EVALUATION OF MICROCYSTIN ENZYME-BASED ANALYTIC TECHNIQUES AND MICROCYSTIN OCCURRENCE IN THE METRO-PHOENIX, ARIZONA WATER SUPPLY SYSTEM

by

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ABSTRACT

Cyanobacteria blooms can produce cyanotoxins, and taste and odor (T&O) compounds in water supplies. Significant 2-methylisoborneol (MIB) and geosmin (T&Os) occurrence in the metro-Phoenix water supply systems has been documented and attributed to blue-green algae. Microcystin (MC) is one of the most common cyanotoxins. Due to the chronic effects of MC, the World Health Organization (WHO) has established a maximum limit of 1 μg-MC/L. The purpose of this thesis was to compare MC monitoring procedures for future use by municipal water laboratories. Microcystin concentrations in three reservoirs and raw water canal systems were monitored over a 14-month period.

Two assays, enzyme-linked immunosorbent assay (ELISA) and protein phoshphatase-2a (PP2A), were chosen to measure MC concentration. ELISA has a method detection limit (MDL) of 0.147 μg-MC/L, has a working range of 0.16-2.5 μg-MC/L, and produced acceptable results ($R^2 > 0.75$) for 100% of assays conducted. PP2A has a MDL of 0.24 μg-MC/L, has a working range that fluctuated based on enzyme reactivity (0.05-2.5 μg-MC/L), and produced acceptable results in 74% of assays conducted. The PP2A assay inconsistency was attributed to the sensitivity of the PP2A enzyme to temperature. Based on PP2A assay variations, ELISA is recommended for future MC monitoring by water laboratories.

Microcystin concentrations in the city of Phoenix (COP) reservoirs were detectable (> 0.147 μg-MC/L) from June to October. Comparison of MC occurrence and water quality parameters indicated no linear relationships between MC and a single

parameter. The change in MC concentration over time was compared with temporal changes in secchi disc depth, MIB and chlorophyll-a concentrations. Microcystin concentrations peaked one to two months immediately following the greatest observed secchi disc depth. Microcystin concentration proceeded peak MIB production by one to two months. Chlorophyll-a concentrations showed inconsistent temporal trends when compared with MC.

Powder activated carbon (PAC) absorbed MC at dosages currently used in T&O control (5-50 mg-PAC/L). Three PACs commonly used in T&O removal absorbed MC, with AquaNuchar, a wood-based PAC, showing the greatest MC absorption capacity. Free chlorine oxidized MC. Chlorine residual concentrations as low as 0.1 mg-chlorine/L oxidized MC with a two-week contact time.

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TABLE OF CONTENTS

CHAPTER Page

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Is water taste and odor an indicator of water quality? The human nose is often relied upon to determine the safety of a substance for consumption. Municipalities in the Phoenix Metro area have received numerous customer complaints regarding the taste and odor (T&O) of the city's drinking water. Consumers typically associate these odors with unsafe drinking water. The main source of the Phoenix T&O problem is blue-green algae (cyanobacteria). Many species of cyanobacteria release T&O causing compounds into the water supply. Over the past three years, the City of Phoenix (COP) has joined with Arizona State University (ASU) and American Water Works Association Research Foundation (AWWARF) in an effort to address T&O concerns in the local water supply. Research efforts included the identification of T&O producing organisms and compounds, monitoring of water quality parameters in the COP water supply, and treatment recommendations [1, 2]. Although the actual T&O compounds have not been shown to be harmful, several genera of cyanobacteria release algal toxins that promote tumors or cause liver cancer [3].

WATER SUPPLY SOURCE

The COP water supply comes from a combination of sources including the Verde and Salt Rivers and the Central Arizona Project (CAP) (Figure 1.1). Lake Pleasant,

Bartlett and Saguaro Lakes are terminal reservoirs of this system. Water is released from the reservoirs and conveyed through open-channel, concrete lined canals. Canal walls provide miles of surface area for periphytic (attached) cyanobacteria to proliferate. During the warmer months, reservoirs thermally stratify approximately 10 m from the surface. As water temperatures decrease in the fall of each year, wind facilitates destratifcation (turn-over) bringing nutrients from below to the surface. Reservoirs are operated differently, and are subject to various upstream impoundments that may affect the growth of MC-producing cyanobacteria [4].

Lake Pleasant is an off-stream reservoir that stores a combination of CAP and Aqua Fria river water. During the months of October through April, Lake Pleasant is filled with Colorado River water from the CAP canal. Then between April and October water is released back into the canal. The Aqua Fria river provided less than 5% of the total inflow to the reservoir during the past 3 years. Based on controlled water release, Lake Pleasant water levels and storage volume vary significantly (approximately 50%) throughout the year.

Bartlett Lake is an on-stream reservoir on the Verde River. The outlet is located near the bottom of the reservoir. Snowmelt at higher elevations is the source of the majority of the water entering the reservoir. Water is stored in Bartlett Lake during the summer months and released after the first of October and continued through the winter.

Saguaro Lake is located along the Salt River and is the lowest of 5 reservoirs. Saguaro reservoir is operated in series with an upstream reservoir to produce hydroelectricity during the high energy demanding summer. Water is released from

Saguaro during the day, and then pumped back to the preceding reservoir during the night. This procedure creates a relatively short hydraulic retention time for the reservoir. Saguaro Lake is typically filled to capacity throughout the year. Most releases from this reservoir occur during the summer (May through September) and stop in early October. Table 1.1 provides a summary table of the physical characteristics of Bartlett, Saguaro, and Lake Pleasant during the 3-year span of the T&O research conducted as ASU.

Table 1.1

Reservoir physical characteristics and hydrologic data at maximum pool elevations

	Bartlett Lake	Saguaro Lake	Lake Pleasant
Surface area (km^2)	11.2	5.1	40.3
Volume (m^3)	$2.2x10^8$	$0.85x10^8$	$10.5x10^8$
Depth (m)	38	33	43
Surface Elevation (m)	532	466	519
Hydrology during study			
Average Annual Inflow (m^3)	$2.8x10^8$	$3.7x10^8$	$7.2x10^8$
Average Annual Outflow (m^3)	$2.6x10^8$	$3.6x10^8$	$7.0x10^8$
Average HRT (days)	139	80	438

Source: [1]

The year round average air temperature in Phoenix is 23 degrees Celsius. Table 1.2 provides a summary of the average water temperatures near the surfaces of Lake

Pleasant, Bartlett and Saguaro Lakes. The water temperature averages and ranges were calculated using data from the top 10 m of the reservoirs. Based on warm temperatures and clear skies, sunlight is readily available for photosynthetic process of cyanobacteria. The physical characteristics of the COP water supply in part account for the large amount of cyanobacteria growth.

Table 1.2

Water temperatures near the surface of three reservoirs for the Phoenix metro area (Aug 1999- Jun 2002)

Reservoir	Water Temperature (degrees Celsius)		
	High (August)	Low (January—February)	Average
Bartlett Lake	29	9	18.9
Lake Pleasant	29	11	19.4
Saguaro Lake	29	9	20.1

Source: [1]

TOXIC CYANOBACTERIA

Toxic cyanobacteria blooms in a public water supply may pose significant health concerns. The cyanotoxins include neurotoxins, hepatotoxins, endotoxins, and general cytotoxins. Based on typical exposure pathways, it is unlikely for consumers to be subjected to acute, lethal poisonings by any of these toxins [3]. However, in 1996 the

first reported episode of acute lethal poisonings of humans occurred when water containing the hepatotoxin microcystin (MC) was used during dialysis treatment in Brazil [5]. These toxins are not only harmful to man, but also to birds, fish and other mammals [6]. Microcystin contaminated water has been reported to have a chronic effect on humans as a tumor promoter. Research has indicated that one of the risk factors for the high occurrence of primary liver cancer in China was consumption of MC in drinking water [7]. Based on this and other research, the World Health Organization (WHO) has established a guideline concentration of 1 μg-MC-LR/L in drinking water to protect consumers from these chronic effects [8]. The guideline is based on total MC, the sum resulting from both intracellular and dissolved MC concentration.

The Phoenix water supply is likely to contain toxin-producing cyanobacteria. Watersheds with significant T&O problems are often found to contain microcystin-producing cyanobacteria. Research conducted at Wright State University [9] indicated a correlation between T&O producing algal blooms and toxin producing blooms for a wide range of water utilities in North America. Of the waters sampled, approximately 80% of the samples reporting T&O occurrence also contained MC. Additionally, three T&O producing genera of cyanobacteria are known MC producers. These genera include *Anabaena*, *Microcystis*, and *Oscillatoria*. During the three-year AWWARF T&O project, several species of each of these cyanobacteria genera were identified, indicating the likely presence of MC.

OBJECTIVES

In 2001 the COP, AWWARF, and ASU began a collaborative project to develop in-house capabilities for monitoring MC in the water supply in an effort to address public health concerns regarding the esthetically unpleasing water. The primary purpose of this thesis is to provide a source of reference for future MC monitoring, and to examine the behavior of MC in the matrix of the COP water supply. The specific objectives of this research are enumerated below:

- Develop and recommend MC detection methods for future monitoring of the COP water supply.
- Monitor extracellular MC concentrations in Lake Pleasant, Bartlett and Saguaro Lakes to determine MC seasonal fluctuations in water supply reservoirs.
- Examine relationships between MC concentration and T&O compounds (MIB and geosmin), nutrients (nitrogen and phosphorous), organic carbon, temperature, light penetration (secchi disc depth), and algal biomass (chlorophyll-a).
- Evaluate absorption of MC by three powder activated carbons (PACs) commonly used in removal of T&O compounds.
- Screen oxidation of MC by chlorine.

The secondary purpose of this research is to analyze twenty-one isolated algae from the COP water supply for T&O causing compounds and MC production. The results of this

research are intended to aid in future monitoring and removal efforts of MC from the COP water supply in order to assure that the public is receiving an esthetically pleasing and toxin-free drinking water.

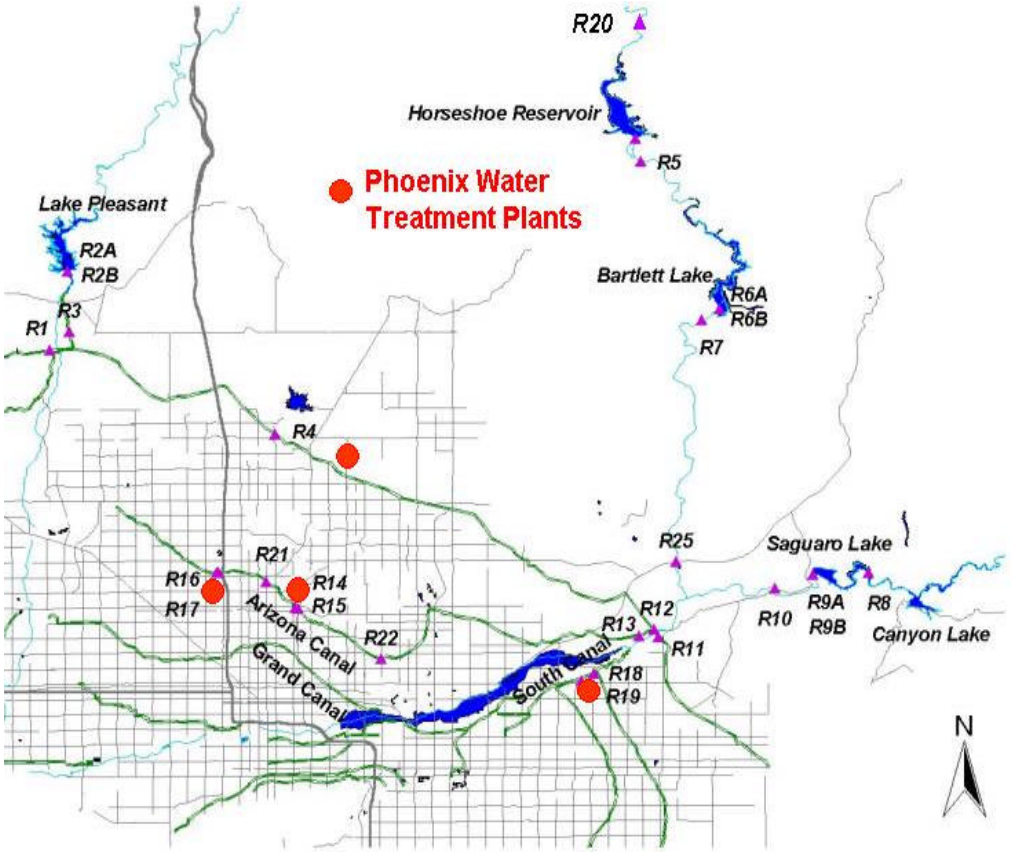


Figure 1.1 Monitoring sites for MC analysis (? R#) and location of COP water treatment plants (?). Samples analyzed for MC concentration included reservoir samples R2, R6, and R9; canal samples R11, R12 and R13; and Deer Valley and Squaw Peak treatment plants (*Source:* [1])

CHAPTER 2

BACKGROUND

Microcystin in the water supply affects several aspects of water quality management. The purpose of this chapter is to provide an overview of cyanobacteria and the cyanotoxin MC. The following topics are included:

- Cyanobacteria
- T&O compounds
- Cyanotoxins
- Microcystin detection methods
- Water treatment plant (WTP) removal options

CYANOBACTERIA

Cyanobacteria, or blue-green algae, are found in a variety of environments. Habitats include hot springs, terrestrial, freshwater, and marine environments. Water quality problems created by cyanobacteria include bloom development, T&O problems, and toxin production. Cyanobacteria are present in the environment as plankton (free floating), periphyton (attached to surfaces), or mats. Historically, there has been some debate over the classification of cyanobacteria as algae or as bacteria. Yoo et al. [3] explained that cyanobacteria are prokaryotic, and have no organized structures including plasmids and mitochondria; thus more closely resembling bacteria

than eukaryotic algae. Cyanobacteria make up one of three groups of pigmented bacteria. The other two groups are purple and green sulfur bacteria. However, unlike purple and green sulfur bacteria, cyanobacteria produce oxygen during photosynthesis. Phycologists view cyanobacteria as prokaryotic algae, because their pigments and photosynthetic mechanisms more closely resemble those of true algae than those of photosynthetic green and purple sulfur bacteria. Often cyanobacteria are classified as a separate, distinct group. Skulberg et al. [10] further discuss the classification debate.

Cyanobacteria range from unicellular forms to colonial unicellular and filamentous forms. The size of the cells varies with individual organisms. They range from only a few micrometers in diameter, to much larger sizes [3]. For example, the individual cells in the filaments of *Oscillatoria princeps* may be as large as 60 mm in diameter. This size represents one of the largest cells among the prokaryotes [3].

Cyanobacteria contain pigments (phycobilins) that reflect both blue and green wavelengths, and are often referred to as blue-green algae. One class of the phycobilins is phycocyanin, which is responsible for the blue appearance of the algae. Phycocyanin traps light energy in the red band of the visible spectrum, and then transfers this energy to the chlorophyll-a pigment that is used in photosynthesis. Chlorophyll-a absorbs a blue wavelength for energy and reflects green. Cyanobacteria use the blue and green pigments to trap light at both extremes of the visible spectrum. Thus blue-green algae utilize deep-penetrating red wavelengths in addition to the blue wavelengths near the surface, allowing cyanobacteria to continue photosynthesis relatively independent of their position in the water column [3]. However, not all cyanobacteria are blue-green in color.

Cyanobacteria colors range from olive-green, gray-green, and yellow-brown to purplish and red.

Gas vacuoles allow some species of cyanobacteria to regulate their depth in the water column. Gas vacuoles are comprised of vesicles that fill and collapse in a cyclic process regulated by the accumulation and synthesis photosynthetic by-products. Advantages of depth regulation include improved photosynthesis through movement to light, and access to nutrients throughout the water column.

Mair, Pepper and Gerba [11] discussed the significance of cyanobacteria in the aquatic environment. Cyanobacteria are primary producers in the microbial planktonic community. Organic carbon and energy produced from cyanobacteria photosynthetic processes are transferred to other organisms as part of the food web. Cyanobacteria are also consumed by zooplankton, which are then consumed by larger organisms as a part of the grazing food chain. Additionally more than 50% of the carbon fixed during photosynthesis of the phytoplankton is released into the surrounding water as dissolved organic matter (DOM). Bacterioplankton utilize this DOM as part of the microbial loop. The bacterioplankton then either mineralizes the organic carbon into carbon dioxide, or produce new biomass. Thus cyanobacteria play a vital role in aquatic environments in primary production [11].

Cyanobacteria are also an essential component in microbial mats. Maier, Pepper, and Gerba [11] further explain that cyanobacteria are the most important source of organic carbon in a microbial mat. Cyanobacteria access sunlight by occupying the top layer of mats. The photosynthetic activity of the blue-green algae creates an oxygen rich

environment for additional microbial growth. Cyanobacteria work with sulfur-oxidizing bacteria to complete a sulfur and nitrogen cycle. Stal [12] explained that cyanobacteria, sediment, and organic debris form the surface layer of the mat preventing excessive ultraviolet radiation from penetrating the mat. Microbial mats are often the only life forms present in very extreme environments.

Cyanobacteria impact the environment in several ways. Blue-green algae blooms lead to undesirable tastes and odors, as well as potential toxin release. Cyanobacteria blooms are defined as prolific planktonic algae in aquatic habitats housing millions of cells per liter [13]. Potential toxicity of a cyanobacteria bloom has been connected to bloom size. Yoo et al. [3] noted that the likelihood of serious cyanobacteria toxicity episode occurring increases with increasing cyanobacteria mass. Nutrient concentrations, water temperatures between 15-30 degrees Celsius, and pH values between 6 and 9, provide conditions favorable to bloom development. The highest concentration of cyanobacteria typically occurs at a depth between 2-9 meters [11]. The fluctuation of T&O compounds 2-methylisoborneol (MIB) and geosmin in the COP water supply may also provide an indicator of MC blooms. However, three of the genera of cyanobacteria that are dominant T&O producers in the COP water supply may also release MC. These genera include *Oscillatoria*, *Anabaena*, and *Microcystis*. Research in Alberta, Canada found no relationship between MIB and geosmin and MC concentrations [14]. Aquatic environments with warm temperatures, and moderate pH values should be monitored for toxins released by cyanobacteria blooms.

TASTE & ODOR COMPOUNDS

Algal blooms are often the source of undesirable T&O compounds in drinking water. Taste and odors common in drinking waters include earthy/musty, sour, woody/hay, metallic, chlorinous, fishy, medicinal and chemical [15, 16]. Geosmin and MIB are two common algal T&O compounds identified in the COP water supply [2]. Geosmin has an earthy odor and MIB a musty odor. Detectable odors for MIB and geosmin are 29 ng/L for MIB and 10 ng/L for geosmin [17]. Water treatment plants that utilize surface water commonly have taste and odor problems caused by algal blooms. In a national survey of public water suppliers; 46% reported algal growth and 22% reported earthy-moldy tastes and odors in their source waters [18]. Cyclocitral (threshold of 5 ng/L) is another algal-produced T&O compound described as fresh grass, hay/woody, and tobacco-like odors [19]. Cyclocitral and geosmin have been identified in the same water body. A significant T&O episode in Appleton, Wisconsin identified geosmin and B-cyclocitral as the culprit compounds [19]. Algal taste and odor problems are typically caused by phytoplankton, but California Metropolitan Water District research indicated that periphyton contributes to taste and odor problems as well [20].

