.41	3.95	0.18						148	7.52	0.58	4.63 <	0.1
37	5	8.5	95	8.52	0.33	3.95	1.56						149	8.58	0.58	4.63	0.16
38	15	8.5	96	8.62	0.26	13.95	3.92						150	8.55	0.61	14.63	2.27
39	15	7.5	97	7.58	0.40	13.95	3.47						151	7.49	0.52	14.63	1.62
40	10	7	98	7.02	0.28	8.95	0.26						152	7.11	0.56	9.63	2.31
blank			99			3.45 <	0.10						153			4.13	0.17
41	15	8	100	8.01	0.32	13.77	3.25						154	8.2	0.53	14.62	2.58
42	10	8.5	101	8.58	0.32	8.77	2.31						155	8.64	0.59	9.62	0.14
43	15	8.5	102	8.61	0.32	13.77	2.04						156	8.69	0.53	14.62	2.55
44	10	8	103	8.1	0.37	8.77	3.95						157	8.1	0.57	9.62	1.57
45	10	7.5	104	7.45	0.35	8.77	1.00						158	7.53	0.54	9.62	0.79
46	15	7.5	105	7.41	0.39	13.77	2.25						159	7.59	0.65	14.62	2.42
47	5	8.5	106	8.64	0.31	3.77	0.13						160	8.54	0.64	4.62	0.28
48	10	7	107	7.16	0.39	8.77	1.68						161	6.98	0.51	9.62	1.85
blank			108			3.27 <	0.10						162			4.12	0.15
		min		min	0.22									min	0.52		
		max		max	0.45									max	0.7		

		0	1
A	pH	7.0	8.5
B	O3	0.6	0.1
C	DOC	4	15

	bottles	avg MC-LR	std dev
1	109, 112, 119	0.15	0.050332
a	110, 120, 133	0.25	0.190526
b	1, 4, 11	0.1	0
ab	2, 12, 25	1.53	1.012785
c	122, 130, 142	1.78	0.869502
ac	146, 150, 156	2.35	0.170978
bc	14, 22, 34	2.46	0.277909
abc	38, 42, 48	2.73	1.095186

		A	B	C	ab	ac	bc	abc	MC-LR	mean	yhat	y - y hat
1	1	0	0	0	1	1	1	0	0.15	1.42	-0.366667	0.51
2	a	1	0	0	0	0	1	1	0.25		-0.366667	0.62
3	b	0	1	0	0	1	0	1	0.10		0.695833	-0.60
4	c	0	0	1	1	0	0	1	1.53		1.700833	-0.17
5	ab	1	1	0	1	0	0	0	1.78		0.695833	1.08
6	ac	1	0	1	0	1	0	0	2.35		1.700833	0.65
7	bc	0	1	1	0	0	1	0	2.46		2.03	0.43
8	abc	1	1	1	1	1	1	1	2.73		2.03	0.70

	A	B	C	ab	ac	bc	abc
effect	0.719167	0.695833	1.700833	0.2575	-0.170833	-0.366667	-0.529167
SS	3.103204	2.905104	17.357	0.397838	0.175104	0.806667	1.680104
DF	1	1	1	1	1	1	1
MS	3.103204	2.905104	17.357	0.397838	0.175104	0.806667	1.680104

Column1	
Mean	0.274565217
Standard Error	0.037599088
Median	0.13
Mode	0.1
Standard Deviation	0.255009425
Sample Variance	0.065029807
Kurtosis	0.951454832
Skewness	1.453350575
Range	0.89
Minimum	0.1
Maximum	0.99
Sum	12.63
Count	46
Confidence Level(95.0%)	0.075728451

DOC	5 mg/L
O3	0.3 mg/L
	0.6 mg/L

2 abnormal values removed.

