2020

Microcystins and Liver Disease Mortality, Insights from an Ecological Study

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Walden University
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Abstract

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MS, University of South Florida, 2015

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Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

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Abstract

Microcystins (MCs) are toxic secondary metabolites produced by freshwater cyanobacteria. Algal bloom subsidence can stimulate MC release, which can impair liver function if orally exposed to in large doses. The purpose of this retrospective, U.S. ecological study was to determine if MC exposure represented an environmental risk factor for liver disease mortality using a socioecological approach. A longitudinal ecological substudy investigated the association between average total MCs in Lake Washington and Lake Manatee and age-adjusted chronic liver disease (CLD)/cirrhosis death rates in Brevard County and Manatee County, Florida (FL). A prediction model of total MCs was deduced by quantifying levels of nitrites and phosphates in Lake Washington and Lake Manatee. According to multiple linear regression analysis, there was a significant positive association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States. Daily sunlight and state of residence also significantly predicted U.S. liver mortality rates. Average total MCs demonstrated predictive value in reference to age-adjusted CLD/cirrhosis death rates in Brevard County, FL. Positive social change can educate the federal and state governments to improve the surveillance of MCs. Results may encourage water treatment plants in Brevard County and Manatee County, FL to monitor and manage cyanobacterial contamination in drinking water sources.

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Dedication

I would like to dedicate this dissertation to my Aunt Mala who lost a battle to breast cancer in November 2016 and my immediate family. She would always tell me how proud of me she was of my academic achievements. Aunt Mala saw me as the mentor to her kids. I am honored to bring a hard copy of my work to her when I complete my doctorate.

Acknowledgments

First and foremost, I want to say how thankful I am for my family. They have watched me grow and transform into a scholar practitioner over the years. I am grateful for their endless love and support. Thanks to my fiancé, Braulia Gil, for the sacrificed weekends, home-made meals, and milestone celebrations. She has been by my side ever since I started on my doctorate. Thank you, Brandon Lopez-Dueñas, for assisting me on the canoe trips at Lake Manatee and set-ups in the laboratory. Also, I would like to recognize my committee members who have guided me in becoming an expert in my research. I cannot thank Dr. Raymond Panas, Dr. Leslie Elliott, and Dr. Simone Salandy enough for their contribution throughout my dissertation journey. I would have not written a quality dissertation without their critiques, expertise, and guidance. Finally, I want to extend my gratitude to my friends and relatives who have cheered me on and witnessed my academic successes.

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Chapter 1: Introduction to the Study

Introduction

Chronic liver disease (CLD) and cirrhosis is a significant cause of mortality worldwide. Globally, these long-term liver conditions cause 2 million deaths annually (Marcellin & Kutala, 2017). In the United States, more than 36,000 deaths in 2013 resulted from CLD/cirrhosis (Xu, Murphy, Kochanek, & Bastian, 2016). Liver disease-related mortality has been underestimated in the past 20 years (Asrani, Larson, Yawn, Therneau, & Kim, 2013). A more updated estimation on liver mortality in the United States is 66,007 deaths per year (Asrani et al., 2013).

In 2016, CLD/cirrhosis was the 8th leading cause of death in Florida (FL). CLD/cirrhosis was the 10th leading cause of death in 2017 (Centers for Disease Control and Prevention [CDC], 2018). Age-adjusted CLD/cirrhosis death rates have risen in Brevard County and fluctuated in Manatee County over the last decade. Aside from age-adjusted CLD/cirrhosis death rate variations, toxic harmful algal blooms (HABs) are increasing throughout FL waters. Specifically, microcystin (MC) production via HABs in recreational waters has captured public health attention.

MCs are potent algal toxins released by cyanobacteria in surface waters (Dokulil & Teubner, 2000). The oral route via contaminated drinking water is the primary route of MC exposure. Alternative exposure routes include consumption of aquatic foods and dietary supplements, dermal contact, and inhalation of aerosols (Drobac et al., 2013). Scholars have demonstrated potential associations between MCs and liver cancer/disease (Chen, Xie, Li, & Xu, 2009; Li et al., 2015; Zhang, Lee, Liang, & Shum, 2013; Zheng et

al., 2017; Zhou, Yu, & Chen, 2002). Yet, no scholar has factored in climatic variables and liver disease risk factors with potential MC-related age-adjusted CLD/cirrhosis death rates. Climate is a major determinant of cyanobacterial bloom formation and toxin production (Rastogi, Madamwar, & Incharoensakdi, 2015). Recently, Ventura-Cots et al. (2018) demonstrated an inverse relationship between average temperature, sunlight hours, and alcohol consumption.

The overarching goal of this study was to evaluate the hypothesis that U.S. age-adjusted CLD/cirrhosis death rates are predicted by average total MCs. The results of this study may lead to increased environmental awareness on MCs and liver disease mortality. Chapter 1 is a synopsis of the study. It begins with the introduction, background, and problem statement. The problem statement is presented, follow by the research questions and hypotheses, conceptual/theoretical framework, and nature of the study. Chapter 1 includes definitions and ends with a discussion on the assumptions, scope and delimitations, limitations, and study significance.

Background

CLDs comprise a global health problem, affecting 844 million people (Marcellin & Kutala, 2017). Approximately 2 million people die from CLDs every year (Byass, 2014). Mortality rates due to CLDs and the prevalence of CLDs compare to those of cardiovascular and pulmonary diseases and diabetes. The worldwide estimation of people with cardiovascular and pulmonary diseases is 540 million and 650 million, respectively (Marcellin & Kutala, 2017). Global mortality from cardiovascular (17.7 million deaths) and pulmonary diseases (6.17 million deaths) is relatively high compared to CLDs

(Emerging Risk Factors Collaboration et al., 2010; Ward, Schiller, & Goodman, 2014). The prevalence of people with diabetes is 422 million, with a mortality rate of 1.6 million deaths (Emerging Risk Factors Collaboration et al., 2010). Worldwide estimations of diabetes mortality reflect those of CLDs.

Although CLDs pose health problems in Western countries, they are often ignored in public health (Marcellin & Kutala, 2018). Nonalcoholic fatty liver disease (NAFLD) is the most common cause of CLD worldwide (Vernon, Baranova, & Younossi, 2011). In the United States, NAFLD has become the most significant cause of CLD in adults, with alcoholic liver disease and Hepatitis C virus being other common etiologies (Setiawan et al., 2016; Younossi et al., 2011). CLD/cirrhosis was responsible for over 36,000 deaths in 2013, making it the 12th leading cause of death in the United States (Xu et al., 2016). Liver mortality was underestimated in the last 2 decades (Asrani et al., 2013). The mortality rate is closer to 66,000 deaths per year (Asrani et al., 2013). Although CLDs represent a neglected public health problem, action is required to aid in the prevention, diagnosis, management, and treatment of liver disease.

CLD/cirrhosis mortality varies by sociodemographic characteristics, including age, sex, and ethnicity/race. Disparities in CLD/cirrhosis death rates among individuals aged 45-64 years in the United States increased 31% (20.1 to 26.4 per 100,000 individuals) from 2000 to 2015 (CDC, 2017). Liver mortality rates for men in the same age group increased 21% (29.8 to 36.2 per 100,000 people) and increased 57% (10.8 to 17) for women (CDC, 2017). CLD/cirrhosis death rates for men aged 25-44 years decreased 10% (6.1 to 5.5) and for women in that age group increased 18% (2.8 to 3.3;

CDC, 2017). Liver mortality rates increased 3% among persons aged ≥ 65 years (CDC, 2017). There are age and sex are risk factors for CLD/cirrhosis mortality and prevalence (CDC, 2017).

Ethnic/racial disparities in CLD/cirrhosis prevalence are recognized (Kemmer & Neff, 2008; Nguyen & Thuluvath, 2008). However, data are limited to each racial/ethnic groups in the United States (Nguyen & Thuluvath, 2008; Setiawan et al., 2016). Black men are more likely to have Hepatitis B or Hepatitis C compared to Mexican American and White men (Flores et al., 2008). Mexican American men have a higher risk of being heavy/binge drinkers than White men (Flores et al., 2008). White women are less likely to be diabetic or obese and have Hepatitis B or Hepatitis C compared to Black women (Flores et al., 2008). Mexican American women have a higher risk of being obese or diabetic than White women, but a lower risk for being heavy/binge drinkers (Flores et al., 2008).