CYANOTOXINS

Production of toxins harmful to humans and animals have been associated with cyanobacteria blooms. Cyanotoxins have been linked with liver damage, gastrointestinal

disturbances, and neural damage. The cyanobacterial hepatotoxin MC-LR was attributed to the lethal acute poisonings of humans in Caruaru, Brazil in 1996 when MC contaminated water was used in dialysis treatment [5]. A study in China found a correlation between the occurrence of primary liver cancer and drinking water heavily infested by toxic cyanobacteria [7]. Water supplies prone to algal and cyanobacteria blooms should be monitored to detect cyanotoxins. Based on the toxicity of MC, the WHO has set a limit of 1 μg/L for MC in drinking water [21]. The 1994 Canadian guideline for MC-LR (most common form of MC found in water supplies) specifies a maximum acceptable concentration of 0.5 μg/L of total MC-LR or 1 μg/L of total MC in drinking water [9]. Carmichael [9] tested 677 water samples from water utilities in both the United States and Canada for MC from June 1996 through January 1998. Of these samples 80% were positive for MC, with 4.3% of the samples at MC concentrations greater than 1 μg/L. Based on the acute and chronic dangers of MC, MC and other cyanobacteria toxins are listed on the contaminant candidate list by the US Environmental Protection Agency [22].

Currently it is unclear why cyanobacteria release toxins. Cyanobacteria respond differently to changing environmental conditions [23]. Jones et al. [24] concluded that light and nitrogen availability had the greatest affect on toxin production. They further noted that toxin production varies over time and may be explained by a combination of environmental factors, including ecological changes of dominating cyanobacteria over time.

Microcystis is the number one toxin producing genera of cyanobacteria [11], but is not the only genus that produces the toxin MC. Table 2.1 summarizes confirmed MC producers. The most often listed producers include *Microcystis aeruginosa, Aphanizomenon flos-aquae, Anabaena flos-aquae, Oscillatoria agardhii,* and *Lyngbya* spp. [3]. The toxin MC may be present in several different forms. Figure 2.1 illustrates the structure of nine different MC congeners. These variants include MC-LR, -LA, -YA, YM, -RR, and –YR.

Table 2.1

Summary of identified microcystin producers

Organisms Producing Only Hepatotoxic Microcystins or Nodularians	Organisms Producing Both Hepatotoxins and Neurotoxic Anatoxins or PSPs
Microcystis aeruginosa Kütz.	*Anabaena circinalis* Rabenh.
Microcystis viridis (A. Br.) Lemm	Anabaena flos-aquae (Lyngb.) Breb.
Microcystis wesenbergii Kom.	*Anabaena spiroides* var. *contracta* Kleb.
Nodularia spumigena Mertens	*Anabaena variabilis* Kütz.
Nostoc rivulare Kütz.	*Aphanizomenon flos-aquae* (L.) Ralfs
Oscillatoria acutissima Kuff	*Oscillatoria agardhii* Gom..
Oscillatoria agardhii/rubescens group	
Oscillatoria nigro-viridis Thwaites	

Source: [3]

Several cyanobacteria were identified and isolated as part of the COP/ASU T&O project conducted over the past years (Table 2.2). Potential MC producers identified include *Anabaena* and *Oscillatoria***.** A large variety of cyanobacteria exist in reservoir phytoplankton. Table 2.2 is not inclusive of all observed cyanobacteria. However, the

identified and isolated species allow characterization of the planktonic make up of the COP source waters, and indicate the presence of potential MC producers.

Table 2.2

Comprehensive list of taxa (excluding diatoms) observed in phytoplankton in the City of Phoenix water supply system

Chlorophyta (30)	Chlorophyta (cont.)	Cyanophyta (17)	Other (10)
Ankistrodesmus sp.	*Pandorina sp.*	*Anabaena sp.*	*Ceratium sp.*
Chlamydomonas sp.	*Pediastrum sp.*	*Aphanothece sp.*	*Cryptomonas sp.*
Chlorella sp.	*Platymonas sp.*	*Chroothece sp.*	*Dinobryon sp.*
Chlorococcum sp	*Pyramimonas sp.*	*Cylindrospermum sp.*	*Euglena sp.*
Cladophora sp.	*Rhizoclonium sp.*	*Gloeocapsa sp.*	*Mallomonas sp.*
Closterium sp.	*Scenedesmus sp.*	*Gomphosphaeria sp.*	*Ophiocytium sp.*
Coleochaete sp.	*Selenastrum sp.*	*Merismopedia sp.*	*Peridinium sp.*
Cosmarium sp.	*Spirogyra sp.*	*Microcystis sp.*	*Phacus sp.*
Eudorina sp.	*Staurastrum sp.*	*Oscillatoria agardhii*	*Synura sp.*
Franceia sp.	*Tetracystis sp.*	*Oscillatoria sp.*	*Vaucheria sp.*
Golenkinia minutissima	*Tetrahedron sp.*	*Oscillatoria splendida*	
Golenkinia sp.	*Ulothrix sp.*	*Oscillatoria tenuis*	
Gonium sp.	*Zygnema sp.*	*Phormidium sp.*	
Microspora sp.		*Pseudanabaena sp. #1*	
Mougeoutia sp.		*Pseudanabaena sp. #2*	
Oedogonium sp.		*Pseudanabaena sp. #3*	
Oocystis sp.		*Spirulina sp.*	

Source: [1]

MICROCYSTIN DETECTION

Several methods are available for monitoring MC in the water supply. These techniques include: high performance liquid chromatographic (HPLC), microcystin

enzyme linked immunosorbent assay (ELISA), and protein phosphatase-1 or -2A (PP1 or PP2A) inhibition assay.

HPLC is an effective method in determining MC concentration. This method has been used as a standard in development of detection techniques. The principle behind HPLC is separation of toxins from other co-extracted compounds based on compound molecular weight. HPLC is typically followed by UV absorbance or mass spectrometry to identify MC. These methods are highly sensitive. Nicholson and Shaw [25] were able to achieve a detection limit of 0.02 μg/L using HPLC-MS. Extraction of the cyanobacterial mass prior to HPLC may create quantification and MC recovery problems [25]. Fischer et al. [6] summarized several of the limitations of the HPLC methods. Natural organic matter (NOM) may mask toxins in HPLC testing. Also, HPLC is capable of detecting single variants of microcystin, but quantification of all present congeners is difficult. Individual MC variants may be present at concentrations below chromatography detection limits, but the possibly significant combined toxicity of these variants would not be measured [6]. The biochemical assays, ELISA, PP1 and PP2A, are less qualitative than HPLC, but are as sensitive, account for combined toxicity, and provide more rapid results [26]. For these reasons HPLC was not examined as a monitoring method for the COP and will not be employed in this study.

The commercially available ELISA kits are a second MC detection method. These kits operate based on the competition of MC in the sample with an enzyme-labeled MC for a limited number of antibody binding sites. The concentration of MC is then determined colorimetrically (Figure 2.2). In Step #1 an assay well is coated with an

antibody specific to MC. Step #2 involves the injection of MC standards and samples to the well. Microcystin present in the standards and samples binds to the antibodies. An enzyme-linked antigen is added in Step #3 and binds to any unoccupied antibody binding sites. In Step #4 an enzyme substrate is added to the well. The substrate binds with the antigen and produces a color. The amount of color present indicates the amount of MC present in the standard or sample. The darker the color of the well, the less MC is present. The cross reactivity of ELISA varies from good (MC-RR) to poor (MC-LA) for the MC congeners. ELISA tests may underestimate MC concentration due to poor cross-reactivity among variants [25]. Cross-reactivity is a function of structural similarity that does not necessarily represent similar toxicity [25]. Thus ELISA may be best employed as a semi-quantitative assay, or a screening tool. Rivasseau et al. [27] found organic interferences that lead to false positives in MC spiked water. Based on these interferences, the limit of quantification for the assay was 0.2 μg/L. EnviroLogix Inc™., the manufacturer of the kits employed in the ASU research, lists a limit of detection for their MC ELISA kits at 0.147 μg/L. These kits were used at ASU to measure MC concentrations in the water supply reservoirs, and as a standard to refine the methods used in the PP2A assay.

The third method for determining MC concentration is the protein phosphatase assay (PP1 and PP2A). Nicholson and Shaw [28] explained the principles behind these assays. The hepatotoxins, MC and nodularins, inhibit enzymes that control the dephosphorylation of intracellular phosphoprotiens. These enzymes are Type 1 (PP1) and Type 2, including a subset of Type 2 called protein phosphatase-2A (PP2A). Based

on this process, the inhibition of these enzymes by MC and nodularins provides a measure of toxin concentration. The assay measures the phosphate release from a phosphorylated protein in the presence of PP1 or PP2A enzyme and an inhibitor (MC) [28]. Complications arose in the measurement of the phosphate released due to the short half-life of the ^{32}P isotope. In order to overcome this challenge, the substrate p-nitrophenyl phosphate was used in the assay, and the color released from the substrate in the presence of MC and PP2A was measured [29] (Figure 2.3). The sensitivity of this assay is in the part per billion range [29]. Heresztyn and Nicholson [30] reported a working range for the assay of 0.05-1.0 μg/L. The PP2A enzyme is preferred over PP1 because it has been reported to be 50 times more sensitive in inhibition of MC [31, 32]. PP2A is being employed and adapted by researchers at ASU to develop a detection method that incorporates current lab equipment and techniques used by the City of Phoenix water utilities.

Solid phase extraction with C18 cartridges provide a means of improving the MC detection capabilities. ELISA and PP2A are able to detect MC in the part per billion range. Carmichael and An [26] provided three reasons for using C18 cartridges in toxin recovery. They were used to:

(1) Lower pigments in sample extracts

(2) Remove inorganic compounds that also act as protein phosphate inhibitors and would result in false positives for MC

(3) Concentrate MC and nodularin toxins in samples.

The addition of a C18 concentration step in the ASU research provides greater detection sensitivity (part per trillion range), as well as removal of potential matrix interference effects.

MICROCYSTIN REMOVAL

Several strategies for MC removal in water treatment processes are available. Miller et al. [33] summarized the various methods researched for cyanotoxin removal. A brief summary of this report is given. Nicholson, Rositano and Burch [34] reported that chlorine is capable of oxidizing MC-LR and nodularin. Ozone treatment has been shown to be effective in reducing purified MC-LR to below detection limits. However, this destruction also appears to be pH dependent, and is less effective at the pH values usually experienced in a bloom. Granulated and activated carbons are effective in removal of toxins from water, but organic compounds found naturally in water can inhibit removal. Nanofiltration was effective in removal of toxins, but this technique requires a great deal of maintenance to avoid filter clogging [33]. Past research was used in the development of experimental methods to test the MC removal capabilities by powder activated carbon and oxidation with chlorine.

Absorption

The PAC currently being used by the COP water utilities to remove T&O compounds may be sufficient to remove MC from the water supply. The octanol/water distribution coefficient (log Dow) for MC is 2.18 to –1.76 for pH values of 1 to 10 [35]. Several T&O compounds in the COP water supply are initially introduced into the system by cyanobacteria blooms in contributing reservoirs. According to the National River Authority in London, 50% of all cyanobacteria blooms produce the toxin MC [36]. Additionally, blooms that are not currently toxin producers may become toxic over time. Based on this large percentage and the dynamic nature of cyanobacteria blooms, the ability of the PAC employed by the COP in T&O compound removal to absorb MC will be examined further.

The optimum PAC for T&O removal is not necessarily optimal for MC removal. The pore size distribution of PAC strongly influences the ability of the PAC to absorb compounds [37]. Pore sizes of PAC are classified as: micro <2nm, meso 2-50 nm, and macro >50 nm [38]. Micro pore size PAC is most effective for removal of the T&O compounds MIB and geosmin. MC molecules are larger than MIB and Geosmin and size prevents it from entering micro pores. Due to the raw material properties used for activated carbon, PAC is invariably micro porous. However, PAC with a larger volume of mesopores, more suitable for MC removal, is less common. Thus PAC being utilized to remove T&O compounds may or may not be able to absorb MC.

One of the objectives of this thesis was to examine three different PACs tested for T&O absorption to determine their ability to remove MC from surface water. Three PACs available for compound absorption are AquaNuchar, Norit HDB, and Norit 20B. AquaNuchar is currently being used by the City of Tempe for T&O removal based on its ability to remove T&O compounds. Norit HDB and Norit 20B are two PACs typically used in T&O compound removal.

Past research involving MC removal by PAC was used in determining the experimental parameters for further PAC testing. Table 2.3 summarizes the basic components and results of recent PAC-MC research. The majority of these experiments were conducted in an effort to determine the most efficient PAC type for removing MC. The detention times in each of these experiments were significantly longer than the contact of PAC with water in treatment plants. These long contact times were chosen to allow for absorption equilibrium to be reached between the MC and the PAC. Although these parameters where used with a different endpoint in mind, the ratio of absorbed MC to PAC dosage was useful in choosing parameters this study. For a much shorter detention time, the amount of MC absorbed per gram of PAC was expected to be much lower than the 7-15 mg/g carbon range reported in the literature. A five-hour retention time typically employed in the Tempe WTP is greater than the two-hour contact time used by Drikas et al [39]. Thus the absorption values of the PAC were expected to be between the values determined by Drikas et al (2 hour detention time), and the values found by Mohammed et al. [40] (5 day detention time), and Newcombe et al. [41] (7 day

detention time). Quantitatively, the PAC absorption capability is expected to be between 0.72 and 15 mg-MC/g-carbon.

Table 2.3

Removal capabilities of microcystin by powder activated carbon

PAC Dose (mg/L)	MC Dose (µg/L)	Total Time	Percent Removed	Absorbed MC (mg/g carbon)	Reference
15	18	120 min	60	0.72	[39]
2	100	5 days	15	7.5	[41]
6	100	5 days	65	11	[41]
10	100	5 days	70	7	[41]
100-500	2000	7 days		7-15	[40]
2.5-200	2500	3-4 days		280	[37]*

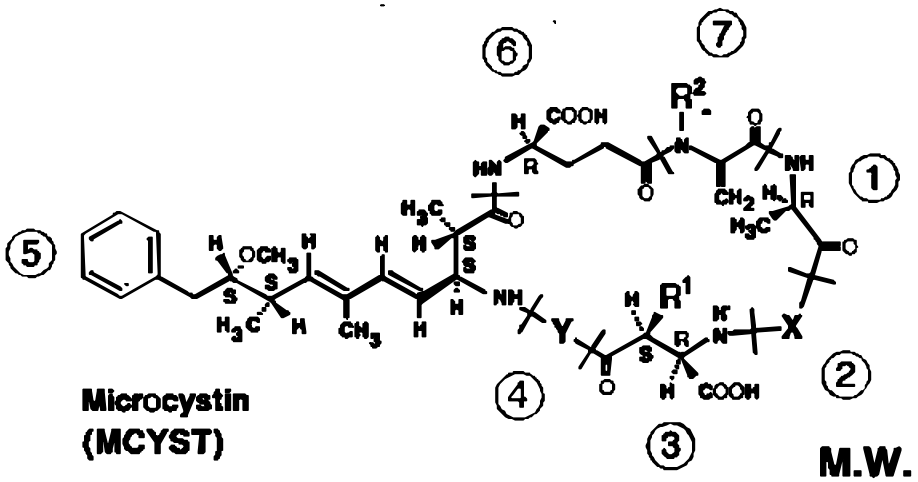
*For the research examining multiple PAC types the results of the best removal are reported

Natural organic matter present in surface water competes with organic compounds for binding sites on activated carbon. To account for this competition, all absorption tests were conducted in SRP water. The amount of MC spiked into the SRP water tested may influence the absorption ability of the PAC. A MC dosage of 7 µg-MC/L was chosen based on past research, desired MC residual, and anticipated naturally occurring MC concentrations.

Oxidation

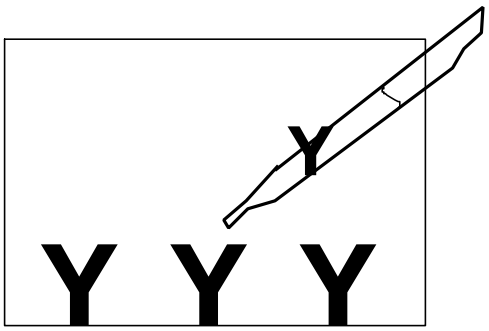
Chlorine is currently being applied as a disinfectant in several WTP in the Metro area and is capable of oxidizing MC. Nicholson et al. [34] examined the effects of

chlorine and chloramines on MC in both freeze-dried and intact material. They found that MC was destroyed under conditions with a minimum chlorine residual of 0.5 mg-chlorine/L present after a 30-minute contact time. Chloramines had no noted effects on the toxin. Nicholson et al. further noted the level of NOM in the water influenced chlorine effectiveness. Drikas et al. [39] studied the various water treatment options for MC removal including the use of chlorination in the destruction of residual dissolved MC. Their research also concluded that chlorine effectively destroys MC with a minimum dose of 0.5 mg-chlorine/L residual after 30 minutes.

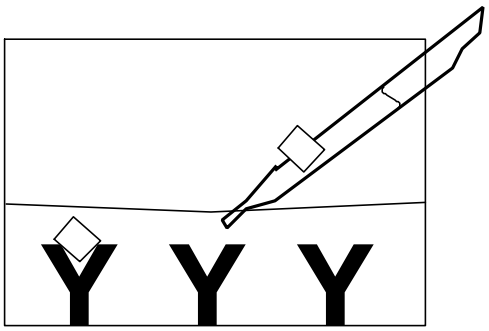


		M.W.
	MCYST-LA: X = Leu; $R^1 = CH_3$; Y = Ala; $R^2 = CH_3$	909
	MCYST-YA: X = Tyr; $R^1 = CH_3$; Y = Ala; $R^2 = CH_3$	959
	MCYST-LR: X = Leu; $R^1 = CH_3$; Y = Arg; $R^2 = CH_3$	994
desmethyl 3-	MCYST-LR: X = Leu; $R^1 = H$; Y = Arg; $R^2 = CH_3$	980
	MCYST-YM: X = Tyr; $R^1 = CH_3$; Y = Met; $R^2 = CH_3$	1035
	MCYST-RR: X = Arg; $R^1 = CH_3$; Y = Arg; $R^2 = CH_3$	1037
desmethyl 3-	MCYST-RR: X = Arg; $R^1 = H$; Y = Arg; $R^2 = CH_3$	1023
desmethyl 3,7-	MCYST-RR: X = Arg; $R^1 = H$; Y = Arg; $R^2 = H$	1009
	MCYST-YR: X = Tyr; $R^1 = CH_3$; Y = Arg; $R^2 = CH_3$	1044

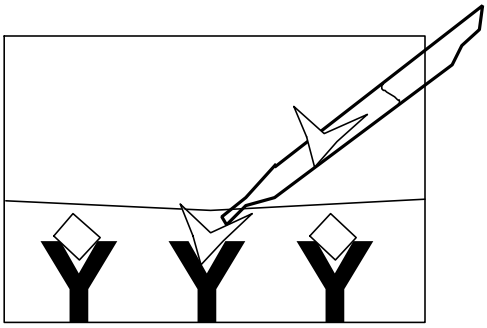
Figure 2.1 Structure of microcystin congeners (*Source:* [3])



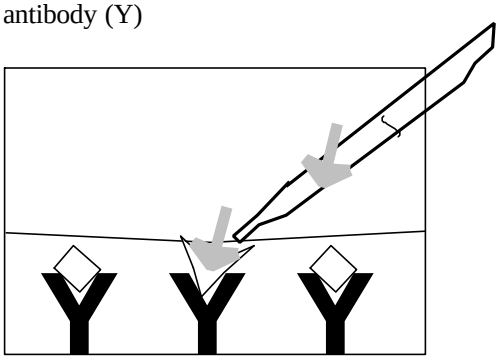
Step #1 Well is coated with antibody (Y)



Step #2 Sample is added and microcystin (◇) is bound by the antibody (Y)

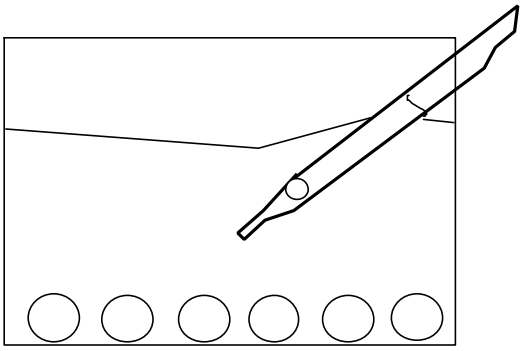


Step #3 Enzyme linked antigen (⩗) is added and binds to the remaining unoccupied sites on the antibody (Y)

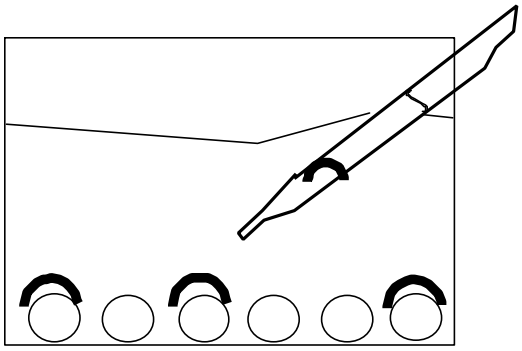


Step #4 Enzyme substrate (⩗) is added to produce color reaction

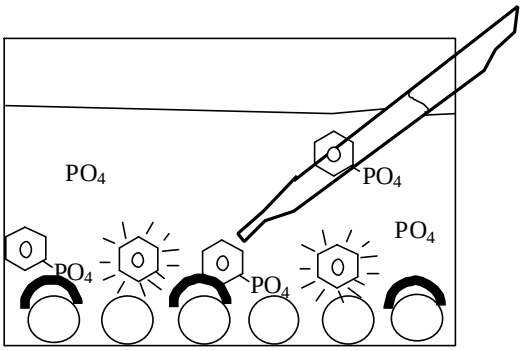
Figure 2.2 Principle of competitive ELISA assay



Step #1 Well is coated with enzyme PP1 or PP2A (◯)



Step #2 Sample is added and microcystin (◠) inhibits enzyme



Step #3 Substrate (⬡ PO_4) is added.
Overtime unihibited enzyme reacts with substrate releasing phosphate (PO_4) resulting in yellow color. Enzymes inhibited by microcystin do not react with substrate and produce no color.