Column1	
Mean	1.517916667
Standard Error	0.197581371
Median	1.41
Mode	2.31
Standard Deviation	0.967947084
Sample Variance	0.936921558
Kurtosis	0.146168735
Skewness	0.617072401
Range	3.85
Minimum	0.1
Maximum	3.95
Sum	36.43
Count	24
Confidence Level(95.0%)	0.408728207

DOC	10 mg/L
O3	0.3 mg/L
	0.6 mg/L

Column1	
Mean	2.00958333
Standard Error	0.21846507
Median	2.205
Mode	0.98
Standard Deviation	1.07025588
Sample Variance	1.14544764
Kurtosis	-0.7266018
Skewness	0.13634679
Range	3.87
Minimum	0.12
Maximum	3.99
Sum	48.23
Count	24
Confidence Level(95.0%)	0.45192942

DOC	15 mg/L
O3	0.3 mg/L
	0.6 mg/L

concentrations of microcystin-LR remaining at 0.1 mg/L ozone compared to concentrations at 0.3 mg/L and 0.6 mg/L ozone at all experimental conditions

Treatment 1

N_1: 48
$df_1 = N - 1 = 48 - 1 = 47$
M_1: 2.4
SS_1: 124.89
$s^2_1 = SS_1/(N - 1) = 124.89/(48-1) = 2.66$

Treatment 2

N_2: 48
$df_2 = N - 1 = 48 - 1 = 47$
M_2: 1.13
SS_2: 57.6
$s^2_2 = SS_2/(N - 1) = 57.6/(48-1) = 1.23$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((47/94) * 2.66) + ((47/94) * 1.23) = 1.94$
$s^2_{M1} = s^2_p/N_1 = 1.94/48 = 0.04$
$s^2_{M2} = s^2_p/N_2 = 1.94/48 = 0.04$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 1.27/\sqrt{0.08} = 4.46$
The t-value is 4.45953. The p-value is .000023. The result is significant at $p < .05$.

concentrations of microcystin-LR remaining at 0.3 mg/L ozone compared to concentrations at 0.6 mg/L ozone at all experimental conditions

Treatment 1

N_1: 48
$df_1 = N - 1 = 48 - 1 = 47$
M_1: 1.13
SS_1: 57.6
$s^2_1 = SS_1/(N - 1) = 57.6/(48-1) = 1.23$

Treatment 2

N_2: 48
$df_2 = N - 1 = 48 - 1 = 47$
M_2: 0.98
SS_2: 50.15
$s^2_2 = SS_2/(N - 1) = 50.15/(48-1) = 1.07$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((47/94) * 1.23) + ((47/94) * 1.07) = 1.15$
$s^2_{M1} = s^2_p/N_1 = 1.15/48 = 0.02$
$s^2_{M2} = s^2_p/N_2 = 1.15/48 = 0.02$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 0.15/\sqrt{0.05} = 0.68$
The t-value is 0.68256. The p-value is .496567. The result is *not* significant at $p < .05$.

concentrations of microcystin-LR remaining at 0.3 and 0.6 mg/L ozone with 5 mg/L or less DOC compared to concentrations of microcystin-LR with 10 or greater mg/L DOC

Treatment 1

N_1: 48
$df_1 = N - 1 = 48 - 1 = 47$
M_1: 0.35
SS_1: 9.4
$s^2_1 = SS_1/(N - 1) = 9.4/(48-1) = 0.2$

Treatment 2

N_2: 48
$df_2 = N - 1 = 48 - 1 = 47$
M_2: 1.76
SS_2: 50.8
$s^2_2 = SS_2/(N - 1) = 50.8/(48-1) = 1.08$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((47/94) * 0.2) + ((47/94) * 1.08) = 0.64$
$s^2_{M1} = s^2_p/N_1 = 0.64/48 = 0.01$
$s^2_{M2} = s^2_p/N_2 = 0.64/48 = 0.01$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = -1.42/\sqrt{0.03} = -8.66$
The t-value is -8.66476. The p-value is < .00001. The result is significant at $p < .05$.

concentrations of microcystin-LR remaining at 0.3 and 0.6 mg/L ozone with 10 mg/L DOC compared to concentration of microcystin-LR with 15 mg/L DOC