In addition to sociodemographic characteristics, lifestyle and environmental factors can promote the rate of liver disease progression. Tobacco smoke, insulin resistance, hepatotoxins, diabetes, central obesity, alcohol intake, and aflatoxins have shown to accelerate liver injury amongst chronic Hepatitis B virus infected patients (Fattovich, Bortolotti, & Donato, 2008). MCs are regarded as hepatotoxins due to their mode of action in the liver. They can inhibit liver serine-threonine protein phosphatases following ingestion. At low doses, a slower onset of kidney and liver failure could occur, whereas at high doses, acute liver necrosis, intrahepatic hemorrhage, and shock may result (Puschner & Moore, 2013).

Scholars have identified MC as potential liver cancer/disease risk factors. CLD/cirrhosis was a primary health outcome in reference to MCs (Melaram, 2019). However, not a single epidemiological study in FL investigated the possibility of drinking water sources contaminated by MCs and counties with high liver mortality rates. CLD/cirrhosis was the 10th leading cause of death in FL in 2017, amounting to an age-adjusted death rate of 11.2 deaths per 100,000 people (CDC, 2018).

During the past 15 years, age-adjusted CLD/cirrhosis death rates in Brevard County, FL have gradually increased. In 2000, the age-adjusted CLD/cirrhosis mortality rate peaked at 15.0 deaths per 100,000 people (Florida Department of Health [FDOH], 2019a). Two years later, the age-adjusted CLD/cirrhosis death rate was 16.5 deaths per 100,000 people (FDOH, 2019a) A sharp increase occurred between 2015 and 2016, followed by an abrupt decline from 2016 to 2017 (FDOH, 2019a). Age-adjusted CLD/cirrhosis death rates in Brevard County have surpassed overall CLD/cirrhosis mortality rates in FL since 1998 (Figure 1).

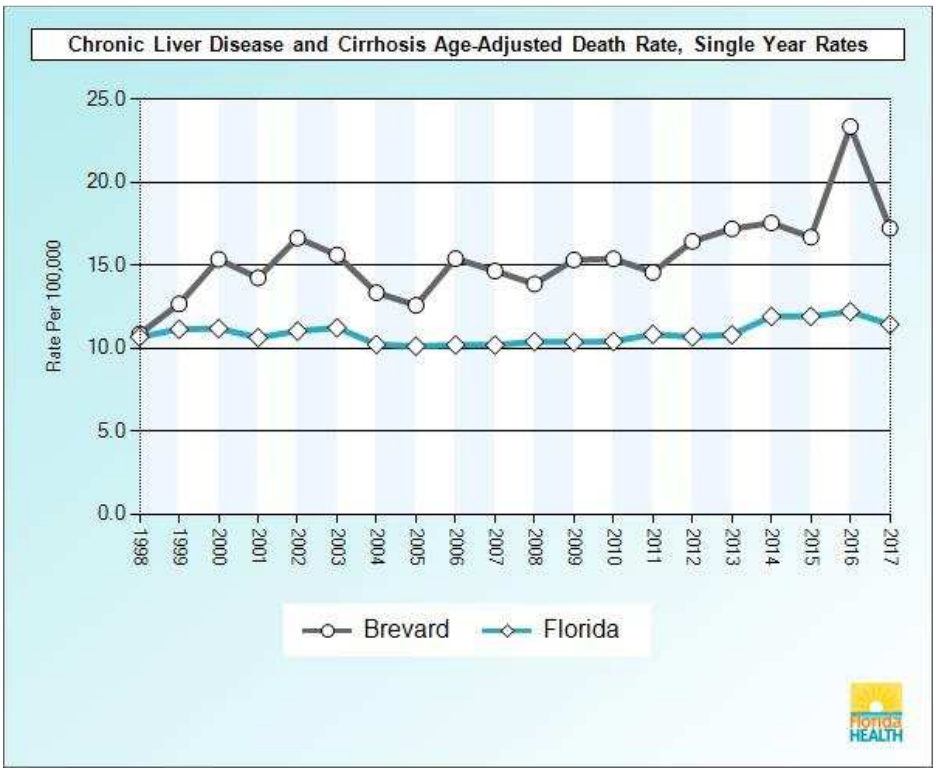

Figure 1. Chronic liver disease and cirrhosis age-adjusted death rate, single year rates, Brevard. Adapted from Florida Community Health Assessment Resource Tool Set, Retrieved from http://www.flhealthcharts.com/charts/DataViewer/DeathViewer/DeathViewer.aspx?indNumber=0091. Copyright 2019 by Florida Department of Health.

Sporadic trends in age-adjusted CLD/cirrhosis death rates manifested from 1998 to 2017 in Manatee County, FL. In 2010, the age-adjusted CLD/cirrhosis death rate was 13.9 deaths per 100,000 persons (FDOH, 2019b). Single year rates decreased steadily after dipping to 8.1 deaths per 100,000 persons in 2012 (FDOH, 2019b). From 2016 to 2017, age-adjusted CLD/cirrhosis death rates increased slightly (10.3 to 11.9; Figure 2).h

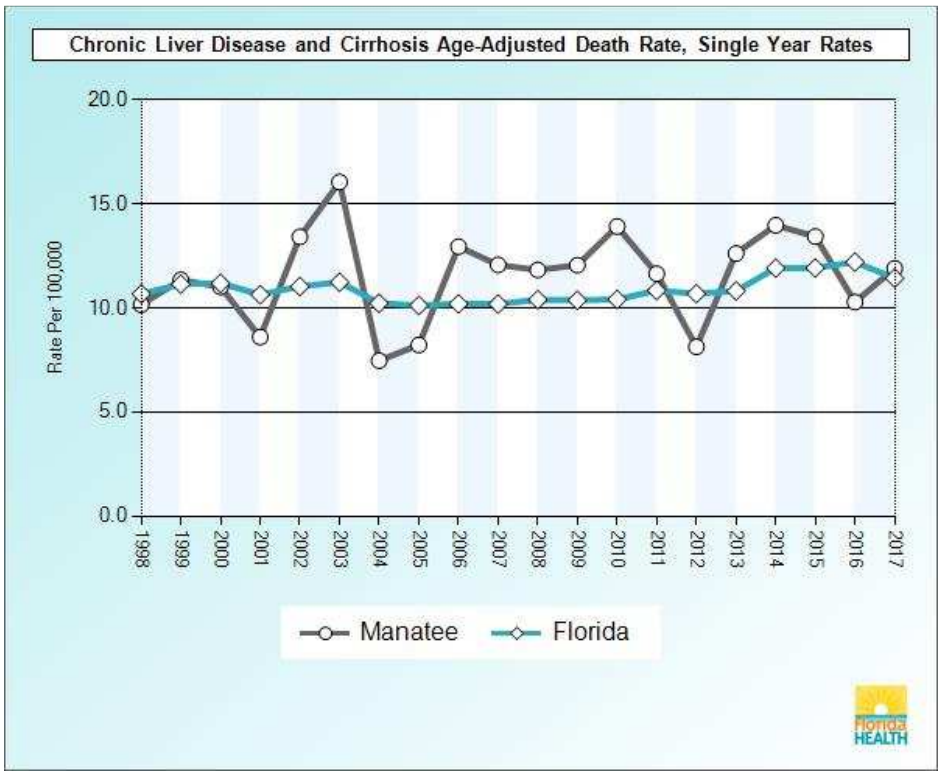

Figure 2. Chronic liver disease and cirrhosis age-adjusted death rate, single year rates, Manatee. Adapted from Florida Community Health Assessment Resource Tool Set, Retrieved from http://www.flhealthcharts.com/charts/DataViewer/DeathViewer/DeathViewer.aspx?indNumber=0091. Copyright 2019 by Florida Department of Health.