Figure 2.3 Principle of PP2A assay

CHAPTER 3

METHODS

City of Phoenix (COP) samples were collected for MC analysis. Lake Pleasant, Bartlett and Saguaro Lakes as well as CAP and SRP canals were sampled. Sampling sites at each of the reservoirs were chosen near reservoir outlets coincident to sampling sites used in the COP/ASU T&O project for data comparison. Limited canal sampling was conducted during a MC bloom episode from July through September 2002. Canal sites included the CAP canal downstream from Lake Pleasant, the SRP south canal above the cross-connect with the CAP canal, the South canal downstream of the cross-connect, and the influent to the Squaw Peak and Deer Valley WTP (Figure 3.1).

Lake samples were collected monthly from September 2001 through October 2002. Bimonthly sampling began in July 2002 and ran through October 2002 in response to large algal growth anticipated from increasing MIB and geosmin concentrations. Data gathered at the sites included: water depth at sampling location, temperature and dissolved oxygen profiles, secchi disc readings, and lake color and odor. Collected samples were analyzed for algae, and chlorophyll-a, MIB and geosmin, nitrogen, phosphorous, organic carbon, and MC concentrations. Methods for determining nutrient concentrations as well as analysis of algae and chlorophyll-a samples are specifically discussed by Sommerfeld et al [1]. Table 3.1 and Table 3.2 provide a summary of the parameters, method measurement and equipment used in sampling.

Table 3.1

Sample volumes, pretreatment/preservation and analytical methods and equipment

Parameter	Volume Collected	Pretreatment/ Preservation	Method	Equipment
Microcystin	1 L in NG	GF/F	ELISA PP2A	Molecular Devices kinetic microplate reader
MIB & Geosmin (ng/L)	40 to 250 mL in AG	NONE	GC/MS	Varian Star 3400CX
Total Organic Carbon (mg/L)	250 mL in AG	HCl Acidification	Medium-temperature catalytic oxidation (SM 5310 B)	Shimadzu TOC 5050
Dissolved organic carbon (mg/L)		GF/F; HCl acidification		
UV Absorbance (m^{-1})			Ultraviolet absorption at 254 nm (SM 5910)	Shimadzu UV160A
Total Phosphorus (µg/L)	1L in NG	GF/C; HCl	Modified SM 4500-P	TrAAcS 800 Autoanalyzer Bran-Luebbe
Dissolved Phosphorus (µg/L)				
Total Nitrogen (µg/L)		GF/C; HCl	SM 4500-N C	
Dissolved Nitrogen (µg/L)				
Nitrate (µg/L)			SM 4500-NO_3^- F	
Ammonium (µg/L)			SM 4500-NH_3 G	
Specific conductance (uS/cm)	1 L in NG		SM 2510	
Chlorophyll-a (µg/L)		GF/C	SM 10200 H	Beckman Instruments Model DU-64

Source: [1]
(AG = amber glass bottle; NG = Nalgene plastic bottle; GF/F = filtration, ashed Whatman GF/F)

Table 3.2

Field measurement and equipment

Parameter	Method	Equipment
pH (pH unit)	Probe (SM 4500-H^+)	YSI Model 60 pH meter
Temperature (^oC)	Probe (SM 2550 Temperature)	
Dissolved Oxygen (mg/L)	Membrane probe (SM 4500-O G)	YSI Model 50B DO meter
Secchi Disc Depth (m)	N/A	Ben Meadows Inc.

Source: [1]

Glass and plasticware used in sample collection and analysis were cleaned thoroughly before use. The bottles were washed with phosphate-free detergent (Citranox, Alconox Inc), rinsed with distilled water and rinsed again with Super Q™ water. A Kemmerer sampler was used to collected reservoir samples. The samples analyzed for MC concentrations were taken roughly 5 m below the surface. Canal samples were collected as grab samples with a 1-liter Nalgene bottle attached to an expandable rod approximately 0.2 m below the surface. The samples were collected and transferred to clean bottles, stored in coolers with ice, and transferred back to the laboratory. Analytical testing was typically performed within two weeks of collection. MC reservoir samples were collected in triplicate with replicate samples stored in a freezer at negative 20 degrees Celsius and brought to room temperature before analysis.

EXPERIMENTS

Powder Activated Carbon (PAC)

Procedures developed for testing PAC absorption of T&O compounds were employed in MC absorption experiments. This protocol specifies carbon dosages of 15 and 25 mg-PAC/L. In order to establish a more complete picture of the absorption of MC by PAC, dosages of 1, 3, 5, 8, 12, 20 and 50 mg/L were tested. These PAC dosages include the PAC dosages (5-50 mg-PAC/L) anticipated at the COP water treatment plants during algal blooms.

The detection limitations of the PP2A assay were a significant factor in determining the MC dosage. The detection limits of the PP2A assay varies, but was typically between 0.24 and 2.5 µg-MC/L. In order to detect remaining MC concentration, sufficient MC needed to be spiked into the SRP water samples. A residual concentration of approximately 1 µg-MC/L was targeted.

Blooms of toxic cyanobacteria typically produce concentrations in the range of 50 µg-MC/L [28]. As the MC travels to the treatment plant it is diluted and MC concentration at the WTP inlet will vary based on the contributing watershed characteristics. The concentrations observed over a 14-month period in the COP water supply were below 0.5 µg-MC/L. Therefore, a low MC concentration of MC stock (Calbiochem-Novabiochem, Corporation) spiked into SRP water was used to represent MC concentrations anticipated in forthcoming years. An initial concentration of 7.5 µg-

MC/L was selected based on the expected PAC absorption capabilities, the desired MC concentration for PP2A detection, and naturally occurring MC levels.

Slurries of each of the PAC samples were prepared at a concentration of 2500 mg PAC/L Super Q™ water. Slurries were allowed to hydrate for 24 hours at room temperature. Water from the Salt River system was collected and filtered (Whatman GF/F). The water was then spiked with MC-LR to obtain an initial concentration of 7.5 µg/L. Treatments were conducted in 40 ml glass vials at PAC doses of 1, 3, 8, 12, 20, and 50 mg PAC/L. Control samples containing the MC spiked SRP water were also run with the PAC treated samples. The vials were shaken with a wrist shaker (Multi-wrist® shaker, Lab-line, Melrose Park, IL) for 5 hours. The shaking duration was chosen based on the conservative hydraulic retention time provided by the City of Tempe for past PAC/T&O tests. After 5 hours the PAC was removed from the samples by syringe filtering (Acrodisc® 32 Supor ® 0.2 um syringe filters, Pall Corporation, Ann Arbor, MI). The concentration of MC left in solution was determined through PP2A assays.

Chlorine

The ability of various residual chlorine concentrations to oxidize MC was examined. Super Q™ water was spiked with MC stock solution (Calbiochem-Novabiochem, Corporation) to obtain a MC concentration of 12 µg/L. Forty-milliliter vials were filled completely with MC solution. Chlorine was injected into the vials with a syringe to produce seven samples with chlorine concentrations of 0, 0.02, 0.05, 0.1 and

1.0 mg-chlorine/L. Samples were allowed to sit at room temperature 14 days to allow time for the residual chlorine to be oxidized. Remaining MC concentrations were determined with PP2A and ELISA assays.

Algae Cultures

Two known MC producing cyanobacteria species were selected for use in the development of intracellular MC extraction techniques. Two *Microcystis aeruginosa* cultures were purchased from the University of Texas at Austin (Appendix). Algal cells from the UTEX cultures were transferred into autoclaved, glass 250 mL flasks containing approximately 150 mL of media J (Appendix). Foam stoppers were used to prevent culture contamination and allow air passage. Cultures were placed on a platform shaker table (Lab-Line Instruments, Inc) and continually mixed at a speed of 100 rpms under a fluorescent light providing approximately 20 uE/m^2/s. As the cultures matured, approximately 2 mL of the culture were transferred to a new 250 mL flask of media J to keep cultures viable.

Intracellular Extraction

Three methods of intracellular extraction were compared to determine the procedures for obtaining maximum MC extraction (Figure 3.2). Two milliliters of

Microcystin aeruginosa culture were passed through a syringe filter (ashed GF/C Whatman). The eluent was set aside for extracellular MC analysis with ELISA.

Method A consisted of extraction solely through beadbeating (mini-beadbeater 3110BX BioSpec Products, Inc). The biomass captured on the filter paper was washed into a beadbeater tube. Silicon beads of 0.1 mm diameter were used in the beadbeating process. Methanol (MtOH) was added to the beadbeating vial to insure there was no headspace remaining. The MtOH MC mixture was decanted and diluted with Super Q to create solution containing less than 20% MtOH to avoid MtOH interference with ELISA. Method B measured the extraction of intracellular toxins through cellular membrane disruption by MtOH followed by beadbeating. For both methods B and C, the GF/C filter was soaked in MtOH and Super Q™ water for 24 hours. In B 1.5 mL of the mixture was removed and passed through the same beadbeating and dilution process as discussed in A. Method C was cellular contact with MtOH for 24 hours. For method C the remaining 10 mL of MtOH/Super Q™ were centrifuged to remove cellular and filter debris from the solution. The supernate was decanted then blown down to 2 mL with nitrogen. The solution was then diluted with Super Q™ water. For samples containing sufficient color, C18 extraction may be used (Figure 3.3). ELISA was used to analyze the MC concentrations in each extract.

Solid Phase Extraction

General MC recovery capabilities of C18 cartridges were examined using extractions of Super Q™ water spiked with MC-LR run in triplicate. A standard of 1.2 µg-MC/L was prepared for C18 concentration using MC-LR stock (Calbiochem-Novabiochem, Corporation). Six milliliters of the standard were set aside, then the remaining 3 L were passed through three DSC-18 cartridges (Supelco, Supelco Park, Bellefonte, PA, U.S.A. 505 m^2/g specific surface area, 57 µm average particle diameter, 67 angstroms average pore diameter). The C18 concentration process is illustrated in Figure 3.2. Step #1 illustrates the conditioning of the C18 cartridges by gravity feeding MtOH and Super Q™ water through the cartridge. In Step #2 one liter of the standard was passed through each C18 cartridge. The cartridge was then rinsed in Step #3 to remove any color introduced by natural organic matter. The cartridges were eluted in Step #4 using 10 mL of 80% methanol solution and collected in 40 mL vials. The solution was then blown down to 2 mL in Step #5. Finally, in Step #6, the MC concentration in the reservoir samples and the concentrated standard solution were determined with PP2A and ELISA assays. Lake samples passed through C18 cartridges were filtered using a GF/F filter then concentrated using the same C18 procedures described above.

ANALYTICAL METHODS

Enzyme-linked Immunosorbent Assay (ELISA)

Recommended methods for the EnviroLogix™ ELISA kit were followed for determining MC concentration in various samples (Appendix). The ELISA kit consisted of an assay plate of 96 antibody coated wells, MC standards of 0.16, 0.6, and 2.5 µg MC/L, a negative control, assay diluent, MC-enzyme conjugate, wash solution, substrate, and stop solution. Initially, the plate, reagents and standards were allowed to come to room temperature. Using a multi-channel pipette, 125 µL of MC assay diluent were added to each of the wells, followed by 20 µL of the standards, negative control, and samples in their respective well. Each of the controls, standards, and samples were tested in triplicate to account for result variations and experimental errors. The plate was then covered with Parafilm to prevent evaporation and placed on a platform shaker (New Brunswick Scientific) for 30 minutes. After mixing, 100 µL of MC-enzyme conjugate were added to each well. The plate was then placed on the shaker and stirred for another 30 minutes. After the second mixing period, the Parafilm was removed and the well contents were shook into a sink. The wells were next flooded with the wash solution then emptied by inverting the plate and shaking the contents into the sink. This flooding and shaking procedure was repeated four times. Removal of as much wash solution as possible was accomplished by slapping the plate on a paper towel. Then 100 µL of substrate were added to each well. The plate was again covered and placed on the shaker

for 30 minutes. Finally, 100 μL of stop solution were added to each of the wells. The optical density (OD) of the wells was measured using a microplate reader (Molecular Devices kinetic microplate reader). The wavelength of the plate reader was set at 450 nanometers with a reference wavelength of 650 nanometers. A column of wells was filled with 200 μL of DI water to provide a zero for the microplate reader. Microcystin concentrations were determined by developing a semi-log curve of $\%B_o$ (Equation 3.1) versus MC concentration of the standards (Figure 3.4). The ELISA standards are chosen to target the linear range of this curve. The equation of best-fit line for the linear range is then used to calculate MC concentrations in the samples. For the assay data used in developing Figure 3.4, the R^2 value of 0.9976 indicates a tight correlation between the trendline and the $\%B_o$ values.

$$\%B_o = \frac{B}{B_o} \times 100\% \qquad \text{Equation 3.1}$$

Where: B = optical density of sample or standard
B_o = average optical density of negative control

Protein Phosphatase –2A Assay (PP2A)

The procedures outlined by Carmichael and An **[26]** for PP1 analysis were combined with research using PP2A enzyme conducted by Heresztyn and Nicholson **[30]** to develop a PP2A assay (Appendix). This method involves the preparation of a diluent buffer, enzyme reagent, and substrate. The diluent buffer was prepared using 4 mL of 1

M Tris-HCL (pH=8.6), 2 mL of 1 M KCl, and 3 mL of 1 M $MgCl_2$. Intermediate solutions A, B, and C were prepared by adding 4.6 mg Dithiothreitol (DTT), 15 mg bovine albumin (BSA), and 15 µL of 1 M $MnCl_2$ to 10 mL of diluent buffer respectively. Solutions A, B, and C were then combined creating a total reagent volume of 30 mL. The enzyme reagent was then prepared by spiking 5.3 µL of PP2A stock enzyme (Promega Ppase-2A) into 4 mL of reagent. Next 63 mg of phophatase substrate (Sigma 104 Phosphatase) was added to 6 mL of reagent creating the substrate solution. A 96-well plate (Corning Inc Costar, cell culture cluster, flat bottom Tissue Culture treated) was used for the PP2A assays. Next 40 µL of enzyme reagent were added to each well, followed by 10 µL of MC standards, DI water, and unknowns in triplicate in their respective wells. The plate was covered with Parafilm and placed on the platform shaker for 20 minutes. After 20 minutes 50 µL of substrate were added to each well. Bubbles in the wells were popped using a wire, then 100 µL of DI water were added to empty wells to be read as blanks by the microplate reader, and the plate was placed in the microplate reader. The absorbance at 405 nanometers for each of the wells was measured at 5-minute intervals for 1 hour. The maximum optical density reading changes over time, V (mOD/min), were recorded. The ratio of sample color change (Vi) over color change in the zero MC wells (Vo) was calculated. MC concentration versus the ratio of Vi/Vo was then plotted to develop a standard curve.

SUMMARY

A variety of methods were used to develop MC monitoring procedures for future use by municipal water laboratories, and examine MC behavior in the COP water matrix. The key points for the monitoring and analytical techniques include:

- Sampling:
  - Monthly samples collected from Lake Pleasant, Bartlett and Saguaro Lakes from September 2001 through October 2002.
  - Monthly water quality parameters also monitored as part of the COP/ASU T&O project.
- Experiments:
  - PAC tests conducted using dosages of 1, 3, 5, 8, 12, 20 and 50 mg-PAC/L spiked with 7.5 μg-MC/L.
  - Residual chlorine tests conducted using concentrations of 0.02, 0.05, 0.1, and 1.0 mg-chlorine/L spiked with 12 μg-MC/L.
  - *Microcystis aeruginosa* cultures obtained from UTEX were cultured for use in intracellular extraction method development.
  - Three methods for intracellular extraction were tested using *Microcystis aeruginosa* cultures. These methods included:
    a) Direct beadbeating of filtered biomass.
    b) Soaking biomass in MtOH for 24 hours, followed by beadbeating.

c) Soaking biomass in MtOH for 24 hours.

- Solid phase extraction using C18 cartridges to concentrate MC in a sample, and to remove matrix interferences.

- Analytical Methods:
  - Determination of MC concentration using ELISA.
  - Determination of MC concentration using PP2A.