Treatment 1

N_1: 24
$df_1 = N - 1 = 24 - 1 = 23$
M_1: 1.52
SS_1: 21.55
$s^2_1 = SS_1/(N - 1) = 21.55/(24-1) = 0.94$

Treatment 2

N_2: 24
$df_2 = N - 1 = 24 - 1 = 23$
M_2: 2.01
SS_2: 26.35
$s^2_2 = SS_2/(N - 1) = 26.35/(24-1) = 1.15$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((23/46) * 0.94) + ((23/46) * 1.15) = 1.04$
$s^2_{M1} = s^2_p/N_1 = 1.04/24 = 0.04$
$s^2_{M2} = s^2_p/N_2 = 1.04/24 = 0.04$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = -0.49/\sqrt{0.09} = -1.67$
The t-value is -1.66916. The p-value is .101879. The result is *not* significant at $p < .05$.

concentrations of microcystin-LR remaining at 0.3 mg/L ozone and 5 or less mg/L DOC vs. concentrations of microcystin-LR remaining at 0.6 mg/L ozone and 5 or less mg/L DOC

Treatment 1

N_1: 24
$df_1 = N - 1 = 24 - 1 = 23$
M_1: 0.54
SS_1: 7.45
$s^2_1 = SS_1/(N - 1) = 7.45/(24-1) = 0.32$

Treatment 2

N_2: 24
$df_2 = N - 1 = 24 - 1 = 23$
M_2: 0.16
SS_2: 0.21
$s^2_2 = SS_2/(N - 1) = 0.21/(24-1) = 0.01$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) =$
$((23/46) * 0.32) + ((23/46) * 0.01) = 0.17$
$s^2_{M1} = s^2_p/N_1 = 0.17/24 = 0.01$
$s^2_{M2} = s^2_p/N_2 = 0.17/24 = 0.01$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 0.38/\sqrt{0.01} = 3.23$
The t-value is 3.23203. The p-value is .002275. The result is significant at $p < .05$.

concentrations of microcystin-LR remaining at 0.3 mg/L ozone at 10 or greater mg/L DOC vs. concentrations of microcystin-LR remaining at 0.6 mg/L ozone and 10 or greater mg/L DOC

Treatment 1

N_1: 24
$df_1 = N - 1 = 24 - 1 = 23$
M_1: 1.72
SS_1: 33.33
$s^2_1 = SS_1/(N - 1) = 33.33/(24-1) = 1.45$

Treatment 2

N_2: 24
$df_2 = N - 1 = 24 - 1 = 23$
M_2: 1.8
SS_2: 17.38
$s^2_2 = SS_2/(N - 1) = 17.38/(24-1) = 0.76$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) =$
$((23/46) * 1.45) + ((23/46) * 0.76) = 1.1$
$s^2_{M1} = s^2_p/N_1 = 1.1/24 = 0.05$
$s^2_{M2} = s^2_p/N_2 = 1.1/24 = 0.05$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = -0.08/\sqrt{0.09} = -0.27$
The t-value is -0.27218. The p-value is .786699. The result is *not* significant at $p < .05$.

	microcystin-LR remaining at 5, 10, 15 mg/L DOC and 8.0, 8.5 pH	
	at 0.3 mg/L ozone	at 0.6 mg/L ozone
	1.07	2.3
	0.62	0.1
	1.13	3
	0.79	3.01
	0.78	0.32
	2.62	1.41
	0.98	0.12
	0.64	1.9
	0.67	0.1
	0.13	0.13
	3.99	2.24
	1.56	0.16
	3.92	2.27
	3.25	2.58
	2.31	0.14
	2.04	2.55
	3.95	1.57
	0.13	0.28
average	1.698889	1.343333
std. dev.	1.295003	1.118978