The relationship between MCs and liver cancer/disease remains unclear. Water quality issues and concomitant health-related effects lack appreciable scientific interest (Dias, Paulino, & Pereira, 2015). Small freshwater lakes in FL receive little attention regarding algal bloom occurrences. Lake Washington (Melbourne, FL) and Lake Manatee (Bradenton, FL) are two prime examples and supply drinking water to communities.

Lake Washington had MC concentrations six times greater than drinking water standards in 2001 (Waymer, 2016a). Detection of MCs in Lake Washington resulted in

periodic screening of the cyanotoxin. Between 2012 and 2014, increased nitrogen levels from human activities in Lake Washington was reported (Waymer, 2016b). Nitrogen is an essential nutrient for cyanobacterial growth; thus, algal blooms can emerge unexpectedly. Cyanobacteria in Lake Manatee (Bradenton, FL) caused taste and odor issues in drinking water. City officials proclaimed the water was safe although blue-green algae were present (Rogers, 2017). The coexistence between toxic cyanobacteria in municipal water sites and county-level age-adjusted CLD/cirrhosis death rates called for study on a neglected environmental health issue.

Problem Statement

Cyanobacteria are productive blue-green algae in aquatic ecosystems. Most cyanobacterial species are found in freshwater lakes, ponds, and rivers (Schmidt, Wilhelm, & Boyer, 2014). Cyanobacteria may propagate under ideal conditions stirring up algal blooms. These dense accumulations can induce toxin release among harmful cyanobacteria. MC is one cyanobacterial toxin of public health concern (Vesterkvist, Misiorek, Spoof, Toivola, & Meriluoto, 2012).

Epidemiology scholars have documented potential health effects of MC exposure (Chen et al., 2009; Labine et al., 2015; Li et al., 2015; Pilotto et al., 1997; Stewart et al. 2006; Zhang et al., 2013; Zheng et al., 2017; Zhou et al., 2002). In an epidemiological survey, Ueno et al. (1996) associated MC contaminated water sources to primary liver cancer (PLC). In a retrospective cohort study, Zhou et al. (2002) determined a correlation between MCs in drinking water and colorectal cancer (CRC) incidence. Lévesque et al. (2014) showed that humans living near lakes affected by cyanobacteria and MCs

experienced acute health effects. In a county ecological study, Zhang, Lee, Liang, and Schum (2015) demonstrated a link between the geographic distribution of cyanobacterial blooms and NAFLD.

The epidemiology of MCs is meager. Scholars who have studied cyanotoxins focused on known risk factors of gastrointestinal and respiratory diseases. Scholars have not concurrently investigated climatic and liver disease risk factors relative to MCs in recreational waters. In this study, I filled this gap by associating total MCs concentrations in tandem with climatic and identified liver disease risk factors to age-adjusted CLD/cirrhosis death rates. The study was threefold in nature. I assessed the ecological association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, related average total MCs to age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties and deduced a model of MC predictors in Lake Washington and Lake Manatee.

Purpose of the Study

The purpose of the study was to determine if MC exposure was an environmental risk factor of liver disease mortality. The study was composed of three objectives. In a retrospective, U.S. ecological study, I examined the association between climatic factors, liver disease risk factors, average total MCs, and U.S. age-adjusted CLD/cirrhosis death rates. In a longitudinal ecological substudy, I explored the relationship between age-adjusted CLD/cirrhosis death rates and average total MCs in Brevard and Manatee counties, FL. A model of MC predictors was derived by measuring nitrites and phosphates in Lake Washington and Lake Manatee.

Research Questions and Hypotheses

RQ1: What is the association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes?

$H_0$1: There is no association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes.

H_a1: There is an association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes.

RQ2: What is the association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes?

$H_0$2: There is no association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes.

H_a2: There is an association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes.

RQ3: What is the association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee?

$H_0$3: There is no association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee.

H_a3: There is an association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee.

Conceptual/Theoretical Framework

The socioecological model (SEM) served as the lens for the study. Originally, the framework was developed to explain the role of environmental and social influences on child growth and development (Kilanowski, 2017). Bronfenbrenner (1986), the founder of bioecological theory, organized ecological systems as nested circles to understand their influences on childhood. The expansion of the bioecological theory led to new hierarchical dimensions, including the political, community, organization, and interpersonal levels. The SEM was appropriate for a dissertation in environmental epidemiology because it includes the social, ecological, and physical constructs of algal blooms and environmental health. The complex interplay between anthropogenic, climatic and environmental factors, and social beliefs may contribute to variable MC concentrations in eutrophic lakes. In an investigation of the natural environment, I attempted to explain the public health impact of MC exposure.

Nature of the Study

The nature of the study was quantitative. Two ecological study designs were applied to meet the research objectives: an ecological study and a longitudinal study. A retrospective, ecological study was employed to assess the association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States. A longitudinal

ecological substudy was performed to evaluate the relationship between average total MCs and liver mortality rates in Brevard and Manatee counties. Nutrients in Lake Washington and Lake Manatee were measured to construct a predictive model of total MCs.

I incorporated primary and secondary data sources. Surface water samples were collected monthly over the potential peak algae bloom season (June-August) from Lake Washington and Lake Manatee. Aliquots of grab samples were necessary to make a set of composite samples. The Abraxis QuikLyse system was implemented to extract total MCs and nodularins. Enzyme-linked immunosorbent assay (ELISA) detected total MCs after sample preparation. Colorimetric assays measured nitrites and phosphates in raw water.

Secondary data were gathered from governmental organizations. Total MCs data were acquired from the United States Environmental Protection Agency (USEPA) National Lakes Assessment (NLA). The Centers for Disease Control and Prevention Wide-ranging Online Data for Epidemiological Research (CDC WONDER) stored archival environmental and age-adjusted CLD/cirrhosis mortality data. Crude prevalence data on diabetes and alcohol consumption were obtained from the Behavioral Risk Factor Surveillance System (BRFSS). Data on total MCs, county-level environmental climate measures, age-adjusted CLD/cirrhosis mortality rates, and crude prevalence of alcohol consumption and diabetes were compiled for 36 contiguous states of the United States. The FDOH provided county-level data for age-adjusted diabetes, alcohol consumption, and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee. All secondary data were aggregated, organized, and sorted for analysis.

Definitions

Age-adjusted alcohol consumption death rate: Number of alcohol consumption deaths per 100,000 population, controlling for differences in age distributions.

Age-adjusted chronic liver disease and cirrhosis death rate: Number of chronic liver disease and cirrhosis deaths per 100,000 population, controlling for differences in age distributions.

Age-adjusted diabetes death rate: Number of diabetes deaths per 100,000 population, controlling for differences in age distributions.

Average total MCs: sum of total microcystins concentrations of samples divided by total number of samples.

Blue-green algae: Synonymous with freshwater cyanobacteria.

Chronic liver disease (CLD): A condition lasting more than 6 months consisting of various liver pathologies.

Cirrhosis: A form of CLD wherein normal healthy tissue is replaced by scar tissue.

Colorectal cancer (CRC): Cancer of the colon or rectum.

Crude alcohol consumption prevalence: Number of adult respondents who have had at least one drink of alcohol within the past 30 days.

Crude diabetes prevalence: Number of respondents who have been told by a doctor that they have diabetes.

Cyanobacterial harmful algal blooms (CHABs): Toxic and nontoxic blue-green algae proliferations in waterbodies.

Cyanobloom: Excess cyanobacterial growths in aquatic waters.

Cyanotoxins: A group of heterocyclic compounds produced by cyanobacteria.

Enzyme-linked immunosorbent assay (ELISA): An immunological technique used to screen antigenic substances.

Eutrophication: A natural process driven by nitrogen and phosphorus in lakes or ponds.

Harmful algal blooms (HABs): Generic term for microorganisms that impair waterways of ecological and human importance.

Hepatocellular carcinoma (HCC): Most common form of primary liver cancer; occurs in persons with CLD/cirrhosis.

Limit of detection (LOD): Smallest measurable concentration or quantity of a substance.

Liquid chromatography-mass spectrometry (LC-MS): A coupled analytic method for separating and detecting small compounds in complex samples.