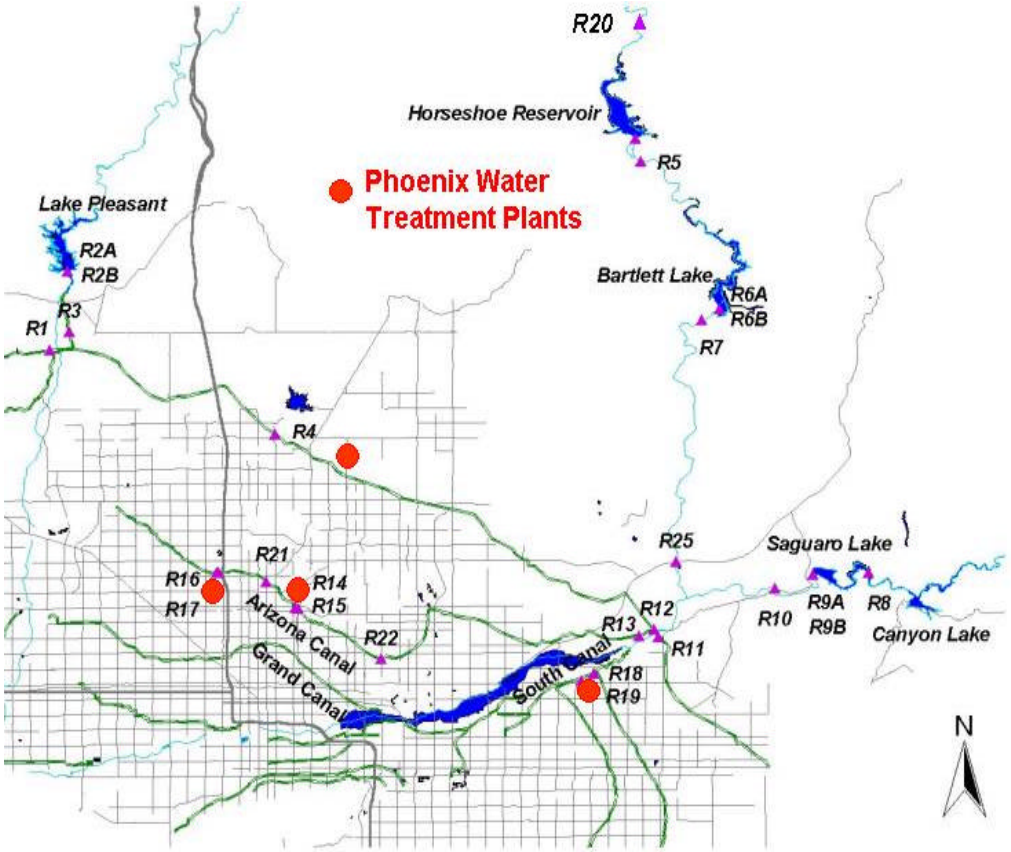


Figure 3.1 Monitoring Sites for MC analysis (? R#) and location of COP water treatment plants (?). Samples analyzed for MC concentration included reservoir samples R2, R6, and R9; canal samples R11, R12 and R13; and Deer Valley and Squaw Peak treatment plants (*Source:* [1])

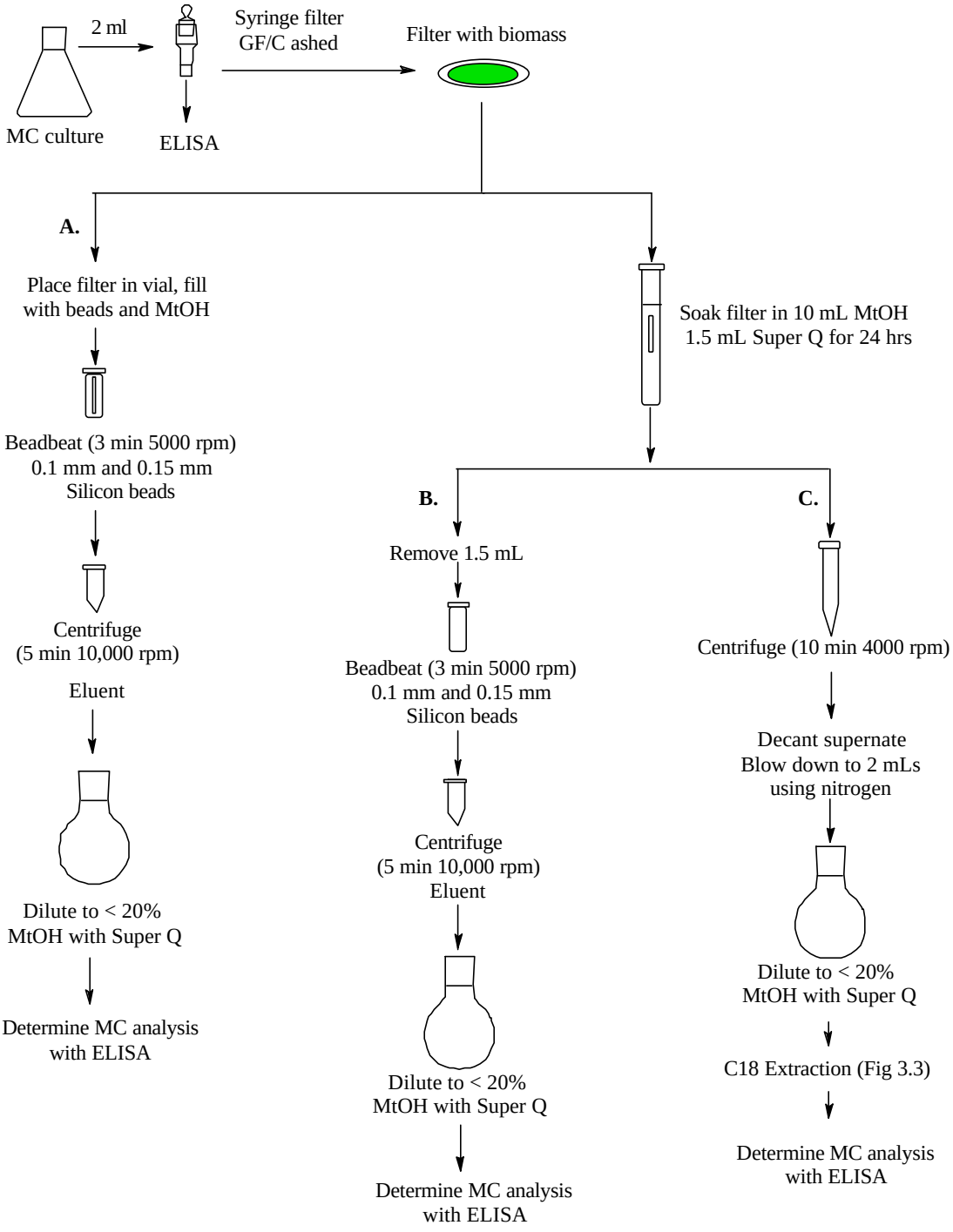


Figure 3.2 Schematic of intracellular extraction methods A, B, and C.

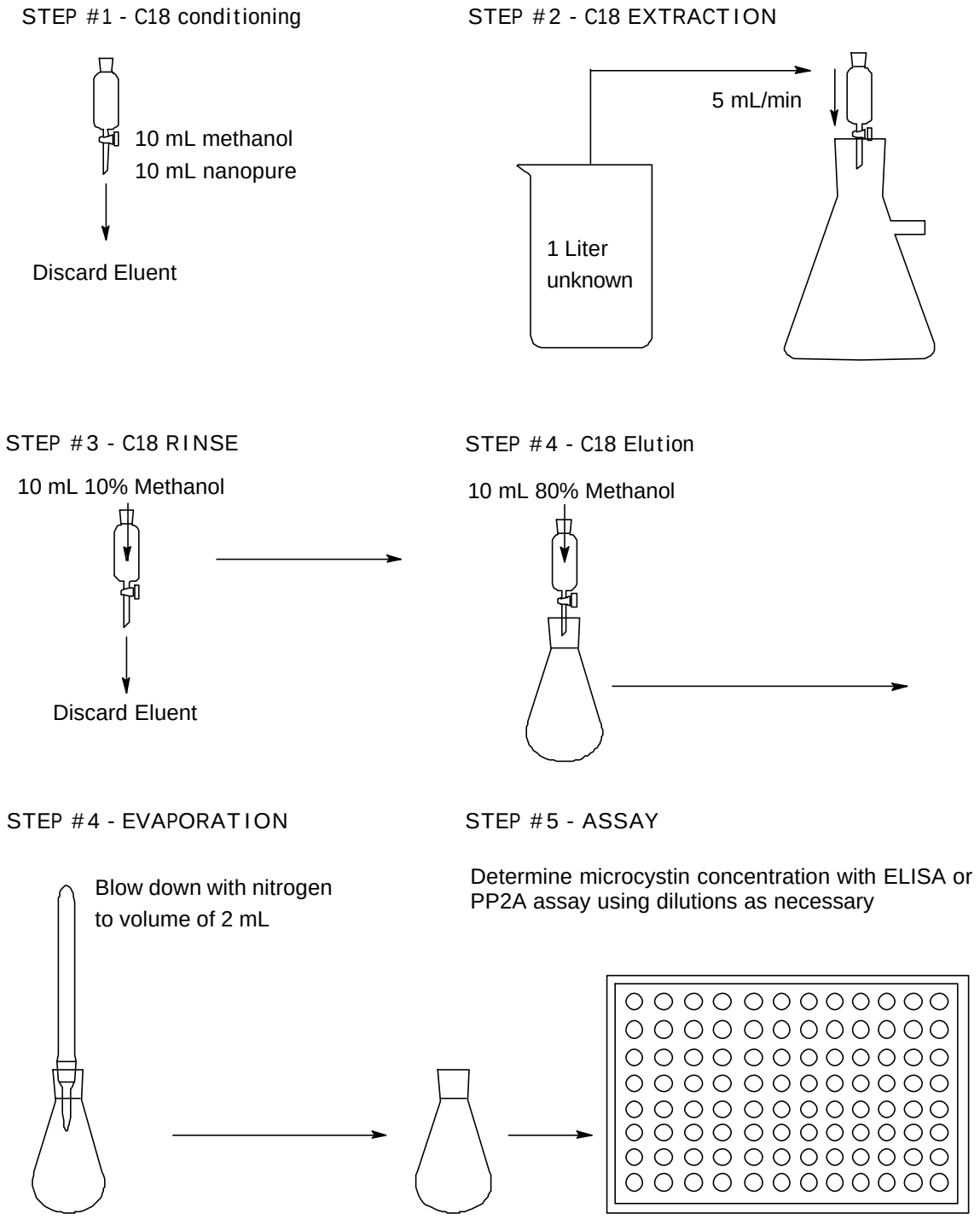


Figure 3.3 Schematic of solid phase extraction procedures

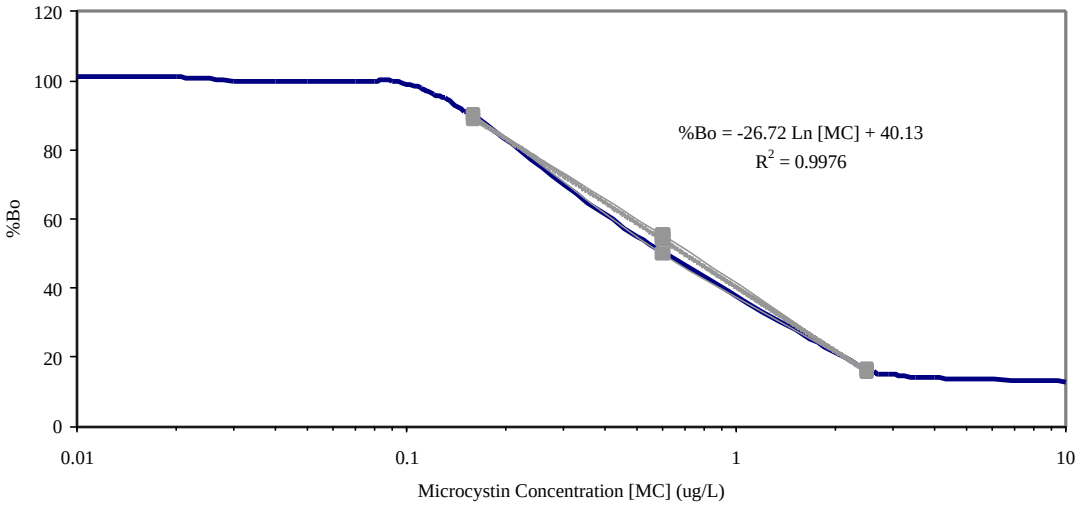


Figure 3.4 Relationship between optical density (%Bo) and microcystin concentration for ELISA standard curve development

CHAPTER 4

PRODUCTION OF TASTE & ODOR BY CYANOBACTERIA

A main objective of the COP/ASU T&O project was identifying MIB/Geosmin producers. However other T&O compounds are of concern in the COP water supply. The secondary objective of this research involved the analysis of twenty-one isolated cyanobacteria for non-MIB/Geosmin T&O producing compounds.

NON-MIB & GEOSMIN TASTE & ODOR COMPOUNDS

Non-MIB/Geosmin T&O compounds in isolated algal cultures were identified by SPME coupled with GC-MS tests conducted at COP laboratories. The cultures examined were from the COP reservoirs, rivers and canals. Isolates were cultured in BG-11 growth media (Appendix) to obtain a highly concentrated solution of potential T&O compounds. The cultures were allowed to grow until the culture was producing an odor detectable by the human nose. The BG-11 algal mixture was then filtered using a GF/C syringe filter to remove cellular material that interferes with GC-MS analysis. The eluent was next delivered to the COP laboratory for SPME analysis to determine the T&O culprit compound. The compounds examined in this testing process were obtained from the "Taste and Odor Wheel" [18] and include: cis-3-hexen-1-ol, cis-3-hexenyl Acetate, 2-Isobutyl-3-Methoxypyrazine (IBMP), 2-Isopropyl-3-Methoxypyrzaine (IPMP), Methylisoborneol, Cyclocitral, Geosmin, and 2,4,6-Tribromoanisole.

The results of the non-MIB or Geosmin T&O compounds are presented in Table 4.1. As indicated in the table, all but one of the compounds (*Leptolyngya*) were positive for cyclocitral. Additionally, the majority of the cultures did not contain any other compounds at concentrations greater than the detection limits of SPME. However the *Plankothrix* was positive for 2,4,6-Tribromoanisole, and the *Lyngbya* was positive for Cis-3-hexen-1-ol. It is important to note that these results should not be interpreted quantitatively. The algal cultures were allowed to grow for various amounts of time, which resulted in varying compound concentrations. The purpose of this test was to determine the presence of T&O culprit compounds; therefore results should be interpreted as positive or negative for the presence of a particular compound. Based on these results, the presence of cyclocitral in the COP water supply may play a significant role in T&O episodes occurring in the Phoenix metro area. Additionally, isolated cyanobacteria were also tested for MC. Microcystin was not found in any of the samples based on analysis using PP2A.

SUMMARY

Cyclocitral can be produced by algae in the COP water supply and may play a significant role in the T&O occurrence. Of the twenty-one isolated cultures examined using SPME, twenty samples were positive for cyclocitral. Future work concerning cyclocitral in the COP water supply should include removal of cyclocitral by the water

treatment processes, specifically PAC, and monitoring of production trends to determine critical T&O production periods throughout the year.

Table 4.1

SPME results for isolated algal cultures from the City of Phoenix water supply system

Taxonomy	Additional Information	Results (ng/L)							
		Cis-3-hexen-1-ol	Cis-3-hexenyl acetate	2-isobutyl-3-methoxypyrazine (IBMP)	2-Isopropyl-3-methoxypyrazine (IPMP)	Methylisobomeol	Cyclocitral	Geosmin	2,4,6-Tribromoanisole
Pseudanabaena	Salt River Saguaro inlet Plankton (05/01)	<50	<5	<2	<2	<2	20	<2	<2
Phormidian	Central Ave Canal	<50	<5	<2	<2	<2	140	<2	<2
Anabaena	CAP canal at cross-connect (08/01)	<50	<5	<2	<2	<2	109	<2	<2
Pseudanabaena	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	134	<2	<2
Pseudanabaena	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	576	<2	<2
Plectonema might be ***Limnothrix* (*Oscillatoria*)**	Plankton from Saguaro (09/01)	<50	<5	<2	<2	<2	10	<2	<2
Pseudanabaena	Periphyton from Saguaro (09/01)	<50	<5	<2	<2	<2	6	<2	<2
Pseudanabaena	From 19th Ave Canal	<50	<5	<2	<2	<2	9	<2	<2

Table 4.1 (continued)

SPME results for isolated algal cultures from the City of Phoenix water supply system

Taxonomy	Additional Information	Results (ng/L)							
		Cis-3-hexen-1-ol	Cis-3-hexenyl acetate	2-isobutyl-3-methoxypyrazine (IBMP)	2-Isopropyl-3-methoxypyrazine (IPMP)	Methylisobomeol	Cyclocitral	Geosmin	2,4,6-Tribromoanisole
Lyngbya	Sediment from Saguaro (05/01)	<50	<5	<2	<2	<2	4	<2	<2
Symploca	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	15	<2	<2
Limnothrix (Oscillatoria)	Plankton from Saguaro (09/01)	<50	<5	<2	<2	2	83	<2	<2
Leptolyngya (Oscillatoria)	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	<2	<2	<2
Microcoleus	Plankton from Saguaro (09/01)	<50	<5	<2	<2	<2	11	<2	<2
Limnothrix (Oscillatoria)	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	45	<2	<2
Leptolyngya (Oscillatoria)	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	3	<2	<2

Table 4.1 (continued)

SPME results for isolated algal cultures from the City of Phoenix water supply system

Taxonomy	Additional Information	Results (ng/L)							
		Cis-3-hexen-1-ol	Cis-3-hexenyl acetate	2-isobutyl-3-methoxypyrazine (IBMP)	2-Isopropyl-3-methoxypyrazine (IPMP)	Methylisobomeol	Cyclocitral	Geosmin	2,4,6-Tribromoanisole
Microcoleus	Floating pad from Saguaro (08/01)	<50	<5	<2	<2	<2	2	<2	<2
Plankothrix (Oscillatoria)	Periphyton from Saguaro (05/01)	<50	<5	<2	<2	<2	19	<2	5
Pseudanabaena	Plankton from Saguaro (09/01)	<50	<5	<2	<2	<2	11	<2	<2
Pseudanabaena	Plankton from Saguaro (08/01)	<50	<5	<2	<2	<2	10	<2	<2
Lyngbya	Plankton from Saguaro (08/01)	285	<5	<2	<2	<2	514	<2	<2

CHAPTER 5

MICROCYSTIN METHODS VALIDATION & COMPARISON

The primary objective of this thesis is to develop MC monitoring methodologies for the COP water supply. Several aspects of the ELISA and PP2A assays were considered for analysis including:

- Detection range and method detection limits (MDL)
- Matrix interference
- Freezing effects
- Assay consistency
- C18 cartridge recovery capabilities
- Intracellular extraction techniques

ASSAY DETECTION RANGE & LIMITS

The EnviroLogix™ ELISA kit specifies a MDL of 0.147 µg/L for MC. The MDL was calculated using an interpolation at three standard deviations from the mean (81.3% B_o) for negative water samples (Appendix). The MDL values calculated using the procedures outlined in the Code of Federal Regulations (1986) were 0.21 µg-MC/L (3 times the standard deviation for eight replicate samples), and 0.24 µg-MC/L (based on 95% confidence using the F-ratio method). The kit MDL of 0.147 µg-MC/L was used in reporting sample MC concentrations. Standard curves developed for ELISA ranged from

0.16 to 1.6 μg-MC/L in kits used from September 2001 through August 2002. In succeeding months EnviroLogix™ increased the range of the standard curve from 0.16 to 2.5 μg-MC/L.

The ELISA assay provided a consistent, reliable method for monitoring MC concentration in the reservoirs. Table 5.1 summarizes the ELISA assays run over the past 14 months. EnviroLogix's™ kits provide removable rows of wells with each tray. When an assay is run, the user determines the number of rows needed for the assay, therefore more than one assay may be performed using the same kit. The column Kit No. in Table 5.1 indicates the kit number assigned by ASU to track any trends specific to an individual kit. For example, in the initial assays (#1-4), the 50% B_o was less than 0.6 μg-MC/L, the 50% B_o value determined in assays #5-11. Kits (#1 and #2) were near the manufacture's specified expiration date, and this variation may be due to some degradation of the substrates, enzymes and antibodies. The ELISA assays provided consistent readings as seen in the similar slopes and high R^2 values. All of the ELISA assays were considered acceptable based on high R^2 values (all greater than 0.94), and consistent %Bo standard values. The Envirologix™ kit is a reliable method for determining MC concentration.

Table 5.1

Summary of ELISA assays conducted

Assay No.	Date	Kit No.	50%Bo (µg/L)	Slope	R^2	Acceptable	Assay Range (µg/L)	Comments
1	11/27/02	1	0.4	-28.8	0.957	Yes	0.16-1.6	Calibrators
2	1/22/02	1	0.2	-22.3	0.947	Yes	0.16-1.6	less than
3	2/02/02	2	0.2	-22.7	0.961	Yes	0.16-1.6	recommended
4	5/15/02	2	0.2	-24.3	0.947	Yes	0.16-1.6	%Bo ranges. Near expiration date
5	6/25/02	2	0.5	-28.3	0.982	Yes	0.16-1.6	
6	8/28/02	3	0.6	-27.0	0.993	Yes	0.16-1.6	
7	9/12/02	3	0.6	-24.1	0.975	Yes	0.16-2.5	
8	9/19/02	4	0.6	-22.6	0.989	Yes	0.16-2.5	
9	10/10/02	5	0.6	-27.6	0.992	Yes	0.16-2.5	
10	10/17/02	6	0.6	-26.7	0.997	Yes	0.16-2.5	
11	10/25/02	7	0.6	-26.8	0.987	Yes	0.16-2.5	

The MDL and range of the PP2A assay is dependent on protein phosphatase-2A enzyme reactivity. Initially the MDL for PP2A was 0.05 µg-MC/L calculated according to procedures outlined in the Code of Federal Regulations (1986), using three sample concentrations of 0.15, 0.3, and 0.02 µg-MC/L. The initial MDL tests were conducted using a PP2A assay range from 0.05 to 0.25 µg-MC/L and are valid for assays within this range. However, enzyme reactivity was inconsistent from assay to assay. As a result PP2A assay ranges varied with each assay performed. A more typical range was from 0.24 to 2.5 µg-MC/L. The MDL for assays in this range was 0.24 µg-MC/L.