combined average	1.521111
combined std. dev.	1.223183

Appendix 3.2

Experiment with *Microcystis aeruginosa* cells

#	doc	pH	cells (mL)	Bottle #	pH	0.30 O3 residual	DOC	toxin T	toxin E	toxin I	CT
15	15	8	10	1	7.86	0.34	15.39	7.10	1.00	6.10	5.10
4	5	8.5	10	2	8.39	0.24	4.89	4.96	4.85	0.11	3.60
										0.00	
7	10	8	10	3	8.05	0.25	10.39	3.39	1.00	2.39	3.75
5	5	8.5	10	4	8.36	0.29	4.55	4.94	5.06		
				4			4.55	4.65	4.55	0.10	4.35
11	10	8.5	10	5	8.51	0.35	10.00	6.37	2.16	4.21	5.25
										0.00	
12	10	8.5	10	6	8.61	0.35	10.00	5.58	1.00	4.58	5.25
9	10	8	10	7	7.80	0.42	10.39	2.49	1.00	1.49	6.30
6	5	8.5	10	8	8.39	0.35	4.89	3.50	6.53		
							4.89	4.70	4.04	0.66	5.25
13	15	8	10	9	8.01	0.26	15.39	5.41	1.00	4.41	3.90
blank			0	10	7.60	-	4.82	ND	ND		
negative			1	11				860.83	ND		
8	10	8	10	12	8.16	0.28	10.39	5.41	1.00	4.41	4.20
1	5	8	10	13	8.23	0.30	4.89	4.63	2.38	2.25	4.50
16	15	8.5	10	14	8.63	0.33	15.39	5.52	1.00	4.52	4.95
2	5	8	10	15	7.94	0.40	4.77	3.95	2.94	1.01	6.00
14	15	8	10	16	8.12	0.25	15.27	3.95	4.40		
								5.10	5.19	0.00	3.75
18	15	8.5	10	17	8.34	0.27	15.27	4.85	1.00	3.85	4.05
3	5	8	10	18	8.18	0.29	4.77	4.29	3.05	1.24	4.35
17	15	8.5	10	19	8.48	0.23	15.27	4.40	1.00	3.40	3.45
10	10	8.5	10	20	8.57	0.39	10.27	5.86	4.74	1.12	5.85
blank			0	21	7.48	-	4.89	ND	ND		
negative			54	22				615.47	ND		

#	doc	pH	bottle #	pH	0.6 O3 residual	DOC	toxin T	toxin E	toxin I	CT
15	15	8	23	8.20	0.59	15.00	7.48	5.61	1.87	8.85
4	5	8.5	24	8.8	0.54	4.55	7.51	7.69		
						4.55	4.95	4.67	0.28	8.10
7	10	8	25	8.06	0.56	10.00	6.21	3.13	3.08	8.40
5	5	8.5	26	8.46	0.67	4.82	6.35	3.68	2.67	10.05
11	10	8.5	27	8.71	0.72	10.32	5.55	7.67		
						10.32	5.93	4.88	1.05	10.80
12	10	8.5	28	8.45	0.72	10.32	5.79	5.58	0.21	10.80
9	10	8	29	7.97	0.53	10.00	6.41	1.25	5.16	7.95
6	5	8.5	30	8.61	0.59	4.55	6.52	5.42	1.10	8.85
13	15	8	31	8.12	0.57	15	7.52	1.00	6.52	8.55
blank			65	7.5	-	4.55	ND	ND		
negative			66				860.83	ND		
8	10	8	32	8.08	0.53	10.32	7.38	1.33	6.05	7.95
1	5	8	33	8.12	0.57	4.82	4.28	3.79	0.49	8.55
16	15	8.5	34	8.52	0.71	15.32	7.75	0.99	6.76	10.65
2	5	8	35	8.09	0.60	4.77	3.39	4.63		
						4.77	4.01	2.83	1.18	9.00
14	15	8	36	8.26	0.64	15.32	6.29	0.29	6.00	9.60
18	15	8.5	37	8.44	0.53	15.32	6.52	1.00	5.52	7.95
3	5	8	38	8.2	0.54	4.77	3.39	2.05	1.34	8.10
17	15	8.5	39	8.55	0.53	15.27	6.20	0.70	5.50	7.95
10	10	8.5	40							
blank			41	7.55		4.771	ND	ND		
negative			42				615.47	ND		