Microcystins (MCs): Toxins produced by cyanobacteria that target mammalian liver.

Microcystin (MC-LR): Most potent microcystin variant with leucine and arginine residues.

Nitrogen fixation (N_2 fixation): Biological mechanism where atmospheric nitrogen is converted into a more useable form.

Nodularins: Toxins produced by cyanobacteria that closely resemble microcystins.

Nonalcoholic fatty liver disease (NAFLD): Liver disease not caused by alcohol consumption.

Total MCs: Sum of detectable congener-independent, intracellular, and extracellular microcystin and nodularin in a sample.

Assumptions

The quantitative study had some important assumptions. The first assumption was that secondary data were collected with care and reflected the U.S. population. It was assumed the toxin data of the USEPA NLA were analyzed by ELISA and represented the exposure in the retrospective, U.S. ecological study. In the longitudinal ecological substudy, surface water samples were presumed to be collected by the direct grab technique and stored to maintain the integrity of MCs. Samples were supposedly representative of total MCs to which persons were exposed.

Scope and Delimitations

The purpose of the study was to identify total MCs as one possible risk factor for liver disease mortality in the United States. Secondary exposure and outcome data in the ecological analysis was limited to 36 conterminous states. For the ecological longitudinal substudy, Lake Washington and Lake Manatee were selected as sites of exposure. No human subjects participated in the study, and outcome data were obtained from the FDOH. Ascertainment of water quality indicator data was delimited to nutrients in the form of nitrites and phosphates.

Limitations

A major limitation of the ecological study was the age of the secondary data. The exposure data were gathered in 2007. The ecological fallacy was another limitation of the study. To say total MCs causes liver disease mortality for an individual is theoretically incorrect when using aggregate data. The hypothesized association applied to the population level in which inferences were drawn. A third limitation involved the measurement of one water quality indicator category (nutrients).

Significance

This study can contribute to environmental epidemiology by identifying an environmental risk factor related to liver disease mortality. In this study, I compared average total MCs to age-adjusted CLD/cirrhosis death rates. Hence, the study could add to the existing literature on a potential risk factor for liver disease mortality. The results may offer information on MC predictors in municipal water supplies.

Positive social change can increase environmental awareness, health, and wellness. The study could add to the existing ecological studies in the literature. It may encourage more epidemiological studies investigating the link between MCs and liver cancer/disease mortality. Developing educational opportunities at the community level could inform members about algal blooms and their effects on animals, ecosystems, and health. New or supporting courses may be offered at local institutions to enhance degrees in environmental science, microbiology, toxicology, and public health.

Findings can be shared with the Public Works and Utilities Department of Melbourne, FL and Manatee County Utilities Department to highlight the potential risk

of MCs in Lake Washington and Lake Manatee. The research could be accessible to the Parks and Recreation Department of Melbourne and Bradenton to adopt warning signs of algal blooms. Results may guide policymakers in developing an interim guideline value on MCs in drinking water. Water quality testing on algal toxins might become more common in Brevard and Manatee counties.

Summary

The goal of Chapter 1 was to provide context on CLD/cirrhosis and MCs. The aim of the study was to determine if MC exposure associated with liver disease mortality in the United States. Other research objectives included an evaluation of the hypothesized MC liver disease mortality relationship in Brevard and Manatee counties and model development for predicting detectable MCs in Lake Washington and Lake Manatee. The research can contribute to environmental epidemiology as it may uncover an environmental exposure of liver disease mortality.

In Chapter 2, I present the literature on MC epidemiology.

Chapter 2: Literature Review

Introduction

Cyanobacteria, or blue-green algae, are one of the most primitive bacteria on the planet. They have been isolated from almost every environment. The evolutionary success, essential growth requirements, and climatic and ecological factors of cyanobacteria are responsible for extensive cyanobacterial HABs (CHABs) (Anderson, Cembella, & Hallegraeff, 2012). These blooms are capturing public health attention due to their global expansion.

CHABs and their full health-related effects are poorly characterized. Cyanoblooms can release toxic secondary metabolites that contaminate water sources used for agriculture, drinking, and recreation (Saqrane & Oudra, 2009). MCs are the focus of epidemiological studies on recreational use and potential health risks. The biotoxins have been affiliated with liver cancers/diseases and respiratory symptoms.

The literature review is chronologically presented. I start with a discussion on the science behind cyanobacteria and the blooms they cause. The economic, sociocultural, and human impacts of CHABs are described thereafter. Attention shifts to cyanotoxins, emphasizing MCs. I highlight the most pertinent studies on dermal contact and inhalation and consumption of contaminated drinking water relative to MCs. The aim of the study was to assess the ecological association between average total MCs and U.S. age-adjusted CLD/cirrhosis death rates.

Literature Search Strategy

The Walden University Library was the source of databases for scholarly articles on the epidemiology of MCs in drinking and recreational waters. CINAHL and MEDLINE Combined Search and PubMed were chosen to begin the literature search. In CINAHL and MEDLINE Combined Search, *epidemiology* and *microcystins* were keyed as the Boolean/phrase into the search engine. The initial results populated 48 hits. The results were limited to academic journals, publication date (2000-2018), and full text. The quotes narrowed the search from 48 to 23 hits; the articles were sorted by relevance. I used PubMed to locate resources on recreational exposure and MCs. The following was typed in the PubMed search engine: *microcystins* and *recreational exposure*. The search results were restricted to full text, 10 years, and humans. A total of 19 hits appeared, but only the scholarly works in the best matches for MCs and recreational exposure were selected for the literature review.

Background information on freshwater HABs and MC epidemiology was searched using Google Scholar. The advanced search was used to reduce many publications on the topics. Articles were found with the words *microcystin epidemiology* and *freshwater harmful algal blooms*. The advanced search returned articles dated between 2013-2018. Bibliographies of sources were reviewed to embed relevant material on the subject matter. The EPA and World Health Organization were referenced when information on MCs was scarce.

Conceptual Model

The lens for the study was the socioecological model (SEM). The conceptual model is based on the bioecological theory of human development. Bronfenbrenner (1986) was the founder of the ecological theory and explained the developmental process of a child in the context of enduring environments. These were the immediate settings or upper layers. The immediate settings had three dimensions: the social influences of people, the physical environment, and the engaged activities of these people and the child (Kilanowski, 2017). The supportive layer social systems were adjacent to the upper or immediate settings, and they encompassed the institutional (social systems), geographical, and physical environments (Ashiabi & O'Neal, 2015). Culture was implied in the form of social systems in the supportive layer (Ashiabi & O'Neal, 2015).

Bronfenbrenner (1986) revised the bioecological theory and organized the environments as nested circles to further understand socioecological effects on child growth and development. Various systems encased the individual level, with the closest one exerting the strongest effect. They included the macrosystem, exosystem, mesosystem, and microsystem. These dimensions paved the SEM backbone (Ashiabi & O'Neal, 2015).

The SEM is used to examine political, community, organizational, and interpersonal factors on human health. Interrelationships between each level may enhance shared understanding of how connections and patterns among different systems affect public health. Many determinants can increase exposure to waterborne chemicals in freshwater, like individual behavior, culture, social factors, and the physical environment.

The contacts between human activities, ecological factors, and social views may increase exposure to MCs in potential bloom-forming lakes. MC exposure might relate to liver disease mortality among persons who used the water for drinking and recreation. The SEM model was practical because it explored the environmental and social conditions of freshwater HABs.

Cyanobacteria

Cyanobacteria are a unique group of prokaryotic microorganisms. They are gram-negative bacteria, although they contain a relatively thick peptidoglycan layer. Cyanobacteria lack histone associated proteins, membrane-bound organelles, and true nuclei (Ananya & Ahmad, 2014). Morphologically, the bacteria can appear colonial, filamentous, or unicellular (Ananya & Ahmad, 2014). Cyanobacteria perform oxygenic photosynthesis like most plants and true algae. Cyanobacteria possess chlorophyll α and blue phycobiliproteins (Ananya & Ahmaad, 2014). Hence, cyanobacteria are usually referred to as blue-green algae (Ananya & Ahmad, 2014).