Enzyme stability proved to be a hindrance with PP2A analysis. Table 5.2 summarizes the PP2A assays run over the past 14 months. The Enzyme No. column indicates the ASU assigned number to separate assays run with the same enzyme.

Protein phosphatase-2A is stable at –20 degrees Celsius. As the enzyme warms, it activates and should promptly be used in analysis. The enzyme stock used in the lab (Promega PPTase-2A, V631A) was difficult to keep below this temperature. The enzyme variations resulted in unpredictable 50% Vi/Vo values, and various assay ranges. The MC concentrations of several samples were undetermined due to the inconsistent assay ranges. Assays were considered unacceptable based on inconsistent calibrator results, lack of color production, or poor R^2 values (<0.75). These concentrations may have been detectable with ELISA, or with different protein phosphatase-2A enzyme. Figures 5.1-5.3 are calibrator curves developed using three different PP2A enzymes. Figure 5.1 was developed using enzyme #1 that was initially warmed to room temperature before use in the assay. The high R^2 value inidicates consistency between calibrators. The MDL for this assay was 0.05 μg-MC/L. Figure 5.2 was developed using enzyme #4. Again a high R^2 value is observed. The MDL for this assay was approximately 0.24 μg-MC/L, and the assay range is much larger than in Figure 5.1. Enzyme #4 was kept at –20 degrees Celsius and was not allowed to warm resulting in greater enzyme activity and thus the increased assay range. Figure 5.3 was developed using a fresh enzyme (#5) that was also stored at –20 degrees Celsius. The R^2 value is lower than expected and there is an usual amount of scatter between calibrator replicates. The inconsistency of PP2A enzyme proved to be a problem. It was difficult to select appropriate calibrator concentrations when such large variations in assay ranges occur and are difficult to anticipate. Therefore, the ELISA assay is recommended for future monitoring based on its consistent assay results.

Table 5.2

Summary of PP2A assays conducted

Assay No.	Date	Enzyme No.	V/Vo= 50% µg/L	Slope	R^2	Acceptable	Assay Range (µg/L)	Comments
1	4/26/02	1	0.15	-3.12	0.9988	Yes	0.05-0.30	Enzyme at room temperature
2	6/06/02	1	0.15	-3.36	0.9415	Yes	0.05-0.25	
3	6/13/02	1	0.15	-3.03	0.9143	Yes	0.05-0.30	
4	6/20/02	2	0.15	-2.93	0.7110	Yes	0.05-0.25	Poor R^2 value
5	6/27/02	2	NA	NA	0.2489	No	NA	New enzyme used, calibrators
6	6/27/02	2	NA	NA	0.3110	No	NA	not in range of Vi/Vo < 1
7	7/10/02	2	NA	NA	NA	No	NA	
8	7/12/02	2	0.6	-0.54	0.9276	Yes	0.2-1.2	
9	7/12/02	3	0.8	-0.55	0.9741	Yes	0.2-1.2	
10	7/24/02	3	0.6	-0.52	0.9656	Yes	0.2-1.2	
11	7/24/02	3	0.7	-0.53	0.8232	Yes	0.2-1.5	
12	7/24/02	3	0.7	-0.52	0.9626	Yes	0.2-1.5	
13	7/24/02	3	0.9	-0.51	0.9306	Yes	0.2-1.5	
14	8/02/02	3	NA	NA	0.0085	No	NA	Enzyme was inactive from overuse at room temperature
15	8/02/02	4	1.1	-0.51	0.9027	Yes	0.2-1.6	
16	8/08/02	4	0.6	-1.03	0.9297	Yes	0.2-0.9	
17	8/21/02	4	1.4	-0.43	0.9588	Yes	0.3-1.8	
18	8/28/02	4	1.0	-0.47	0.9917	Yes	0.2-1.5	
19	9/12/02	4	1.0	-0.53	0.9310	Yes	0.4-1.5	
20	9/19/02	5	1.5	-0.19	0.7516	No	0.5-2.5	Inconsistent calibrator results
21	9/25/02	5	NA	NA	NA	No	NA	No color produced
22	9/26/02	4	1.2	-0.36	0.9416	Yes	0.5-2.1	
23	10/03/02	4	1.4	-0.35	0.9077	Yes	0.5-2.5	

MATRIX EFFECTS

The potential influences of the COP water matrix on ELISA and PP2A assays were examined. Three samples from each lake were filtered (Whatman GF/F) and spiked with MC stock solution to obtain MC concentrations of 0, 0.5 and 1.0 μg-MC/L. Control samples were prepared similarly in Super Q™ water spiked with the same MC concentrations. Samples were analyzed using ELISA and PP2A to determine potential matrix influences. No distinct trends were identified for a particular water matrix (Table 5.3). The MC concentrations presented in Table 5.3 are the average of three assay wells. No trend is noted between ELISA and PP2A results. That is, ELISA results are neither consistently greater than nor less than the PP2A results. The Super Q™ sample for the 0.5 μg-MC/L dose was undetectable according to ELISA and at a lower concentration than similarly spiked lake samples. There was no apparent reason for this low value. The amount of deviation between the different samples of the same dose illustrates the variability within the assays. Based on these results, matrix effects of natural waters in Arizona on the PP2A and ELISA assays appear to be negligible.

Table 5.3

ELISA and PP2A results (μg/L) for microcystin spiked lake water and Super Q™ samples

	C (1/S)	DOC (mg/L)	UVA (1/cm)	Approximate Initial MC Dosage: No spiked MC ELISA	No spiked MC PP2A	0.5 (μg/L) ELISA	0.5 (μg/L) PP2A	1.0 (μg/L) ELISA	1.0 (μg/L) PP2A
Lake Pleasant	1046	0.0475	0.0475	Blank	Blank	0.59	0.40	1.04	0.78
Bartlett Lake	665	0.0319	0.0319	Blank	Blank	0.43	0.53	0.94	0.85
Saguaro Lake	1974	0.00571	0.0571	Blank	Blank	0.47	0.28	1.52	0.78
Super Q™	NA	NA	NA	Blank	Blank	<0.147	0.28	0.98	0.64

MC concentrations were obtained by averaging the results from three wells (n = 3) for each assay. Where C is the conductance of the water sample.

FREEZING EFFECTS

Collected water samples were frozen and stored at –20 degrees Celsius. Cell lyses may occur due to freezing of the water samples [3]. The largest MC toxin fraction is contained within cyanobacterial cells [3]. Thus cell lysis encouraged through freezing should result in increased MC concentrations. During the increased MC production period observed from May through October, extracellular MC concentrations measured initially were not consistent with the concentrations measured after freezing. In all cases the concentrations decreased. Based on the inconsistent results between unfrozen and frozen samples, an experiment was conducted to determine possible freezing effects on MC concentration in natural water. The tests were performed using MC stock solution.

Any cell lyses occurring would be accounted for within the specific lake water matrix. One-liter samples from each lake and Super Q™ were spiked with MC stock to obtain MC concentrations of approximately 1.0 µg-MC/L. Samples were analyzed using PP2A, frozen and stored in a freezer for two weeks, then analyzed using ELISA. Results are presented in Table 5.4. Based on a Student t-test with an a-value of 0.05, there is a freezing effect on MC concentration in the samples. For the individual matrices, Pleasant and Saguaro samples had the greatest difference between frozen and unfrozen MC concentrations. In nine of the twelve samples, the frozen samples resulted in lower concentrations than the unfrozen samples. This phenomenon has not been documented in the literature and may be attributed to differences in the specific sample matrices, enzyme activity, or PP2A and ELISA producing different concentrations. Further research is required to determine what characteristics of the matrices are causing the decrease in MC concentration when the samples have been frozen. Variability in ELISA and PP2A assays are discussed in the next section of this report.

Table 5.4

ELISA and PP2A results for frozen, microcystin spiked lake water and Super Q™ samples

Lake	Sample Number	Unfrozen MC concentration (μg/L)	Frozen MC concentration (μg/L)
Pleasant	1	1.00	0.85
	2	0.96	0.89
	3	1.14	0.99
Bartlett	4	1.01	0.87
	5	0.94	0.99
	6	0.88	0.96
Saguaro	7	1.72	0.85
	8	1.31	0.85
	9	1.52	0.99
Super Q™	10	0.97	0.82
	11	1.1	0.80
	12	0.89	0.95
Mean		1.12	0.90
Standard Deviation		0.25	0.07

Frozen samples were allowed to sit in freezer at –20 degrees Celsius for two weeks. MC concentrations were obtained by averaging the results from three wells (n = 3).

ASSAY COMPARISONS

The calibration standards from an ELISA kit were tested to determine the accuracy of PP2A analysis in detecting MC concentration. The ELISA calibrator concentrations were 0.16, 0.5, and 1.6 μg-MC/L. The 0.5 and 1.6 μg-MC/L calibrators were diluted 10:1 to bring the MC within the detection limits of PP2A (0.05 to 0.25 μg-MC/L for this enzyme). The calibrators were treated as unknown samples and a PP2A assay was used to determine MC concentration. The ELISA calibrators were tested on

this PP2A plate to compare the MC concentration measurements of the two tests (Table 5.5). The PP2A test reasonably predicted the MC concentration of the calibrators with the tightest correlation for the 0.16 µg-MC/L calibrator and the greatest variability for the 0.5 µg-MC/L calibrator. The 0.16 µg-MC/L calibrator was already within the detection limits of the PP2A assay and therefore no dilution was necessary. Dilution of the 0.5 and 1.6 µg-MC/L calibrators may account for the greater variation among the replicates. Figure 5.4 provides a comparison of the two MC concentrations. Perfect agreement between the tests would have resulted in a curve with a slope and R^2 value of one. In this case the R^2 value is 0.88, indicating some deviation between the tests. In this case the slope was 0.87 indicating the PP2A measured concentrations were less than specified ELISA concentrations. The percent error was calculated by subtracting the PP2A concentration from the ELISA specified concentration and dividing the difference by the ELISA concentration. The percent error column illustrates the larger deviations for the diluted 0.5 and 1.6 µg-MC/L standards. Overall the PP2A assay reasonably predicts MC concentration in undiluted samples.

Several samples were analyzed using both the PP2A and ELISA assays. These samples are compared in Figure 5.5. Slightly greater variability is seen in the PP2A analysis. The 1:1 sloped line provides an indication of perfect correlation between the assay results. The majority of the data falls near this line, indicating reasonable correlation between the MC concentrations determined by the assays.

Table 5.5

ELISA calibrator concentrations predicted by PP2A assay

MC Concentration (μg/L)		Percent Difference (%)
ELISA (C_1)	PP2A (C_2)	$\frac{[C_1-C_2]}{C_1} \times 100\%$
0.16	0.14	12.5
0.16	0.18	12.5
0.16	0.14	12.5
0.5	0.34	32.0
0.5	0.93	86.0
1.6	1.6	0
1.6	1.6	0
1.6	1.1	31.3

SOLID PHASE EXTRACTION

The recovery capabilities of C18 extraction were tested using prepared 1.2 μg-MC/L samples. The detectable range for this PP2A assay was 0.07-0.26 μg/L. Dilutions of both the initial sample and C18 concentrated standards were used in PP2A analysis. Reservoir and Deer Valley samples were not diluted based on previous undetected levels of MC in these waters. Table 5.6 presents the results of C18 extraction of spiked Super Q™ water. The results of the C18 extraction were somewhat varied, recovering 1.2 ± 0.42 μg-MC/L. This variation in recovery capabilities may be attributed to different cartridges and should be considered when examining MC concentrations obtained from extracted samples. Standard #1 showed 68% recovery; Standard #2 showed 92%

recovery, and Standard #3 indicates 133% recovery. Based on the variability seen in these results, future C18 concentrated results should be viewed as approximate concentrations, not exact values. The MC concentration examined using C18 recovery will typically be in the part per trillion range, a thousand times lower than the WHO limits, and therefore the variable recovery among the C18 replicates is not a concern because the approximate values are significantly less than toxic concentrations that pose health risks and are still useful for MC monitoring.

Table 5.6

Microcystin concentration for C18 extracted samples (initial concentration 1.2 μg-MC/L)

Replicate #	MC Concentration (μg/L)
Sample 1	0.82
Sample 2	1.1
Sample 3	1.6
Average (μg/L)	1.2
Variance ($\mu g^2/L^2$)	0.18
Standard Deviation (μg/L)	0.42

Four samples from the COP water supply were concentrated using C18 cartridges and tested for MC using the PP2A assay. The MC concentration in the samples was anticipated to be low based on previous screening work, therefore no dilution of the C18 concentrates were performed. Table 5.7 illustrates the increased detection capabilities C18 concentration provides for MC monitoring of the COP water supply. During the

month of May, Bartlett Lake, Lake Pleasant, and Saguaro Lake have MC concentrations below the detection limits of both the PP2A and the ELISA tests. By using the C18 cartridges to concentrate the MC in the reservoir samples, the amount of MC in the Bartlett, Lake Pleasant, and Saguaro reservoirs was determined to be 2.1, 2.9 and 2.4 ng-MC/L respectively. The Deer Valley sample during the month of April had a concentration of 3.2 ng-MC/L. Thus, through the use of C18 cartridges, MC present at low concentrations are detectable. The results indicate the presence of MC toxin in Bartlett, Lake Pleasant, and Saguaro reservoirs as well as in the canals at part per trillion levels. Thus C18 concentration allows the monitoring of fluctuations in MC reservoir at levels less than the MDLs of ELISA and PP2A assays.

Table 5.7

Microcystin concentrations recovery using PP2A

Sample Site	MC Concentration Average (μg/L)	
	No C18	With C18
May 2002 Bartlett	<0.05	0.00021
May 2002 Saguaro	<0.05	0.00024
May 2002 Pleasant	<0.05	0.00029
April 2002 Deer Valley	<0.05	0.00032

INTRACELLULAR EXTRACTION

Three procedures were tested to determine the most complete MC intracellular extraction technique. The three methods included (A) direct beadbeating of filtered biomass, (B) 24-hours in methanol (MtOH) followed by beadbeating of filtered biomass, (C) and 24-hours in MtOH. The extractions were performed on a culture of *Microcystis aeruginosa* (UTEX) after 15 days of growth. The extracellular concentration of the sample was 0.62 μg-MC/L determined by ELISA, and the intracellular concentration 494 ± 89 μg-MC/L. An additional test was run to further compare method (B) and method (C).

Intracellular extraction tests indicated that cells soaked for 24 hours in MtOH followed by cell lyses through beadbeating (Method B) provided the most robust extraction. In the initial intracellular extraction comparison, Method C, MtOH soaking followed by C18 extraction, measured the highest intracellular concentration of 0.795 μg-MC/OD_{730}. Method B measured a concentration of 0.627 μg-MC/L, and Method A, direct beadbeating, a concentration of 0.533 μg-MC/OD_{730}. A second extraction test was done to further compare Methods B and C.

The second test was conducted on the same *Microcystis aeruginosa* culture this time after 10 and 29 days of growth. Table 5.8 and Table 5.9 present the results for the intracellular extraction methods. The variation in the intracellular concentrations may be explained, in part, by the heterogeneous nature of the culture. Cells tended to form

"clumps" when the culture was disturbed. Thus it is likely that different amounts of biomass were removed during the initial filtration of these samples. Direct beadbeating (A) removed significantly less intracellular MC than both methods B and C. Method B provided the most robust extraction of intracellular MC, however method C produced similar results and is the simplest extraction technique. Based on this analysis, method C is recommended for use in future intracellular extraction. These concentrations were calculated using Equation 5.1.

$$C_{OD} = \frac{C_{ELISA} \times V}{OD_{730}} \qquad \text{Equation 5.1}$$

Where: C_{OD} = MC concentration of sample (μg-MC/OD_{730})
C_{ELISA} = concentration determined through ELISA assay (μg-MC/L)
V = filtered culture volume (L)

In the second extraction, Method B measured the highest intracellular concentration. However, Method C also provided similar intracellular extraction, and is recommended for future extraction based on method simplicity and sufficient extraction.

Table 5.8

Comparison of intracellular extraction methods

Sample Number	Microcystin concentration (μg-MC/OD_{730})		
	Direct Beadbeating	24 hrs in MtOH + Beadbeating	24 hrs in MtOH +C18 extraction
1	0.594	0.895	1.261
2	0.618	1.160	0.619
3	0.652	0.709	1.057
4	0.488	0.981	0.528
5	0.388	1.090	0.510
6	0.472	0.811	
7	0.490		
8	0.512		
9	0.580		
Mean	0.533	0.627	0.795
Standard Deviation	0.084	0.170	0.342

Culture Growth = 15 days
OD_{730} = 1.05 cm^{-1}
Extracellular concentration = 0.62 μg-MC/L
Intracellular concentration = 494 ± 89 μg-MC/L

Table 5.9

Intracellular extraction of *Microcystis aeruginosa* cultures

Sample Number	Microcystin concentration (μg-MC/OD_{730})			
	24 hrs in MtOH + Beadbeating		24 hrs in MtOH	
	10 day	29 day	10 day	29 day
1	0.662	1.042	0.437	>1.036
2	0.732	1.076	0.380	>1.036
3	0.634	0.991	0.408	>1.036
4	0.915	0.762	0.775	0.949
5	0.887	0.758	0.648	0.990
6	0.859	0.275	0.592	0.966
7		0.242	1.056	0.680
8		0.275	0.944	0.874
9			0.958	0.829
Mean	0.782	0.926	0.689	0.881
Standard Deviation	0.121	0.154	0.257	0.116

Culture Growth = 10 days
OD_{730} = 0.142 (1/cm)
Extracellular conc. = >2.5μg-MC/L
Intracellular conc. = 56 μg-MC/L

Culture Growth = 29 days
OD_{730} = 1.81 (1/cm)
Extracellular conc. = >25 μg-MC/L
Intracellular conc. = 838 μg-MC/L

*Gray values were not included in mean and standard deviation calculations because the large difference in their results was accounted to the heterogeneous nature of algae cultures or experimental error, not extraction method.

The amount of biomass present in a sample may be approximated using the OD_{730} of the sample. For the *Microcystis aeruginosa* samples, a relationship was developed between the optical density of a culture and its corresponding biomass. Dry biomass was determined for OD_{730} values for *Microcystis aeruginosa* culture after 21 and 41 days of growth (Table 5.10). The dry mass per liter of culture was also determined. Figure 5.6 compares biomass concentration with OD_{730}. As anticipated, biomass concentration

increases with increasing OD_{730} values. Using a best-fit curve for this figure, biomass concentration may be estimated from the OD_{730} using equation 5.2.

$OD_{730} = 1.694\ (B) + 0.456$ Equation 5.2

Where: OD_{730} = Optical density of sample measured at a wavelength of 730nm (1/cm)

B = biomass concentration per liter of solution (mg-biomass/L)

Table 5.10

Optical density and related biomass for *Microcystis aeruginosa* culture

Culture Growth (days)	OD_{730} (1/cm)	Biomass Concentration (mg-biomass/L)	μg-biomass/OD_{730}
21	0.95	0.356	0.75
21	0.98	0.282	0.58
41	2.22	0.940	0.85
41	2.50	1.270	1.02

Optical density and intracellular MC concentrations changed overtime in algal cultures. The MC producing cultures indicated that the ratio of intracellular MC and OD was very similar when measured over time (Figure 5.7). Figure 5.7 also provides the growth curve of the culture with a lag phase up to day 10, followed by a period of exponential growth. Additionally, the MC concentrations measured using the three extraction methods are indicated in the figure.