			total toxin			extracellular	
	initial	remaining total	percentage destroyed		percentage remaining	remaining extracelluar	percentage remaining
5 mg/L	6.1547	4.96	0.19411182		0.80588818	4.85	0.78801566
5	8.6083	4.65	0.45982366		0.54017634	4.55	0.52855965
	6.1547	4.7	0.23635596		0.76364404	4.04	0.65640892
	6.1547	4.63	0.24772938		0.75227062	2.38	0.38669635
	6.1547	3.95	0.35821405		0.64178595	2.94	0.47768372
	6.1547	4.29	0.30297171		0.69702829	3.05	0.49555624
	8.6083	4.95	0.42497357		0.57502643	4.67	0.54249968
	8.6083	6.35	0.26233983		0.73766017	3.68	0.42749439
	8.6083	6.52	0.24259145		0.75740855	5.42	0.6296249
	8.6083	4.28	0.50280543		0.49719457	3.79	0.44027276
	6.1547	4.01	0.3484654		0.6515346	2.83	0.4598112
	6.1547	3.39	0.44920142		0.55079858	2.05	0.33307879
avg	7.177033		0.33579864	33.57986	0.66420136		0.51380852
stdev			0.09512353	9.512353	0.09512353		0.11642458
10 mg/L	6.1547	3.39	0.44920142		0.55079858	1	0.16247746
10	8.6083	6.37	0.2600165		0.7399835	2.16	0.25092062
	8.6083	5.58	0.35178839		0.64821161	1	0.11616696
	6.1547	2.49	0.59543113		0.40456887	1	0.16247746
	6.1547	5.41	0.12099696		0.87900304	1	0.16247746
	6.1547	5.86	0.04788211		0.95211789	4.74	0.77014314
	8.6083	6.21	0.27860321		0.72139679	3.13	0.36360257
	8.6083	5.93	0.31112996		0.68887004	4.88	0.56689474
	8.6083	5.79	0.32739333		0.67260667	5.58	0.64821161
	8.6083	6.41	0.25536982		0.74463018	1.25	0.14520869
	8.6083	7.38	0.14268787		0.85731213	1.33	0.15450205
avg			0.28550006	28.55001	0.71449994		0.31846207
stdev			0.14622062	14.62206	0.14622062		0.22398973
15 mg/L	6.1547	7.1	0		1	1	0.16247746
15	6.1547	5.41	0.12099696		0.87900304	1	0.16247746
	6.1547	5.52	0.10312444		0.89687556	1	0.16247746
	6.1547	4.85	0.21198434		0.78801566	1	0.16247746
	6.1547	4.4	0.28509919		0.71490081	1	0.16247746
	8.6083	7.48	0.13107118		0.86892882	5.61	0.65169662
	8.6083	7.52	0.1264245		0.8735755	1	0.11616696
	8.6083	7.75	0.0997061		0.9002939	0.99	0.11500529
	8.6083	6.29	0.26930985		0.73069015	0.29	0.03368842
	8.6083	6.52	0.24259145		0.75740855	1	0.11616696
	6.1547	6.2	0		1	0.7	0.11373422
avg			0.14457346	14.45735	0.85542654		0.17807688
stdev			0.09344907	9.344907	0.09344907		0.15437972

% Microcystis Destroyed, 5 mg/L DOC

Column1	
Mean	0.33579864
Standard Error	0.029851947
Median	0.325718557
Mode	#N/A
Standard Deviation	0.103410177
Sample Variance	0.010693665
Kurtosis	-1.401226789
Skewness	0.295385003
Range	0.308693615
Minimum	0.194111817
Maximum	0.502805432
Sum	4.029583684
Count	12
Confidence Level(95.0%)	0.065703692