Evolutionary History

The evolutionary history of cyanobacteria dates to approximately 3.2 to 3.5 billion years ago (bya; Blankenship, 2010). During this period, multilayered deposits or stromatolites comprised Precambrian rocks. The fossils resembled cyanobacterial masses found in natural habitats. The rise in oxygenated levels of Earth's atmosphere roughly 2.3 bya originated from the photosynthetic capacity of cyanobacteria (Soo et al., 2017). Their life course had eased the development of aerobic respiration and multicellular complexity

(Soo et al., 2017). Cyanobacteria are notorious for their broad and rich biological diversity.

Taxonomy

Cyanobacterial taxonomy is a controversial subject. Stanier et al. (1978) proposed to include cyanobacteria in the bacteria nomenclature. The inclusion of cyanobacteria under the rules of the International Committee on Systematic Bacteriology (ICSB)/International Committee on Systematics of Prokaryotes (ICSP) took more than 20 years to establish (De Vos & Trüper, 2000; Labeda, 2000; Tindall, 1999; Walter et al., 2017). A reason for the long efforts to include cyanobacteria as part of the ICSB/ICSP stemmed from historical failures to recognize cyanobacteria as prokaryotes. Molecular-based methods later replaced taxonomic principles. In the 1960s, cyanobacteria had distinguishable prokaryotic features (Percival & Williams, 2014). Presently, there is no consensus on cyanobacteria classification (Hoffmann, Komárek, & Kaštovský, 2005; Oren & Tindall, 2005; Oren & Ventura, 2017; Oren, Komárek, & Hoffmann, 2009; Walter et al., 2017).

Cyanobacteria are members of Kingdom Monera, division Eubacteria, and class Cyanobacteria (Percival & Williams, 2014). Approximately 2000 species comprise 150 genera (Vincent, 2009); an estimated 46 species are toxigenic (Hitzfield, Höger, & Dietrich, 2000). Cyanobacteria genera and species of concern are generally identifiable (Percival & Williams, 2014).

Environments

Cyanobacteria are ubiquitous and inhabit a wide range of environments. They are prevalent in freshwater, including lakes, ponds, and rivers (Schmidt et al., 2014). Cyanobacteria also reside in coastal and estuarine waters (Blάha, Babica, & Maršalek, 2009). Cyanobacteria have been discovered in extreme habitats. Unlike other microalgae, cyanobacteria can occupy Antarctic ice to hot springs (Rampelotto, 2013). Some cyanobacteria may even tolerate high salinity levels of salt marshes. The diverse environments of cyanobacteria reflect their remarkable adaptations.

Photosynthesis

Cyanobacteria are microscopic, unicellular, photosynthetic algae. Photosynthesis among cyanobacteria is oxygenic or anoxygenic. Cyanobacteria are the only bacteria that perform oxygenic photosynthesis (de Beer et al., 2017). Some cyanobacteria participate in anoxygenic photosynthesis and use hydrogen sulfide as their electron acceptor (Cohen, Padan, & Shilo, 1975). Cyanobacteria may function aerobically or anaerobically to generate chemical energy (Cohen et al., 1975; Holland, Schlesinger, & Turekian, 2005; Padan, 1979).

Nitrogen Fixation

Nitrogen (N_2) fixation is one reason for the global distribution of cyanobacteria. The process converts atmospheric nitrogen into ammonia (Paerl, 2017). N_2 fixation operates anaerobically, making it sensitive to aerobic photosynthesis (Postgate, 1998). Cyanobacteria are confronted with a paradoxical situation. N_2 fixation and oxygenic photosynthesis are mechanisms for supporting growth. The mechanisms cannot achieve

maximum results simultaneously. Various biochemical, ecological, and morphological adaptations have enabled cyanobacteria to perform N_2 fixation to curtail the problem of free oxygen (Kellar & Paerl, 1980; Paerl, 2017). Cyanobacteria do not contribute to a thorough and efficient N_2 fixation process, and minimal nitrogen is supplied to nitrogen-deficient waterbodies (Paerl, 2017).

Cyanobacterial Harmful Algal Blooms

CHABs are increased algal growths of harmful cyanobacteria. These phenomena occur worldwide, and mostly flourish in freshwater lakes and ponds (Natural Resources Defense Council, 2019). Nutrient availability, ecosystem disturbance, hydrology, sunlight, and temperature are triggers for cyanobacterial abundance. The biological stimuli must interact with one another to create ideal conditions for cyanobacterial bloom formation (Berger et al., 2008). Causes of toxic algae blooms are displayed in Figure 3.

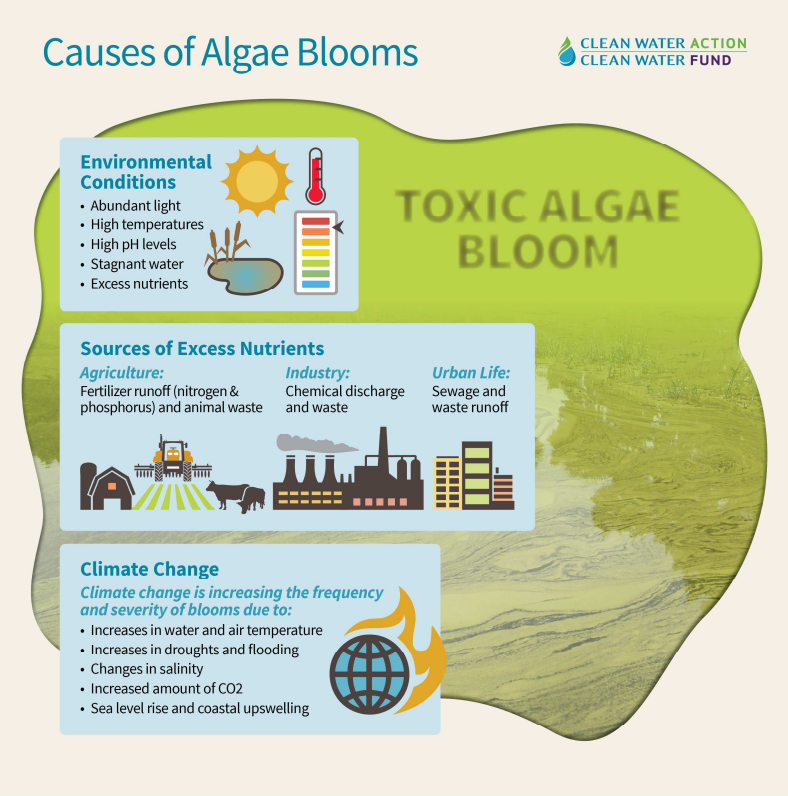

Figure 3. Causes of toxic algae blooms. Adapted from Harmful Algal Outbreaks and Drinking Water, In *Clean Water Action*, 2019, Retrieved from https://www.cleanwateraction.org/features/harmful-algal-outbreaks-and-drinking-water. Copyright 2019 by Clean Water Action.

Dynamics

The dynamics of cyanobacteria are multifaceted. The causal factors driving cyanobacterial blooms remain unclear (Rastogi et al., 2015). Scholars have supported the role of eutrophication and global climate change in cyanobloom expansion and toxin production (Conley, Paerl, Howarth, Boesch, & Seitzinger, 2009; Gehringer & Wannicke, 2014; Kaebernick & Neilan, 2001; Kleinteich et al., 2012; Neilan, Pearson, Muenchhoff, Moffitt, & Dittmann, 2013; O'Neil, Davis, Burford, & Gobler, 2012; Paerl & Paul, 2012; Paerl & Scott, 2010; Rastogi et al., 2015; Smith & Schindler, 2009).