SUMMARY

Assays and intracellular extraction methods were examined for future use in monitoring MC in the COP water supply. The results of these analyses include:

- The ELISA assay has a MDL of 0.147 μg-MC/L, a range from 0.147 to 2.5 μg-MC/L. Assay results were unaffected by water matrices, and 100% of the ELISA assays conducted were considered acceptable based on their high R^2 values. ELISA assays are recommended for future MC analyses.
- The PP2A assay has a MDL 0.05 μg-MC/L for an enzyme range from 0.05 to 0.25 μg-MC/L, and a MDL of 0.24 μg-MC/L for an enzyme range from 0.24 to 2.5 μg-MC/L. The PP2A assay was unaffected by water matrices, and 74% of the assays conducted were considered acceptable. Assays were considered unacceptable based on inconsistent calibrator results, lack of color production, or poor R^2 values (< 0.75). Enzyme activation at room temperature was considered the main source of error in PP2A assays. PP2A is not recommended for future MC monitoring.
- Freezing of samples before MC analysis effects measured MC concentrations, and further research should be conducted to determine the source of this concentration change.
- ELISA and PP2A predicted similar concentrations for three ELISA kit standards.

- SPE with C18 cartridges provides a means of measuring MC in the part per trillion range.
- Method C (24 hours in MtOH) is recommended for future intracellular extraction based on relative ease and completeness of extraction.

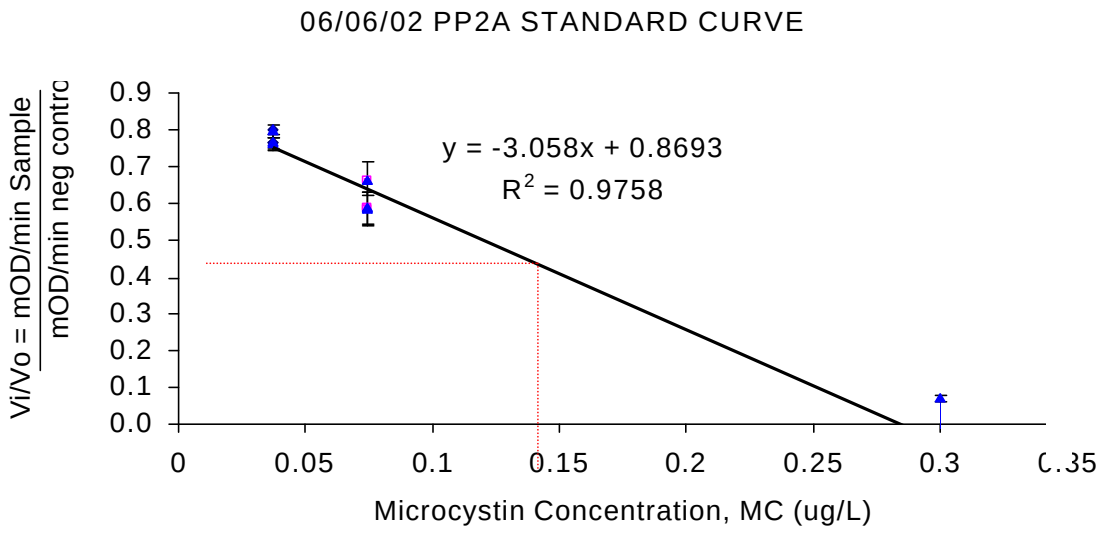


Figure 5.1 PP2A calibration curve using enzyme #1

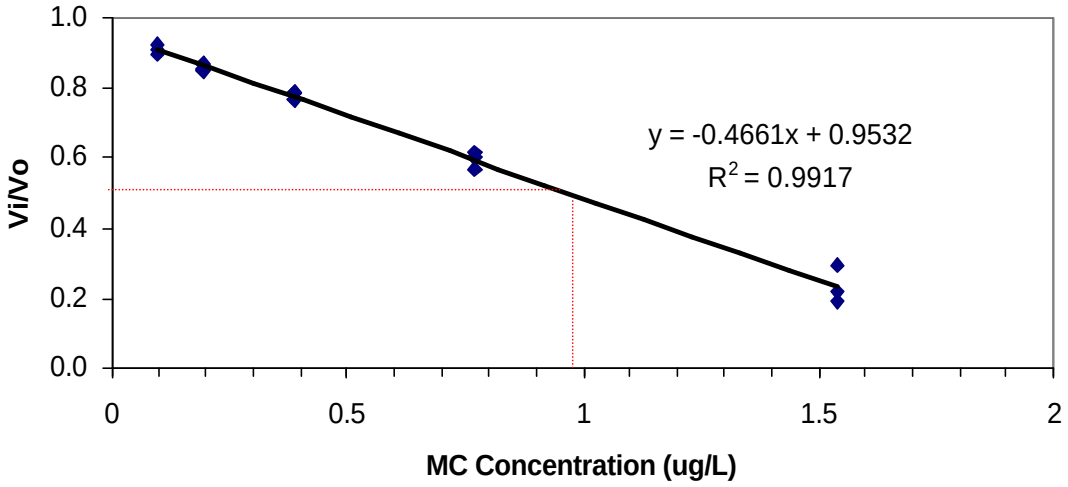


Figure 5.2 PP2A calibration curve using enzyme #4

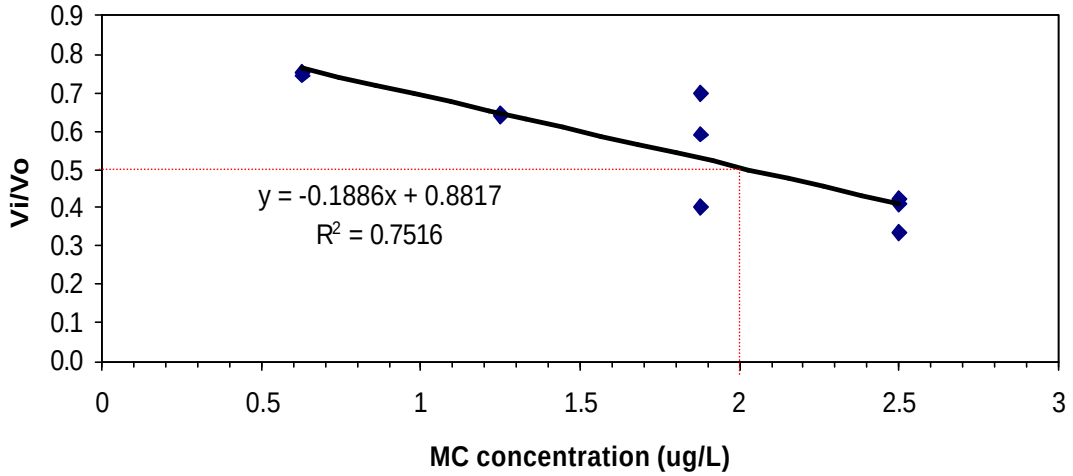


Figure 5.3 PP2A calibration curve using enzyme #5

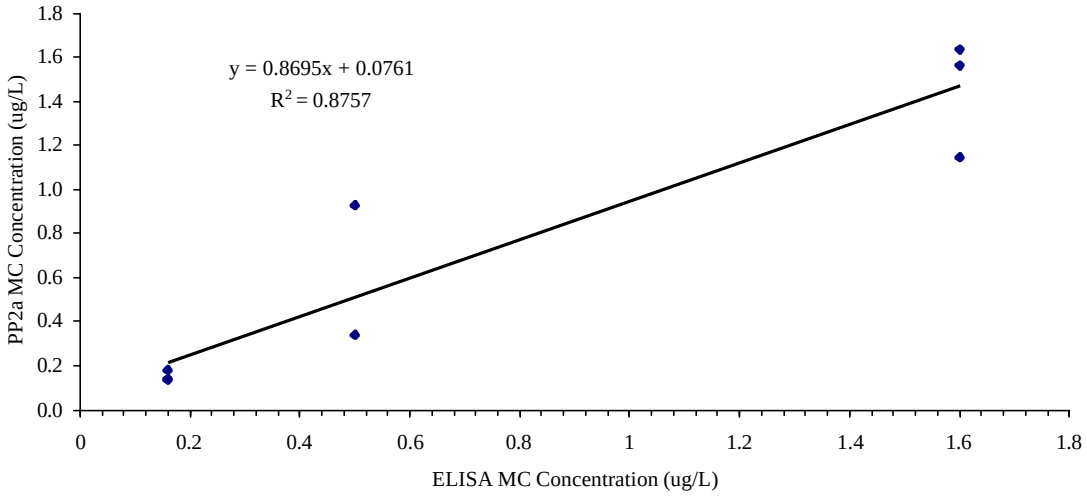


Figure 5.4 ELISA calibrators versus PP2A measured concentrations

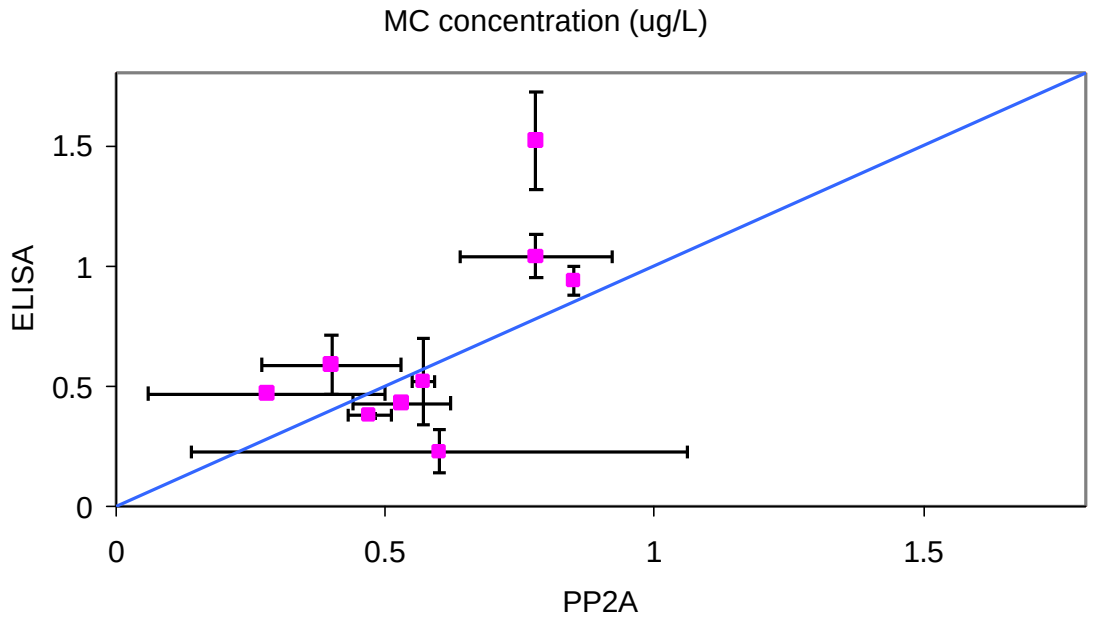


Figure 5.5 Comparison ELISA and PP2A microcystin concentrations

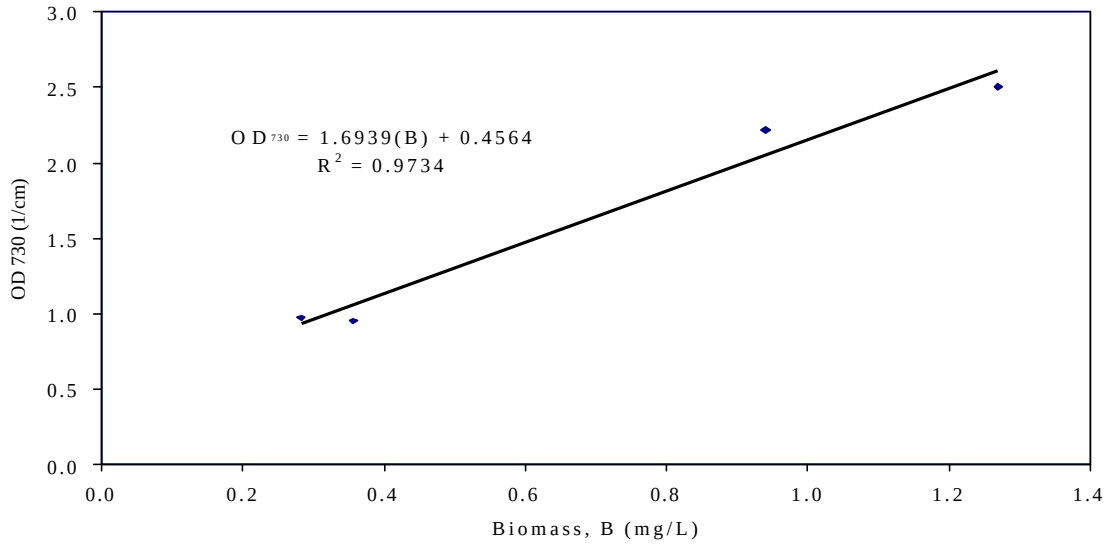


Figure 5.6 Relationship between optical density (OD_{730}) and *Microcystis aeruginosa* biomass

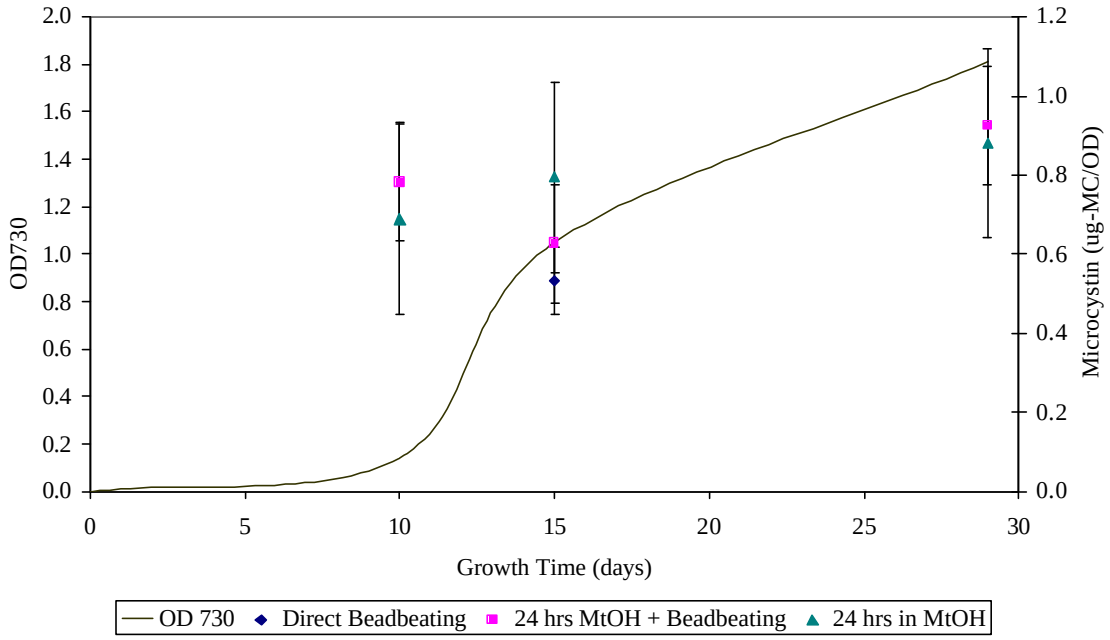


Figure 5.7 Optical density and intracellular microcystin change overtime in *Microcystis aeruginosa* culture

CHAPTER 6

APPLICATION OF MC ANALYTICAL TECHNIQUES

Microcystin concentrations in the Phoenix water supply were monitored using both ELISA and PP2A assays. Concentrations were undetectable from September 2001 through May 2002. In June 2002 the MC concentration in the reservoirs increased and detectable concentrations (>0.147 μg-MC/L) persisted through the end of October. Canal and WTP influent concentrations were monitored during this time frame. Several factors contribute to the production of MC. This chapter presents the observed change in MC concentration and potential correlation between MC concentration and algal biomass (chlorophyll-a), T&O compounds (MIB and geosmin), nutrients (phosphorous and nitrogen), organic carbon and light penetration (secchi disc depth) in the reservoirs over time.

TEMPORAL VARIATION IN MICROCYSTIN CONCENTRATION

The MC concentration in the Phoenix water supply varies with time. Figure 6.1 illustrates the MC fluctuations over the past 14 months, and Figure 6.2 shows the MC fluctuations in the canals and the Squaw Peak WTP for August and September 2002. Values less than the assay detection limit (0.147 for ELISA, 0.05 or 0.24 μg-MC/L for PP2A) are represented as zero. Deer Valley WTP is located at the end of the Arizona Canal and the MC concentration of the influent was monitored through part of the year to

indicate periphytic MC production in the canal (Figure 6.3). The Deer Valley MC concentration closely follows the lake concentrations, indicating little of no periphytic MC contribution. The MC concentration was also measured in the Deer Valley WTP immediately following PAC addition during August 2002. In general the MC concentration decreased after contact time with PAC. Hence PAC used for T&O compound absorption did reduce MC concentration. On August 28, 2002 the MC concentration was tracked through the Deer Valley WTP process (Table 6.1). The influent concentration was 0.32 μg-MC/L, the concentration following PAC was <0.1 μg-MC/L for this PP2A assay, and the effluent concentration was 0.11 μg-MC-L. The effluent concentration may have increased through release of intracellular MC following chlorine addition. The concentration did not appear to vary much following PAC and chlorine addition. At low concentrations of MC, this data is inconclusive. Future research should include the monitoring of MC through WTPs in the metro area.

Table 6.1 Microcystin concentrations (μg/L) in water treatment plants

Water Treatment Plant Removal—August 28, 2002			
	Deer Valley	Squaw Peak	Union Hills
Influent	0.32	0.15	0.12
Following PAC addition	<0.1	0.14	0.15
Following chlorination	0.11	NA	0.15

Microcystin concentrations in the reservoirs varied with time. The highest dissolved MC levels were measured in July. Samples were frozen for one to four weeks

prior to conducting assays from September 2001 through May 2002. As discussed in Chapter 5, MC concentrations may have been higher prior to freezing of the samples. It is therefore possible that MC concentrations were greater than assay detection limits prior to June 2002. Using C18 extraction techniques, MC at the part per trillion level was measured in September of 2001 and again in May of 2002. Hence MC is potentially in the water supply year round, but did not reach detectable levels via ELISA and PP2A assays until June. Dissolved MC levels were below the WHO action level of 1 μg-MC/L for total MC throughout the monitoring period. However, based on the dynamic nature of algal blooms, MC concentrations should continue to be monitored in the future.

Canal samples were monitored for MC throughout August and September of 2002 (Figure 6.2). The purpose of this monitoring was to determine if periphytic algal growth contributed to MC in the water supply. Microcystin concentrations measured in R12 samples include a combination of Saguaro and Bartlett Lake water. The R12 MC concentrations closely follow the lake concentrations, indicating no periphytic MC contribution. Lake Pleasant water flows through site R11 the CAP canal. Lake Pleasant had concentrations of 0.12 μg-MC/L in both August and September. R11 concentrations fluctuated around this range. A MC sample was also taken below the cross connect where CAP and SRP canal water are combined (R13). Based on the flow rates in these two canals on the day of sampling, the R13 sample showed no significant increase or loss of MC. The canal samples indicate no MC contribution by periphytic algae. Microcystin concentrations were also monitored in the Squaw Peak WTP. As indicate in Figure 6.2,

the MC concentration in the Squaw Peak WTP decreases slightly following PAC addition. The MC concentration in the Union Hills WTP (located on the CAP downstream of Lake Pleasant) was also measured on August 8th and 28th. Measured concentrations were 0.24 and 0.12 µg-MC/L, respectively. These concentrations are similar to those measured in Lake Pleasant during the same time period (0.58 to 0.12 µg-MC/L), again indicating little or no periphytic MC contributions.