% Microcystis Destroyed, 10 mg/L DOC

Column1	
Mean	0.285500063
Standard Error	0.046239021
Median	0.278603209
Mode	#N/A
Standard Deviation	0.153357484
Sample Variance	0.023518518
Kurtosis	0.574922769
Skewness	0.464749447
Range	0.547549028
Minimum	0.047882106
Maximum	0.595431134
Sum	3.140500694
Count	11
Confidence Level(95.0%)	0.10302696

% Microcystis Destroyed, 15 mg/L DOC

Column1	
Mean	0.144573455
Standard Error	0.029551192
Median	0.126424497
Mode	0
Standard Deviation	0.098010215
Sample Variance	0.009606002
Kurtosis	-0.935709784
Skewness	-0.034117666
Range	0.285099192
Minimum	0
Maximum	0.285099192
Sum	1.590308008
Count	11
Confidence Level(95.0%)	0.065844158

concentration of total microcystin-LR remaining in all water matrices with 5 mg/L DOC vs. water matrices with 15 mg/L DOC

Treatment 1

N_1: 12
$df_1 = N - 1 = 12 - 1 = 11$
M_1: 0.34
SS_1: 0.12
$s^2_1 = SS_1/(N - 1) = 0.12/(12-1) = 0.01$

Treatment 2

N_2: 9
$df_2 = N - 1 = 9 - 1 = 8$
M_2: 0.18
SS_2: 0.04
$s^2_2 = SS_2/(N - 1) = 0.04/(9-1) = 0.01$

T-value Calculation

$$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((11/19) * 0.01) + ((8/19) * 0.01) = 0.01$$
$s^2_{M1} = s^2_p/N_1 = 0.01/12 = 0$
$s^2_{M2} = s^2_p/N_2 = 0.01/9 = 0$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 0.16/\sqrt{0} = 3.9$
The t-value is 3.90019. The p-value is .000962. The result is significant at $p < .05$.

concentration of extracellular microcystin-LR remaining in all water matrices with 5 mg/L DOC vs. water matrices with 15 mg/L DOC

Treatment 1

N_1: 12
$df_1 = N - 1 = 12 - 1 = 11$
M_1: 0.78
SS_1: 0.28
$s^2_1 = SS_1/(N - 1) = 0.28/(12-1) = 0.03$

Treatment 2

N_2: 9
$df_2 = N - 1 = 9 - 1 = 8$
M_2: 0.22
SS_2: 0.33
$s^2_2 = SS_2/(N - 1) = 0.33/(9-1) = 0.04$

T-value Calculation

$$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) = ((11/19) * 0.03) + ((8/19) * 0.04) = 0.03$$
$s^2_{M1} = s^2_p/N_1 = 0.03/12 = 0$
$s^2_{M2} = s^2_p/N_2 = 0.03/9 = 0$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 0.55/\sqrt{0.01} = 7$
The t-value is 7.00253. The p-value is $< .00001$. The result is significant at $p < .05$.

total toxin at 0.3 mg O3/L	total toxin at 0.6 mg O3/L
7.10	7.48
4.96	4.95
3.39	6.21
4.65	6.35
6.37	5.93
5.58	5.79
2.49	6.41
4.70	6.52
5.41	7.52
5.41	7.38
4.63	4.28
5.52	7.75
3.95	4.01
4.85	6.29
4.29	6.52
4.40	3.39
5.86	6.20
average 4.92	6.06
std. dev. 1.059407	1.219761