Eutrophication

Eutrophication is a driver of cyanobloom formation. More than 40% of lakes and reservoirs in Europe, the United States, and Asia are eutrophic, which provide optimal conditions for cyanobacteria proliferation (Chorus & Bartram, 1999). Eutrophication is a form of nutrient pollution; a waterbody is loaded with minerals and nutrients, primarily nitrogen and phosphorus (Howarth et al., 2000). Although certain bloomforming cyanobacteria fix nitrogen, phosphorus regularly controls freshwater cyanobacteria growth (O'Neil et al., 2012). Phosphorus enrichment can shift the phytoplankton community towards cyanobacterial dominance (O'Neil et al., 2012). Eutrophication is generally destructive to ecosystems and neighboring local economies (Howarth et al., 2000)

Global Climate Change

A contributor to CHAB development is global climate change. Environmental factors promote the profusion of cyanobloom dynamics (Joung, Oh, Ko, & Ahn, 2011; Neilan et al., 2013; Rastogi et al., 2015). Low turbulence, stagnant water conditions, higher pH values, and higher temperatures can augment cyanobacterial distribution (Bláha, Babica, & Maršálek, 2009). Fossil fuel combustion and concurrent changes in air temperature may influence the dilution and portability of contaminants. Droughts and floods in the summer might encourage urban runoff into water sources. Global climate change is expected to favor toxic HABs and increase biological oxygen demand in ecosystems (Whitehead, Wilby, Battarbee, Kernan, & Wade, 2009).

Impacts

Ecological and Socioeconomic

The ecological and socioeconomic costs of HABs are significant. They can have detrimental impacts on agriculture, drinking water, fisheries, food web resilience and habitats, real estate, tourism, water quality, and can lead to anoxia and fish kills (Carmichael & Boyer, 2016). An estimated 25% to 75% of HABs are toxic (Chorus, 2001). At least 35 states have documented CHAB occurrence, with increased cyanobacterial abundance reported in FL, Nebraska, New Hampshire, and New York (Fristachi, Sinclair, Hall, Hambrook Berkman, & Boyer, 2008). Nearly 2.2 to 4.6 billion dollars is spent on freshwater ecosystems in the United States, annually (Dodds et al., 2009).

Animals

Harmful cyanobacteria in freshwater sources impact both animals and humans. Reports on animal health and human health are often viewed separately. Direct, high-intensity exposure to dense cyanobacterial accumulations frequently cause animal deaths and illnesses (Hilborn & Beasley, 2015). Most cyanobacteria-associated poisonings have occurred in dogs, largely because they consume toxic planktonic cyanobacterial scum, drink contaminated water, and lick bloom material from their coats (Codd, Edwards, Beattie, Barr, & Gunn, 1992). Animal companion deaths, such as cats, are seldomly reported (McLeod & Bondar, 1952). Acute effects of animal companions include convulsions, diarrhea, hemorrhage, profuse salivation, sudden death, vomiting, and

weakness (Corkill, Smith, Seckington, & Pontefract, 1989; Hamill, 2001). Freshwater systems bearing harmful cyanobacteria may present human health risks.

Human

Cyanobacterial waterblooms can affect human health. Acute and chronic toxicities may result from planktonic cyanobacteria exposure (Bláha et al., 2009). Short-term health effects include skin irritation, gastrointestinal symptoms, and kidney and liver failure (Costa et al., 2012). Long-term health effects of cyanobacterial blooms remain uncertain (Lopez, Jewett, Dortch, Walton, & Hudnell, 2008). The largest outbreak of MC poisoning occurred in Brazil; dialysate prepared from contaminated water killed more than 50 hemodialysis patients (Azevedo et al., 2002). Furthermore, many persons likely spend time in recreational waters enduring algal blooms. At first, scholars did not report associations between swimming in bloom waters and eye irritation or sore throat. Ensuing research found that one self-reported symptom occurred just one week after exposure to > 5,000 cells per milliliter (ml) of cyanobacteria (Backer, 2012). To date, no irrefutable evidence supports human cyanotoxin lethality in the United States.

Cyanotoxins

Cyanotoxins are toxins of cyanobacteria in brackish, coastal, freshwater, and marine ecosystems (Bláha et al., 2009). They are characterized by their chemical structures and toxicologic effects. Chemically, the cyanotoxins are classified as heterocyclic compounds (alkaloids), lipidic compounds, or peptides. From a toxicological perspective, cyanobacterial toxins are categorized as cytotoxins, dermatoxins, hepatotoxins, irritant toxins, and neurotoxins (Sivonen & Jones, 1999). MCs, a group of

hepatotoxins, are possibly the most widespread cyanotoxins in the environment (Vesterkvist et al., 2012).

Microcystins

MCs are secondary metabolites generated by toxic cyanobacterial species. *Microcystis* is the main producer of MC, but several other cyanobacterial genera can release MCs, including *Anabaena, Hapalosiphon*, *Nostoc*, and *Oscillatoria* (Szlag, Sinclair, Southwell, & Westrick, 2015). Persistent blooms emanating MCs can pose health risks to people who rely on impaired water resources used for drinking water supplies, fisheries, and recreational activities (Harke et al., 2016). MCs are generally the only cyanotoxins screened for by municipal water systems (Chorus & Bartram, 1999).

Biosynthesis

MCs are naturally synthesized monocyclic heptapeptides. The secondary metabolites are produced when MC synthase genes are present in cyanobacterial genera (Puddick et al., 2014). The enzyme is a protein complex encoded by non-ribosomal peptide synthetase and polyketide synthase genes. MC synthase catalyzes heterocyclic formation sequentially. The multi-enzyme complex adds amino acids, malonyl coenzyme A, and *S*-adenosyl-$_{L}$-methionine constituents onto phenylacetate and converts them into a modified peptide chain, which is condensed to create a heterocyclic structure (Moore, Chen, Moore, Patterson, & Carmichael, 1991; Nishizawa, Asayama, Fujii, Harada, & Shirai, 1999; Nishizawa et al., 2000; Puddick et al., 2014; Tillett et al., 2000).

Structure and Variation

MCs are cyclic heptapeptide compounds. The bioactive hepatotoxins contain non-protein and protein components. Archetypal of MCs and the related nodularins is a distinctive β-amino acid Adda, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-dienoic acid (Altaner, Puddick, Wood, & Dietrich, 2017; Carmichael 1997). MCs also contain D-glutamic acid (Glu), N-methyl dehydroalanine (Mdha), D-alanine (Ala), D-erythro-β-methylaspartic acid (Masp) and two variable L-amino acids. (Puddick et al., 2014). Variability at positions two and four in the peptide ring distinguishes MC congeners. The presence of leucine and arginine at the variable sites is representative of microcystin-leucine arginine (Vichi, Buratti, & Testai, 2016, Figure 4). Scholars have characterized about 130 MC variants (Carmichael & Boyer, 2016; Greer et al., 2018).

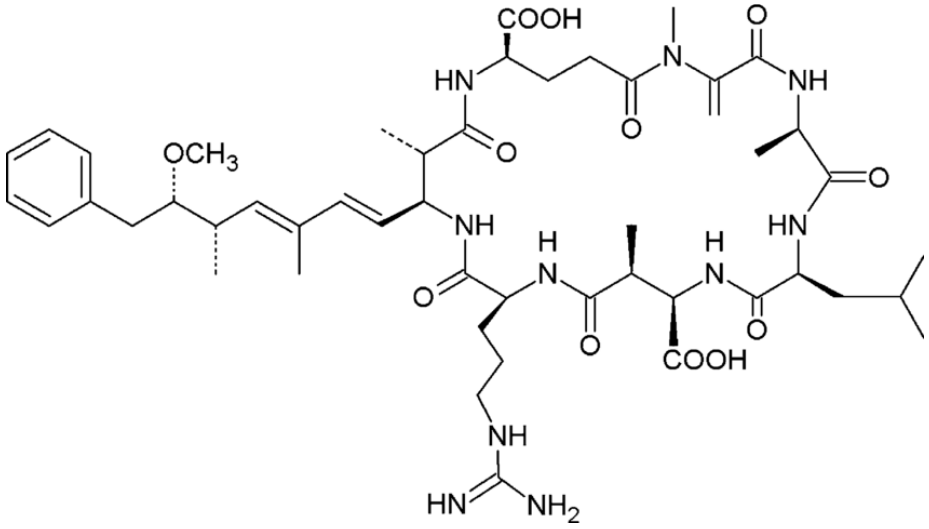

Figure 4. Chemical structure of MC-LR. Adapted from "Genotoxicity and potential carcinogenicity of cyanobacterial toxins: A review," by B. Zegura, A. Štern, and M. Filipic, 2011, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 727*, p. 19. Copyright 2011 by Elsevier.