MICROCYSTIN PREDICTORS

Several factors contribute to the production of MC. Currently the cause of MC production is unknown. As a part of the COP/ASU T&O project, several water quality parameters have been monitored for use in predicting MIB/geosmin production. The parameters investigated as potential MC predictors include:

- Algal biomass (chlorophyll-a)
- T&O compounds (MIB/geosmin)
- Nutrients (total and dissolved phosphorous and nitrogen)
- Light penetration (secchi disc depth)
- Particulate organic carbon (POC)

Correlations between MC and chlorophyll-a, MIB, phosphorous (total and dissolved), nitrogen (total and dissolved), secchi disc depth, and POC were determined by trendlines developed using Excel. The data was linearly fit and the corresponding R^2 value was

used to determine the extent of data correlation. An R^2 value less than 0.5 was selected to indicate insignificant correlation between variables. Using this criterion no linear correlation was found between MC concentration and chlorophyll-a, MIB, nutrients, light penetration and POC.

The change in MC concentration with time compared with the change in MIB concentration with time indicate increase in MC concentration in the reservoirs precede T&O episodes by approximately one month (Figures 6.4-6.6). This relationship may be explained ecologically. Microcystin producing cyanobacteria may flourish in the water column during June under specific pH, temperature, nutrient and light conditions. Then as the matrices change, the MIB-producing cyanobacteria begin to dominate the column.

Microcystin concentrations peaked one to two months following peak light penetration in the reservoirs measured by secchi disc depth (Figures 6.7-6.9). Periods of large light penetration decreases often indicate algal blooms in reservoirs. Microcystin concentrations appear to increase as light penetration drops following peak penetration. Cyanobacteria may be flourishing during these periods of light penetration drops, then as the cells die, MC is released and MC concentrations increase.

Changes in MC concentration overtime were compared with chlorophyll-a concentrations in Bartlett and Saguaro Lakes (Table 6.2). Chlorophyll-a data was available for July through October 2002. Lake Pleasant showed a large increase in chlorophyll-a following the peak MC concentration. The increasing chlorophyll-a values may indicate an increase in competing cyanobacteria and thus a decrease in MC

producing algae. Bartlett and Saguaro Lake MC and chlorophyll-a temporal data showed no distinct trends. The MC concentration decreased at one point as chlorophyll-a decreased and then continued to decrease when chlorophyll-a concentrations increased. Microcystin producers are only a small part of the total algae composition of the reservoirs and therefore MC production is not readily explained by chlorophyll-a data.

Table 6.2

Microcystin and chlorophyll-a concentrations in three water reservoirs

	July '02	August '02	September '02	October '02
Lake Pleasant				
MC (μg/L)	0.53 (0.10)	0.12 (0.15)	0.11 (0.15)	0.16 (0.02)
Chl-a (μg/L)	0.3	0.4	0.9	1.4
Bartlett Lake				
MC (μg/L)	0.43 (0.15)	0.26 (0.20)	0.26 (0.20)	<0.147
Chl-a (μg/L)	7.1	0.7	1.5	4.8
Saguaro Lake				
MC (μg/L)	0.27 (0.12)	0.21 (0.10)	<0.10	0.16 (0.00)
Chl-a (μg/L)	10.7	1.3	3.5	3.1

MC concentrations are an average value (n = 3). Standard deviations are listed in parentheses.

Total MC concentrations were determined for the reservoirs during August and October of 2002. Table 6.3 provides a summary of dissolved and total MC concentrations in Lake Pleasant, Bartlett and Saguaro Lakes. The total MC concentration is just larger than the dissolved concentration. Very little MC in the samples was contained in the cells at the time of sampling. In October the MC concentrations within the cells at the time of sampling, were one order of magnitude greater than those measured in August. Based on similar total and dissolved MC concentrations, samples were probably not taken from a MC producing bloom. In future monitoring dissolved MC concentrations are expected to be similar to total concentrations unless samples come directly from a bloom.

The amount of MC per OD_{730} was calculated for the October samples. These concentrations were >1.52 μg-MC/OD_{730} in Lake Pleasant, 0.076 μg-MC/OD_{730} in Bartlett Lake, and 0.460 μg-MC/OD_{730} in Saguaro. The MC mass per OD_{730} values indicate that a larger percentage of the biomass in the Lake Pleasant sample includes intracellular MC than in Saguaro and Bartlett at the end of October 2002. Based on intracellular and extracellular concentrations, Lake Pleasant appears to be more prone to MC production than Bartlett and Saguaro Lakes.

Table 6.3

Microcystin concentrations in Lake Pleasant, Bartlett and Saguaro Lakes

	Microcystin concentration (µg-MC/L)		
	Extracellular	Intracellular	Total
August 19, 2002			
Lake Pleasant	< 0.20	0.000972	<0.20
Bartlett Lake	0.26	0.000556	0.260556
Saguaro Lake	0.21	0.000506	0.210506
October 30, 2002			
Lake Pleasant (n=3)	0.15	>0.005	0.15
Bartlett Lake (n=3)	<0.147	0.00170	<0.147
Saguaro Lake (n=3)	0.15	0.00175	0.15175

POWDER ACTIVATED CARBON ABSORPTION

Powder activated carbon used for T&O compound removal absorbs MC. The percent remaining of MC toxin after absorption by PAC was determined based on PP2A assay results. The percent remaining was defined as C/Co multiplied by 100%. Where C is the final concentration (µg-MC/L) after contact with PAC, and Co is the initial concentration (µg-MC/L). The percent remaining MC was then plotted versus PAC dosage to compare MC absorption by three PAC brands.

AquaNuchar, Norit HDB and Norit 20B absorbed MC (Figure 6.13). The AquaNuchar removed forty percent of the MC at a dosage of 1 mg-PAC/L, the Norit HDB removed approximately fifty percent, and the Norit 20B removed ten percent. At a PAC dose of 5 mg/L, the AquaNuchar had removed approximately ninety percent of the

MC, while only a slight increase in percent removed was observed for the Norit HDB and the Norit 20B. As the PAC dosage increased, the performance of the Norit HDB and Norit 20B became more similar. By a dose of 8 mg-PAC/L, the AquaNuchar had removed sufficient MC to have a concentration less than the WHO limit of 1 μg/L. A dosage of 20 mg-PAC/L of the Norit HDB achieved a similar result. A dose of 50 mg-PAC/L of the Norit 20B was required to obtain a concentration less than 1 μg-MC/L. At the lowest PAC dosage, the AquaNuchar provided the greatest MC absorption.

The material used to make the PAC provides different absorption capabilities. Both the Norit HDB and the Norit 20B brands of PAC are made with coal. The AquaNuchar is a wood-based PAC. The wood-based PACs have been shown to be better absorbents of MC than the coal based PACs [37, 40]. Donati et al. [37] further explained that this difference in absorption capability was attributed to mesopores volume. As anticipated from the literature, the wood based AquaNuchar showed the greatest absorption capacity for the MC. The AquaNuchar removed sufficient MC to achieve acceptable concentrations (<1μg/L) at a dose of 8 mg-PAC/L. This carbon dosage (8 mg-PAC/L) is less than the PAC dosage applied in the metro area during T&O episodes (5-50 mg-PAC/L) and therefore should absorb MC present in the water as well as T&O compounds. The Norit HDB and Norit 20B also removed sufficient MC (< 1 μg-MC/L), but at PAC dosages greater than those typically employed at the Phoenix Metro water treatment plants. Use of the AquaNuchar or other suitable wood-based PACs is recommended for future MC and T&O compound removal.

CHLORINE OXIDATION

Low levels of chlorine are capable of oxidizing MC with sufficient contact time. Results of the residual chlorine test (Chapter 3) are presented in Figure 6.21. Microcystin concentrations after a 14-day contact time were determined using PP2A and ELISA assays. As seen in Figure 6.14, application of a low chlorine dose (0.1 mg-chlorine/L) oxidized 96% of the initial 13 µg-MC/L. For dosages less than 0.1 mg-chlorine/L, no oxidation of MC appears to be occurring. Glassware and water both have some chlorine demand and may have used up the available chlorine in the 0.02 and 0.05 mg-chlorine/L samples. The COP currently uses free chlorine to provide residual disinfection in the water supply. Based on these results, typical residual chlorine dosages from a WTP should be sufficient to oxidize MC in the drinking water.

SUMMARY

Monitoring of MC concentrations over time indicated:

- July was the peak month for MC production with the highest dissolved concentration in Lake Pleasant of 0.53 µg-MC/L.
- Microcystin was measured in the reservoirs from June through October 2002 at levels greater than 0.147 µg-MC/L, the detection limit for ELISA.

- Continued monitoring of MC in the reservoirs is recommended based on the dynamic nature of cyanobacteria blooms.
- No periphytic MC contribution was observed in the canals.
- The MC concentration measured in the Deer Valley WTP immediately following PAC addition decreased. Hence PAC used for T&O compound absorption did reduce MC concentration. Additional research should be done to determine effectiveness of WTP processes on MC removal.
- No linear correlations ($R^2 > 0.5$) were found between MC concentration and chlorophyll-a, MIB, phosphorous, secchi disc depth, and POC.
- Temporally:
    - MC peak production precedes MIB peak concentrations by one month.
    - MC follows peak light penetration (maximum secchi disc depth) by one month.
    - MC concentrations do not appear to follow chlorophyll-a temporal trends.
- AquaNuchar, a wood based PAC, absorbs MC at dosages currently used by the COP for T&O removal (5-50 mg-PAC/L).
- Low levels of chlorine (0.1 mg-chlorine/L) oxidize MC given sufficient contact time.

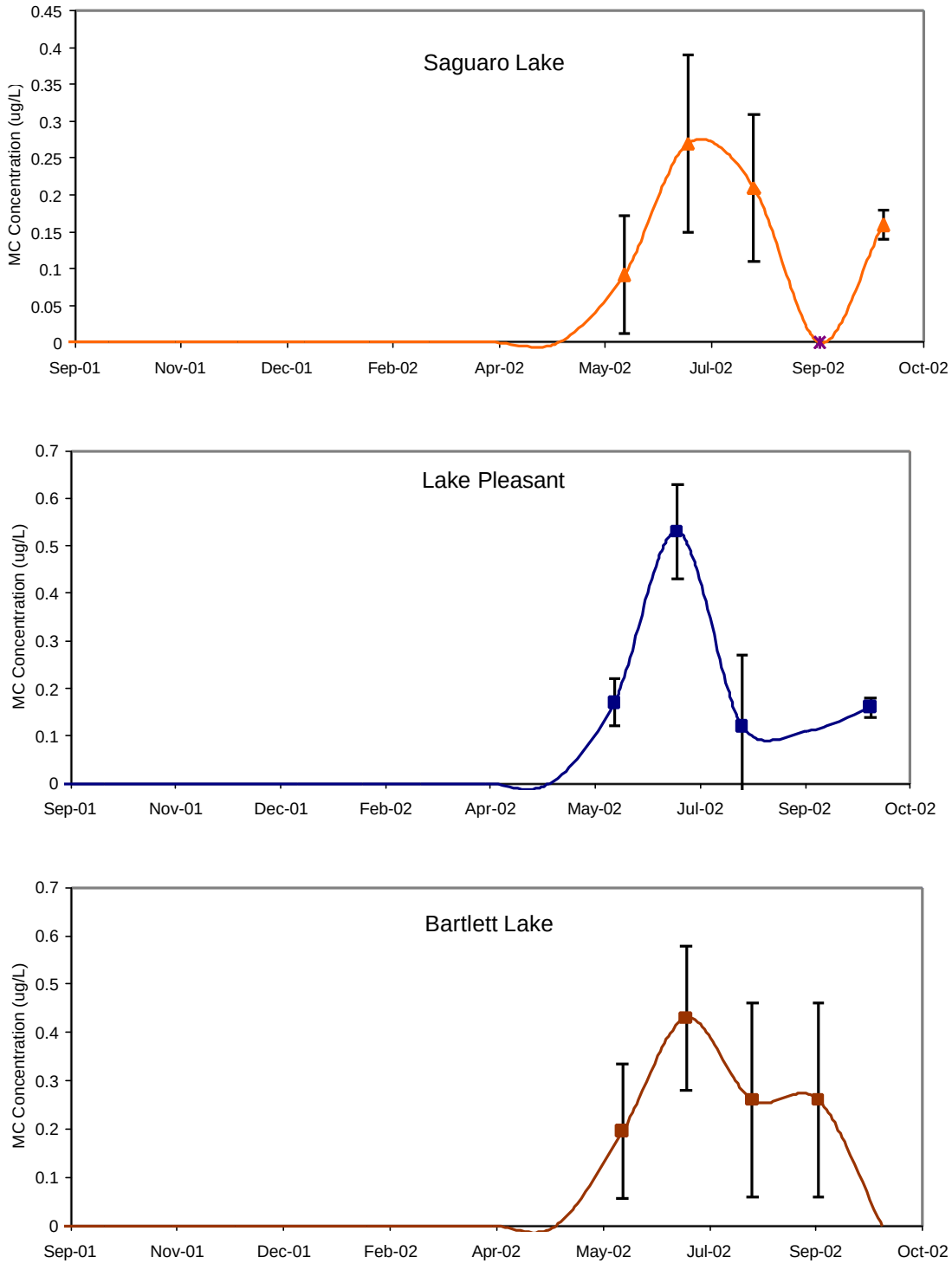


Figure 6.1 Microcystin concentrations with time in three reservoirs

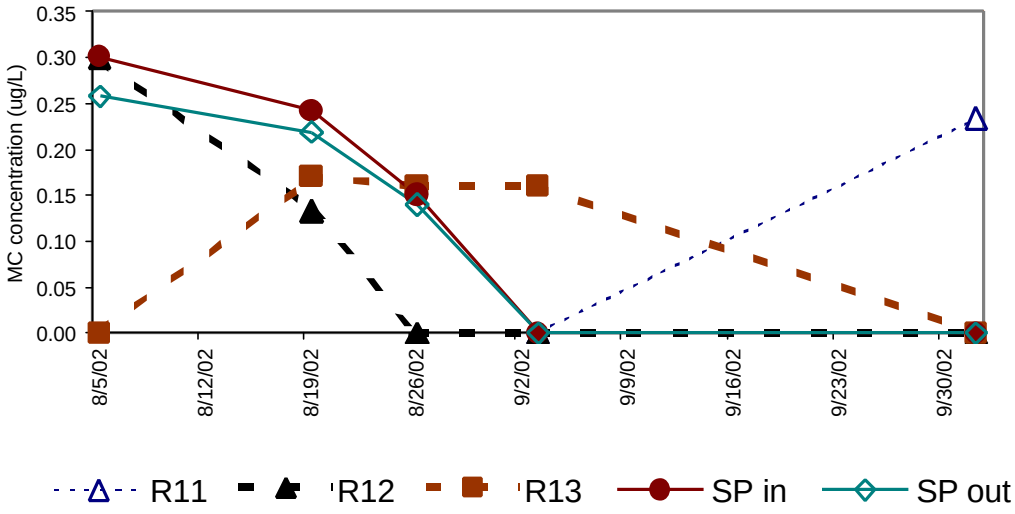


Figure 6.2 Microcystin concentrations with time in canals and Squaw Peak WTP. SP_{in} indicates the MC concentration entering the WTP. SP_{out} indicates the MC concentration following PAC concentration. Each data point represents the average value for three assay wells. Pooled standard deviation for all points was 0.15.

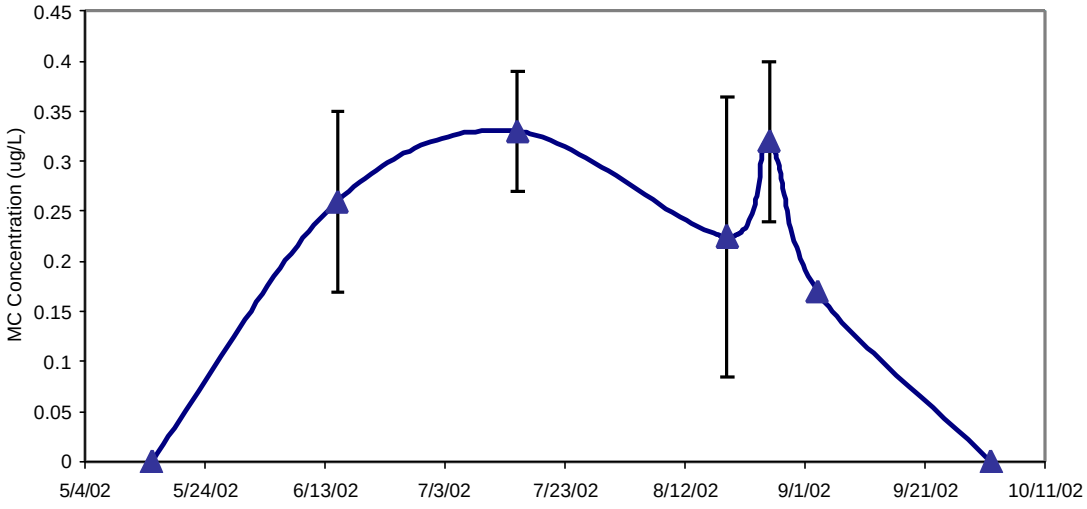


Figure 6.3 Microcystin concentration in Deer Valley WTP prior to PAC addition (n=3).

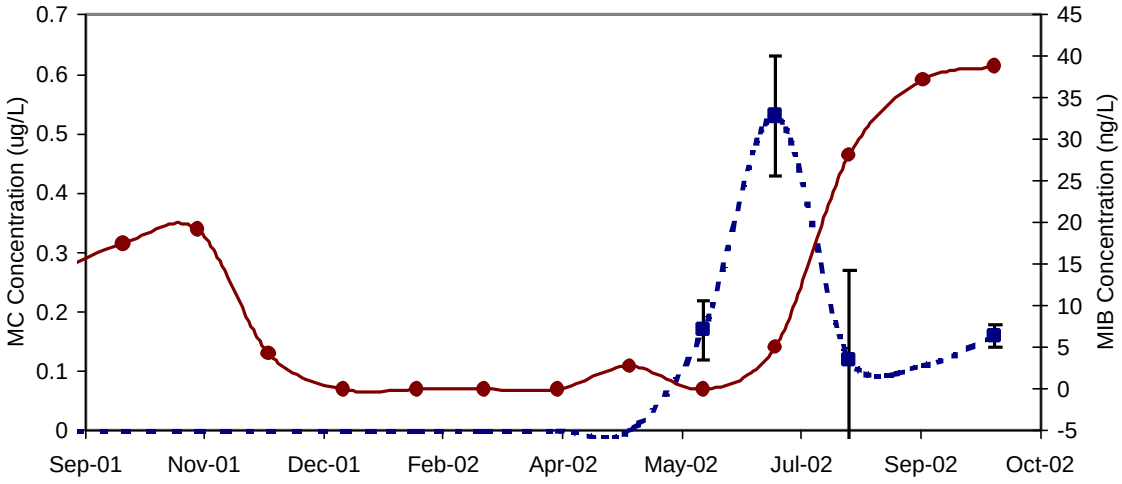


Figure 6.4 Lake Pleasant microcystin and MIB concentration with time. Microcystin is represented as the dashed line.