combined average	5.49
combined std. dev.	1.277232

Appendix 3.3

Experiment with Microcystis aeruginosa cells in absence of DOC

#	cells/mL	mg O3/L	sample ID	total toxin	sample ID	extracell toxin	cell count (/mL) intact	cell count (/mL) DAPV	proportion of initial cell count intact	proportion of initial cell count DAPV	DAPV/cells
6	2000	0.4	1	ND	2	ND	71	44	0.0355	0.022	0.620
10	2000	0.27	3	ND	4	ND	750	770	0.375	0.385	1.027
11	10000	0.25	5	BDL	6	ND	735	155	0.0735	0.0155	0.211
9	100	0.3	7	ND	8	ND	0	2	0	0.02	2.000
8	100000	0.34	9	BDL	10	BDL	6600	10000	0.066	0.1	1.515
12	100000	0.27	11	1.94	12	BDL	7800	10000	0.078	0.1	1.282
13	100000	0	13	4.35	14	ND	108333.3	0			
14	21300000	0	15	1096.55	16	0.74					
7	10000	0.45	65	ND	66	ND	34	8	0.0034	0.0008	0.235
3	10000	0.33	67	ND	68	ND	64	30	0.0064	0.003	0.469
4	100000	0.24	69	ND	70	ND	10000	35000 app	0.1	0.35	3.500
5	100	0.28	71	ND	72	ND	0.333333	0	0.003333	0	0.000
1	100	0.23	73	ND	74	ND	6.666667	0	0.066667	0	0.000
2	2000	0.34	75	ND	76	ND	25	15	0.0125	0.0075	0.600
17	2000	0	77	ND	78	ND	1794	0			
16	22000000	0	79	1040.41	80	ND					
6	2000	0.51	33	ND	34	ND	23	0	0.0115	0	0.000
10	2000	0.49	35	ND	36	ND	47	0	0.0235	0	0.000
11	10000	0.52	37	ND	38	ND	65	20	0.0065	0.002	0.308
9	100	0.45	39	ND	40	ND	4.2	0	0.042	0	0.000
8	100000	0.58	41	ND	42	BDL	195	5	0.00195	0.00005	0.026
12	100000	0.55	43	ND	44	ND	200	0	0.002	0	0.000
13	2000	0	45	BDL	46	ND	2540	0			
14	25000000	0	47	1007	48	BDL					
7	10000	0.63	49	ND	50	ND	24	0	0.0024	0	0.000
3	10000	0.53	51	ND	52	ND	41	0	0.0041	0	0.000
4	100000	0.57	53	ND	54	ND	52	0	0.00052	0	0.000
5	100	0.66	55	ND	56	ND	14.6	0	0.146	0	0.000
1	100	0.59	57	ND	58	ND	57.4	0	0.574	0	0.000
2	2000	0.73	59	ND	60	ND	1.2	0	0.0006	0	0.000
15	2000	0	61	ND	62	ND	303.3333	0			
16	22000000	0	63	945.81	64	1.1					

initial cell count	100	2000	10000	100000
fraction remaining	0	0.0355	0.0735	0.066
	0.0033	0.375	0.0034	0.078
	0.0667	0.0125	0.0064	0.1
	0.042	0.0115	0.0065	0.002
	0.146	0.0235	0.0024	0.002
	0.574	0.0006	0.0041	0.0005

fraction of cells remaining following ozonation at all ozone residuals at an initial cell count of 100 vs. fraction of cells remaining at initial cell count of 100 000

Treatment 1

N_1: 12
$df_1 = N - 1 = 12 - 1 = 11$
M_1: 0.11
SS_1: 0.36
$s^2_1 = SS_1/(N - 1) = 0.36/(12-1) = 0.03$

Treatment 2

N_2: 12
$df_2 = N - 1 = 12 - 1 = 11$
M_2: 0.03
SS_2: 0.02
$s^2_2 = SS_2/(N - 1) = 0.02/(12-1) = 0$

T-value Calculation

$s^2_p = ((df_1/(df_1 + df_2)) * s^2_1) + ((df_2/(df_2 + df_2)) * s^2_2) =$
$((11/22) * 0.03) + ((11/22) * 0) = 0.02$
$s^2_{M1} = s^2_p/N_1 = 0.02/12 = 0$
$s^2_{M2} = s^2_p/N_2 = 0.02/12 = 0$

$t = (M_1 - M_2)/\sqrt{(s^2_{M1} + s^2_{M2})} = 0.08/\sqrt{0} = 1.47$
The t-value is 1.47481. The p-value is .154433. The result is *not* significant at $p < .05$.

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