Properties

MCs are soluble in ethanol and methanol solvents. They are insoluble in acetone, benzene, chloroform, and ether. MCs are stable for seven days in water reservoirs but can endure longer in deionized or filtered water (Belyk et al., 2013). MCs are unaltered in sunlight, unless exposed to photosensitizers (Tsuji et al., 1994; Welker & Steinberg, 2000). Ultraviolet radiation and ozone are effective in removing extracellular MCs (Chorus & Bartram, 1999; Welker, Brunke, Preussel, Lippert, & von Döhren, 2004).

Boiling for several hours is deficient for lysing MCs. The compounds are resistant to chemical hydrolysis or oxidation near neutral pH values. Extreme temperature (40 C) and pH (pH > 12 or pH ~ 1) can degrade over 90% of MCs within ten weeks, owning to slow hydrolysis (Belykh et al., 2013). Increased temperature plus a six molar hydrochloric acid solution may accelerate the process in the laboratory (Belykh et al., 2013).

Mechanism of Action

The mechanism of action of hepatotoxic MCs is phosphate inhibition (Eriksson et al., 1990). Upon accidental ingestion, MCs accrue in the liver via the bile acid transport system (Eriksson, Grönberg, Nygård, Slotte, & Meriluoto, 1990; Falconer, 2005; Runnegar, Berndt, & Kaplowitz, 1995; Pearson, Mihali, Moffitt, Kellman, & Neilan, 2010;). The Adda and Mdha groups facilitate binding of MCs to protein phosphatases, rendering their capacity to remove phosphates in cellular organisms (Schmidt et al., 2014). Cytoskeletal proteins can become hyperphosphorylated, leading to degradation of cytoskeletal elements, hepatocyte disfiguration, and formation of cell contacts and vast

hemorrhages (Honkanen et al., 1990; MacKintosh, Beattie, Klumpp, Cohen, & Codd, 1990). Alterations in biological function can promote hepatomegaly or liver enlargement. Cytokeratin hyperphosphorylation and protein phosphatase inhibition were found to promote cancer development in hepatocyte culture (Ohta et al., 1992; Xing et al., 2006).

Toxicity

Microcystin-leucine arginine (MC-LR) is the most studied of the cyanotoxins (Carmichael, 1992; Greer et al., 2018). MC-LR is a priority algal toxin in the United States (Mekebri, Blondina, & Crane, 2009). The International Agency for Research on Cancer (2006), located in Lyon, France, determined that MC-LR was a possible human carcinogen. MC-LR is believed to cause damage in other mammalian organs, as supported by investigations of embryotoxicity, gastrointestinal disorder, immunotoxicology, kidney impairment, and reproductive toxicity (Beasley, Cook, Dahlem, Hooser, Lovell, & Valentine, 1989; Zhang et al., 2008; Zhou, Yuan, Wu, & Han, 2012). Potential health risks from MC exposure have resulted in provisional guidance values for MC-LR in drinking (1 microgram (μg)/l) and recreational waters (10 μg/l) (World Health Organization, 2013). Detection methods for MC analysis could provide a threshold for MCs in nonregulated areas.

Detection Methods

There are numerous methods for cyanotoxin analysis in cells and water. Sensitivity and specificity are important criteria when analyzing MCs in different matrices. Biochemical, biological, and physiochemical approaches exist, but they differ in their application. Biochemical approaches include enzyme analysis (inhibition of

acetylcholinesterases, protein phosphatases etc.) and immunoanalysis (ELISA). They substitute biological approaches despite being less sensitive than physiochemical approaches. Biochemical methods are practical for monitoring and rapid screening of samples. Biological methods (bioanalysis) quantitatively detect secondary metabolites in living systems. Bioanalysis is simplistic and fast; however, ethical considerations restrict its usage. Several physiochemical methods (chromatographic techniques) can efficiently assess cyanobacterial secondary metabolites qualitatively and quantitatively (Belykh et al., 2013). With many commercially available detection methods, a safe level of MC exposure is determinable.

Routes of Exposure

Human contact to MCs can occur through multiple routes of exposure (Hilborn & Beasley, 2015). The intravenous route is most renowned for poisoning fatalities in a Brazilian hemodialysis center (Azevedo et al., 2002). Ingestion of contaminated drinking water is frequently the watercourse for MCs, contributing to 80% of human exposure (Greer et al., 2018). MC aerosols may induce respiratory symptoms in humans pursuing occupational or recreational activities (Cheng et al., 2007). Dermal contact with MC is possible during bathing, canoeing, or swimming (Drobac et al., 2013). The literature review synthesizes epidemiological scholars investigating cyanobacterial and MC exposure in drinking and recreational waters.

Dermal Contact and Inhalation

Pilotto et al. (1997) evaluated the health effects of cyanobacterial exposures in participants of recreational water activities. Interviews were conducted at water

recreation sites in New South Wales, South Australia, and Victoria. The interviewers visited the locations on designated Sundays during January and February 1995 to invite attendees six years and older to volunteer. Of the 852 participants, 777 had water contact (exposed), and 75 did not have water contact (unexposed) on the day of the interview. Appointments for a telephone follow-up two and seven days after water contacts recorded diarrhea, ear or eye irritations, fevers, flu-like symptoms, mouth ulcers, skins rashes, and vomiting. After two days, the exposed and unexposed groups displayed no significant differences in symptom incidence. Exposure duration and increasing symptom occurrence was statistically significant at seven days ($p = 0.03$). A significant trend was observed among the exposed concerning cyanobacterial density and increased symptom incidence ($p = 0.04$). Individuals exposed to greater than 500 cyanobacteria/ml for over an hour experienced a statistically higher symptom occurrence rate compared to unexposed persons. Symptoms did not correlate with hepatotoxin presence. Time of exposure in toxic cyanobacteria water predisposed subjects to varied health risks (Pilotto et al., 1997).

In an international prospective cohort study, Stewart et al. (2006) examined the incidence of acute symptoms in people exposed to cyanobacteria in lakes and rivers in central and northeast FL, central coast area of New South Wales, and southeast Queensland. Children and adults participating in recreational activities were recruited between 1999 and 2002 at water recreation sites. Enrollment took place for 54 days, mostly during weekdays and holidays of warmer months to maximize recruitment efficiency through attentiveness on peak-use periods of recreational water use. Eligible

subjects included those who planned to engage in recreational activity on the day of recruitment and could be contacted by phone to follow-up.

The scholars employed multivariable logistic regression analyses to study recreational exposure to cyanobacteria and acute illness. Age, clustering by household, hay fever or skin disease, prior history of asthma, region, and smoking were adjusted in the models. Eligibility criteria were met by 3,595 individuals. Of the qualified subjects, 3,193 (89%) consented to participate and 1,331 (37%) completed the questionnaire and follow-up interview. High levels of cyanobacterial exposure resulted in respiratory symptoms reporting compared to low levels of cyanobacteria exposure (odds ratio [*OR*] 2.1, 95% *CI* 1.1-4.0). Likewise, combined reported symptoms produced a higher response of symptoms among individuals exposed to high cyanobacterial levels than individuals exposed to low cyanobacterial levels (*OR* 1.7, 95% *CI* 1.0-2.8).

Stewart et al. (2006) revealed that symptom reporting was common among persons exposed to high cyanobacterial levels in enclosed recreational waters. Respiratory systems were the most apparent of reported symptoms. Low cyanobacterial toxin concentrations were detected in water samples throughout the study. Future scholars should investigate how cyanotoxin levels associate with recreational health effects (Stewart et al., 2006).