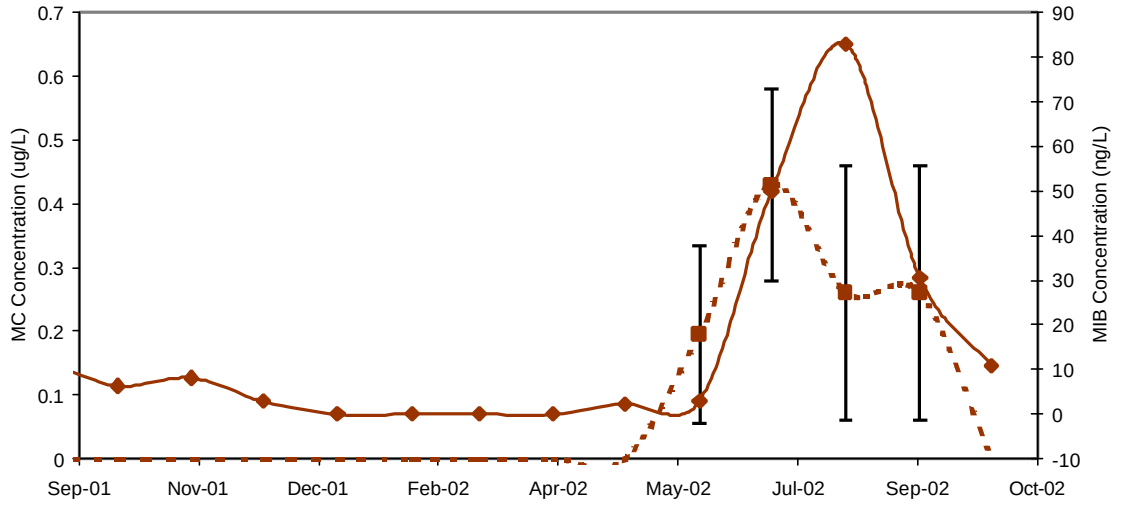


Figure 6.5 Bartlett Lake microcystin and MIB concentration with time. Microcystin is represented as the dashed line.

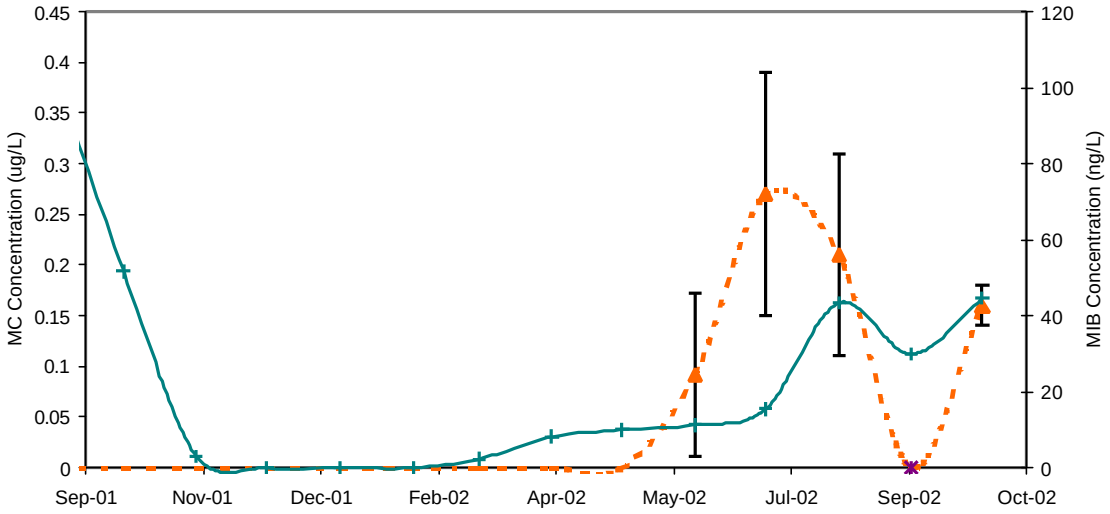


Figure 6.6 Saguaro Lake microcystin and MIB concentration with time. MC is represented as the dashed line.

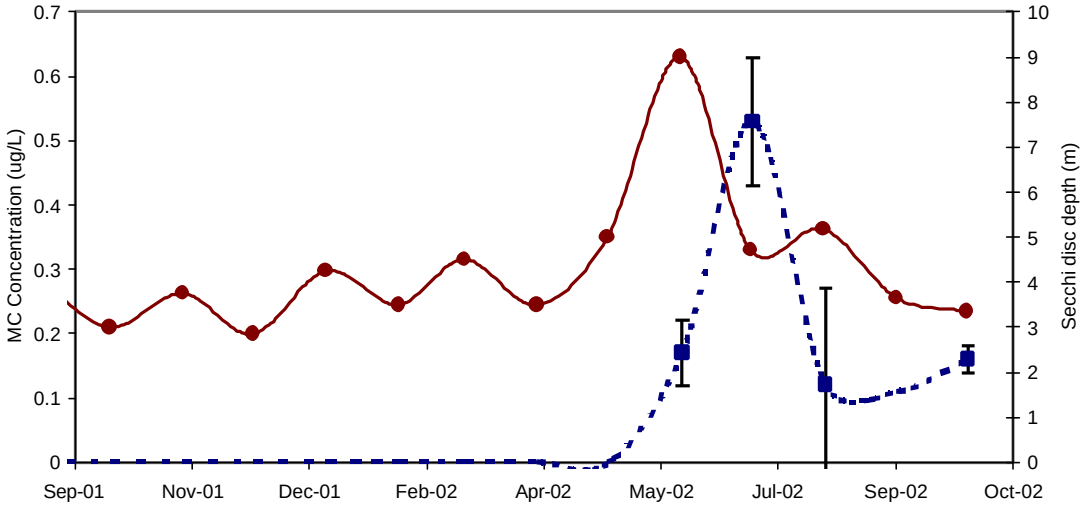


Figure 6.7 Lake Pleasant microcystin concentration and secchi disc depth with time. Microcystin is represented as a dashed line.

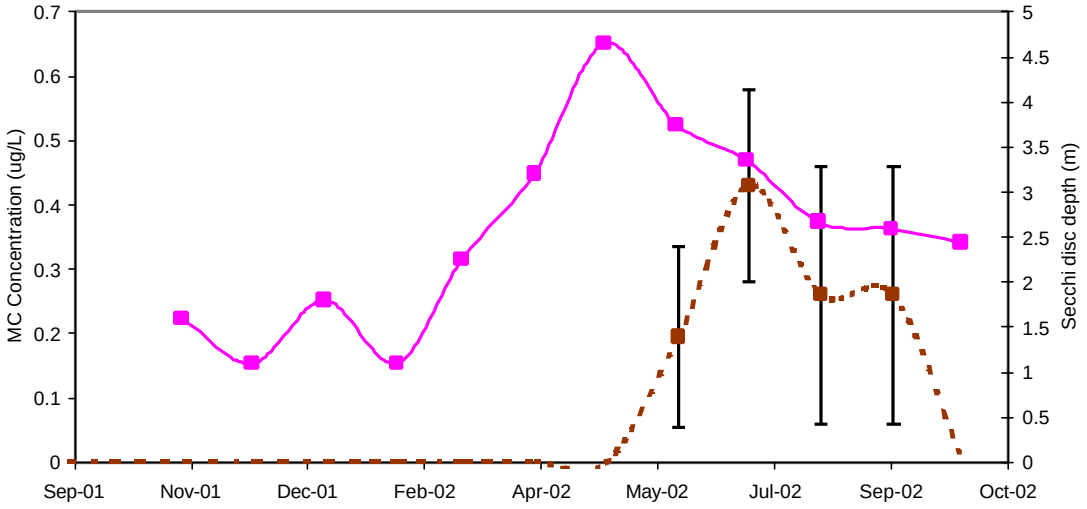


Figure 6.8 Bartlett Lake microcystin concentration and secchi disc depth with time. Microcystin represented as a dashed line.

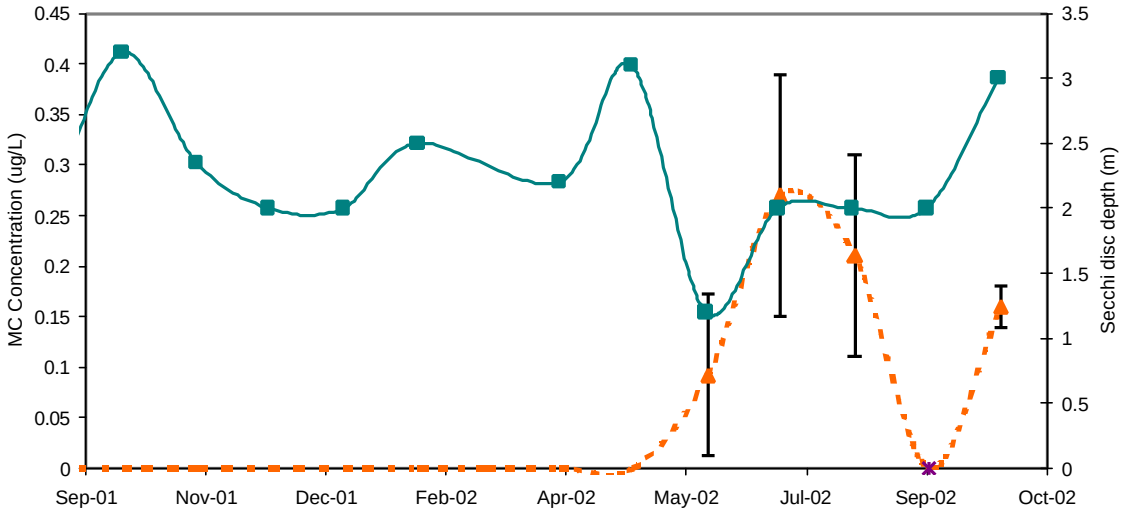


Figure 6.9 Saguaro Lake microcystin concentration and secchi disc depth with time. Microcystin represented as a dashed line.

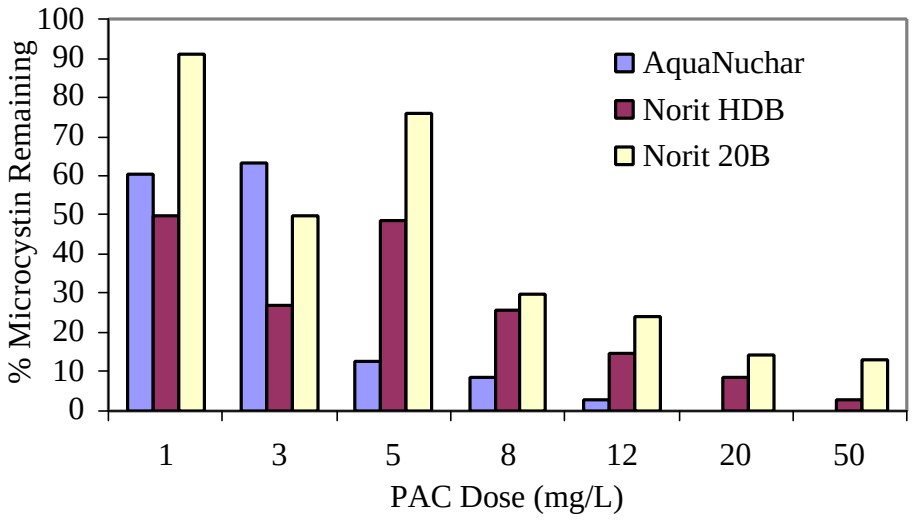


Figure 6.10 Absorption of microcystin by three powder activated carbon. Contact time of 5 hours. Initial microcystin dose of 7.5 µg-MC/L in Salt River Project water.

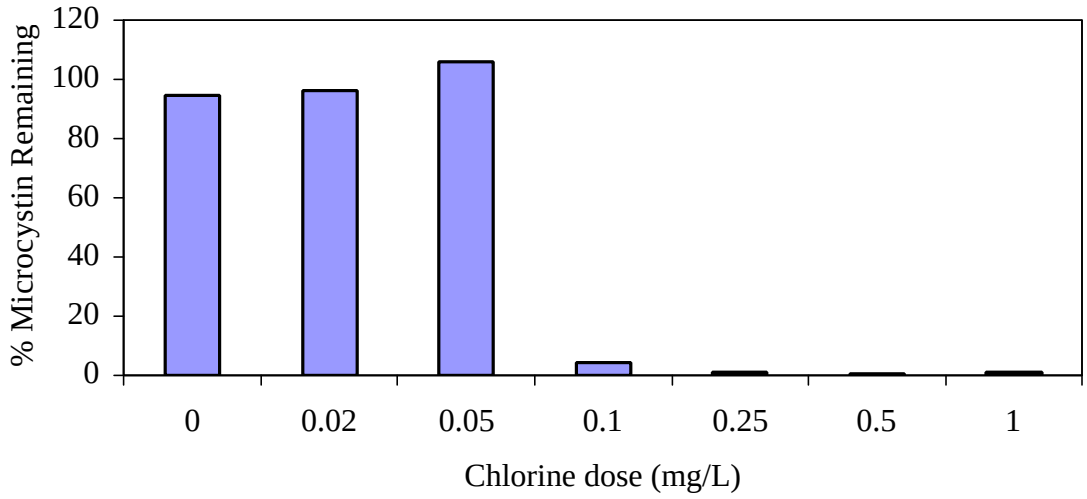


Figure 6.11 Oxidation of microcystin by chlorine after 14-day contact time

CHAPTER 7

SUMMARY AND RECOMMENDATIONS

Cyanobacteria exist in a wide range of environments, including Arizona surface waters. Cyanobacteria are capable of surviving at various depths in a water column due to gas vesicles that allow them to regulate their position and the utilization of multiple pigments during photosynthesis. Cyanobacteria are ecologically significant and act as both primary and secondary producers in the aquatic food chain. An excess of nutrients and compatible temperatures in a body of water may lead to the formation of cyanobacteria blooms. Cyanobacteria blooms lead to T&O problems in a drinking water supply, and may release cyanotoxins that are harmful to humans and animals. The WHO has recommended a limit of 1 μg-MC/L in drinking water to prevent chronic effects of MC. Several methods are available for measuring MC including HPLC, ELISA, and PP1/PP2A assays. Based on detection limits and ease of procedures, ELISA and PP2A assays were selected for further study. Cyanobacteria and cyanotoxins are not easily eradicated from a water supply and various methods have been used in water treatment for their removal. The methods chosen for further study in this research were PAC absorption, and chlorination.

EXTRACELLULAR & INTRACELLULAR DETECTION

The ELISA and PP2A assays were examined for use in future monitoring of the COP water supply. The MDL of the ELISA assay is 0.147 μg-MC/L and varies for PP2A depending on the reactivity of the protein phosphatase-2A enzyme used. The range for ELISA is 0.147 to 2.5 μg-MC/L. The range of the PP2A assays varies, but typically was 0.24 to 2.5 μg-MC/L. Both assay appear to be unaffected by the COP water matrix. Based on the inconsistency of the PP2A assay results, ELISA was used in intracellular extraction method development, chlorine oxidation and lake monitoring after September 2002. ELISA is recommended for future monitoring based on high R^2 values (> 0.9), consistent %Bo values for the standards, and the wide detection range (0.147 to 2.5 μg-MC/L).

Intracellular extraction tests indicated that cells soaked for 24 hours in MtOH followed by cell lyses through beadbeating (Method B) provided the most robust extraction. In the initial intracellular extraction comparison, Method C, MtOH soaking followed by C18 extraction, measured the highest intracellular concentration of 0.795 μg-MC/OD_{730}. Method B measured a concentration of 0.627 μg-MC/L, and Method A, direct beadbeating, a concentration of 0.533 μg-MC/OD_{730}. A second extraction test was done to further compare Methods B and C. In the second extraction, Method B measured the highest intracellular concentration. However, Method C also provided similar

intracellular extraction, and is recommended for future extraction based on method simplicity and sufficient extraction.

TEMPORAL VARIATION & WATER QUALITY RELATIONSHIPS

Microcystin is present in the COP water supply with the highest dissolved levels measured between June and October (0.14 – 0.53 µg-MC/L). Comparison of MC trends with other water quality parameters indicated no linear correlation ($R^2 > 0.5$) between MC concentration and chlorophyll A, MIB, nutrients, light penetration and organic carbon. Temporal MC concentration changes were compared with temporal changes in MIB concentration, light penetration and chlorophyll A concentration. Peak MC concentration proceeds peak MIB production by one to two months. Microcystin peak dissolved concentrations were approximately one month behind peak light penetration. Chlorophyll-a concentrations did not exhibit consistent temporal trends when compared with MC concentration. Both Deer Valley and Squaw Peak WTPs removed some MC through PAC absorption during peak MC production periods. Based on canal samples and MC concentrations in WTP influent, no MC periphytic contributions were observed.

Lake intracellular and extracellular concentrations were measured during August and again in October. August intracellular concentrations were 0.97 ng-MC/L in Lake Pleasant, 0.37 ng-MC/L in Bartlett Lake, and 3.6 ng-MC/L in Saguaro Lake. Corresponding extracellular concentrations for August were <0.20 µg-MC/L in Lake

Pleasant, 0.26 µg-MC/L in Bartlett Lake, and 0.21 µg-MC/L in Saguaro Lake. In October intracellular concentrations were >5 ng-MC/L in Lake Pleasant, 1.6 ng-MC/L in Bartlett Lake, and 1.8 ng-MC/L in Saguaro Lake. Corresponding extracellular concentrations during October were less than the ELISA MDL of 0.147 µg-MC/L for Bartlett Lake and 0.15 µg-MC/L for Lake Pleasant and Saguaro Lake.

POWDER ACTIVATED CARBON ABSORPTION & CHLORINE OXIDATION

The three PAC types examined reduced the initial MC concentration from 7.5 µg/L to below the WHO recommended concentration of 1 µg/L. The AquaNuchar showed the greatest absorption capability and reduce the MC to acceptable concentrations at a dose of 8 mg Carbon/L. This carbon dosage typically less than the PAC dosage range typically applied in the metro area (5-50 mg- carbon/L) for T&O episodes. The Norit HDB and Norit 20B also removed sufficient MC to be within the WHO recommend concentration, but at higher PAC dosages. Use of the AquaNuchar or other suitable wood-based PACs is recommended for future MC and T&O compound removal. Additionally, chlorine residual dosages used by the COP WTPs are expected to oxidize MC at the concentrations anticipated in the water supply.

NON-MIB & GEOSMIN TASTE & ODOR CAUSING COMPOUNDS

The twenty-one isolated T&O producers examined using SPME indicated the presence of cyclocitral in the COP water supply. Based on these results, cyclocitral may play a significant role in T&O episodes occurring in the Phoenix metro area. Future research should be conducted to determine the effectiveness of COP WTP treatment processes in removing the grassy, woody-hay T&O associated with cyclocitral. Additionally no MC was found in the isolated cultures examined. Microcystin producers *Anabaena* and *Oscillatoria* have been identified in the water supply.

FUTURE WORK & RECOMMENDATIONS

The presence of MC in the COP water supply leads to several areas of additional research. Reservoirs should continue to be monitored for both intracellular and extracellular MC concentrations. Because all three reservoirs use hypolomic withdrawl, additional MC monitoring should be conducted on samples from the hypolimnion. Monthly MC monitoring should be continued to insure MC levels remain below the WHO guideline of 1 μg-MC/L. Additional monitoring work should include the tracking of MC concentrations through WTP during peak production months to determine current MC removal capabilities.

Phytoplankton samples should be collected monthly at each reservoir in an effort to isolate MC producers. Isolated MC producers should be studied to determine their growth cycles. The culture should be used in determining when MC production and release occur. The relationship between MC intracellular and extracellular concentrations over time for specific producers. Isolated cultures could also be used to test specific environmental stimuli (example: conductivity) that may inhibit or increase MC production.

If MC concentrations continue to increase, MC producing blooms should be identified through lake sampling. Microcystin blooms are potentially hazardous to individuals or animals that come in connect with them. Intensive lake sampling would aid in the identification of bloom locations.

Finally, the T&O compound cyclocitral is present in the COP water supply. Cyclocitral concentrations should be monitored in addition to monthly MIB and Geosmin concentrations. Future research should include PAC absorption studies, and ozonation oxidations studies, to determine WTP ability to remove cyclocitral from the drinking water supply.

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