Backer et al. (2008) investigated recreational exposure to low aerosol-borne MC concentrations during a harmful cyanobacterial bloom in a small lake. The scholars aimed to detect MC levels in blood samples of individuals at risk for inhaling spray or swallowing water in water-related recreational activities in algal bloom lake water. A

second objective included a correlation between MC in aerosol and water samples and self-reported symptoms. The survey included 104 study participants, 97 persons who planned recreational activities in the water bloom lake (exposed group) and seven members who volunteered to recreate in a nearby lake with no bloom (unexposed group). The field study was completed within one week of detecting a MC concentration of 10 μg/l. Liquid chromatography-mass spectrometry (LC-MS) revealed blood MC levels below 0.147 μg/l for 101 respondents. Only one subject presented detectable MCs. Participants recounted no symptom increases upon low recreational exposure. Backer et al. (2008) were the first to describe a relatively low MC aerosol exposure in pursuit of water-related activities.

Two years later, Backer et al. (2010) studied MC recreational exposure in two Californian lakes (Bloom Lakes). The scholars designated a reservoir without a bloom as the Control Lake. Algal taxonomy, MC concentrations, and potential respiratory viruses (adenoviruses and enteroviruses) in water samples were analyzed. MCs in air samples, blood samples, and nasal swabs of 81 children and adults organizing recreational activities were measured. Participants agreed to an interview, providing demographic and health symptom information. The Bloom Lakes contained highly variable MC concentrations (< 10 μg/l to > 500 μg/l); the Control Lake was free of MCs. No lakes had any respiratory viruses in them. Personal air samples (< 0.1 nanograms (ng)/meter cubed (m^3) [limit of detection (LOD)]-2.89 ng/m^3) and nasal swabs (< 0.1 ng/m^3 [LOD]-5 ng) indicated low MC levels. Blood samples showed detectable MCs below the LOD. Study findings confirmed that water-related activities in algal bloom lakes could stir up

aerosolized MCs, making inhalation a likely exposure route. Collecting nasal swabs for aerosol-borne contaminants could evaluate the possibility of particle deposition in the upper respiratory tract (Backer et al., 2010).

On January 7, 2007, in Salto Grande Dam, Argentina, an acute cyanobacterial intoxication case in recreational water containing MCs was reported (Giannuzzi, Sedan, Echenique, & Andrinolo, 2011). A 19-year-old male drove his jet ski in a bay inundated with cyanobacterial scum and inadvertently submerged himself in the water for two hours. Bloom samples denoted MC-LR concentrations peaking 48.6 µg/l. The teenager suffered abdominal pain, fever, and nausea four hours after exposure. Three days later, dyspnea and respiratory distress were disclosed. The patient was admitted to a hospital for medical treatment since his condition worsened. The initial diagnosis was atypical pneumonia, but hepatotoxicosis developed the following week. Recreational exposure via inhalation in cyanobacterial tainted water demonstrated the progression of respiratory illness to severe liver damage. Giannuzzi et al. (2011) were the first to describe an episode of acute hepatotoxicity in a human engaged in water recreation.

Contaminated Drinking Water

Ueno et al. (1996) established a correlation between contaminated drinking water and PLC in China. Two field studies, one in Haimen City, Jian-Su Province and the other in Fusui County, Guangxi Province, were conducted. Tree trials were completed between 1993-1994 in Haimen City, sampling drinking water from many freshwater sources, including deep wells, ditches, ponds, rivers, and shallow wells. One thousand one hundred thirty-five samples were collected and monitored by ELISA [LOD of 50

picograms (pg)/ml. The first September trial had three ditch water specimens with MC; 11 samples tested negative. MC concentrations ranged between 90-460 pg/ml. *Oscillatoria agardhii*, toxic blue-green algae, were present in some ditches.

The second experiment covered the entire 1994 calendar year and samples were sampled monthly from two deep wells, five ponds/ditches, two rivers, and two shallow wells. Highest MC concentrations appeared in June-September, with a range of 62-296 pg/ml. The last trial in July 1994 comprised 989 different water specimens gathered from distinct freshwater sources. River water samples had the most contamination, trailed by ditch/pond water (17%) and shallow-well water (4%). Deep well water samples had no detectable MC concentations.

A survey of 26 water samples was performed in Fusui, Guangxi Province to assess drinking water quality. Consistent MC contamination occurred in ponds/ditches and rivers. Deep and shallow wells exhibited no traces of MC. These findings paralleled with the first survey where MCs polluted ditch/pond and river waters. The epidemiological surveys suggested a close relationship between contaminated drinking water sources and PLC incidence (Ueno et al., 1996).

In a retrospective cohort study, Zhou et al. (2002) correlated MCs in drinking water to CRC incidence in China. Eight towns were randomly selected as study sites in Haining City, Zhejiang Province. The Cancer Registry of Haining Cancer Research Institute comprised 408 cases (163 colons and 245 rectums) diagnosed from 1977 to 1996. Regarding sex, 184 females and 224 males represented the sample. Data on drinking water sources were gathered by interviewing families of the deceased or living

patients. Indirect competitive ELISA (LOD = 20 pg/ml) was employed to enumerate MC concentrations in pond, river, tap, and well water.

Positive samples for MC concentrations (≥ 50 pg/ml) were detected in pond and river water. The positive rate in pond and river water was 17.14% and 36.23%, correspondingly. Tap and well water had a positive rate of 0. There was a significantly higher concentration of MCs in surface water than groundwater ($p < 0.01$). Spearman correlation analysis indicated a positive association between MCs in river and pond water and incidence of CRC in the study sites ($r_s = 0.881$, $p < 0.01$).

A higher relative risk for CRC was apparent in those who drank from river (7.94), pond water (7.70), and tap (1.88) compared to well water. Zhou et al's (2002) epidemiological study reflected that of Ueno et al. (1996) in which MC contaminated drinking water sources constituted a potential risk factor for PLC incidence. More scholars should focus on testing municipal water treatments to ensure quality drinking water free of MC contamination.

In an ecological pilot study in FL, Fleming et al. (2002) examined proximity to surface water drinking sources and hepatocellular carcinoma (HCC) risk. The pilot study was conducted after a monitoring survey discovered blue-green algae and cyanotoxins in surface drinking water sources. Geographic Information System and environmental databases of diagnosed HCC cancers (1981-1988) were used to explore the association. From 1981 to 1988, there were 4, 741 incident HCC cases, and contributed to a cumulative age-adjusted cancer rate of 1.41 cancer diagnoses per 100,000 cases. The Mann-Whitney Rank Sum Test was applied to 18 surface water treatment services areas

and coincided HCC incident rates against four groups from randomly selected matched control groundwater treatment services areas. The Rank Test indicated no statistically significant differences between incident rates ($p > 0.05$). Increased HCC risk was determined in residents who lived within surface water treatment plant service areas. HCC risk was not as significant for people in nearby sites. Increased risk for HCC was not detected in the randomly chosen groundwater treatment service areas. Fleming et al. (2002) demonstrated a link between HCC risk and residential proximity to surface water drinking sources.

The epidemiology of cancers in Serbia and possible connection with cyanobacterial blooms was studied (Svirčec et al., 2014). There were 13 examined cancers from 1999 to 2008. The cancers included brain cancer, colorectal cancer, gastric cancer, heart, mediastinum and pleura cancer, leukemia, malignant melanoma, ovary cancer, PLC, retroperitoneum and peritoneum cancer, and testicular cancer. The incidences of cancers in 17 Central Serbia districts were analyzed. Five of the districts had cyanoblooms in drinking water reservoirs for more than 2 decades. Results of a *t*-test exemplified a statistical difference in the incidence of 13 cancers in three critical districts, Nišavski, Toplićki, and Šumadijski. The statistically significant findings of cancer incidences in Serbia potentially correlated with drinking water reservoirs contaminated by harmful cyanobacteria (Svirčec et al., 2014).

The geographic association between liver cancer and surrogate markers of cyanobacterial contamination of freshwater lakes was explored in Canada (Labine et al., 2015). Liver cancer incidence data (1996-2004) were sourced from the National Cancer

Registry for Canada. Population-based estimates of age, alcohol abuse, gender, Hepatitis B and C incidence, recent immigrant status, and urban residence were included as risk factors for liver cancer. Surrogate markers of potential cyanobacterial exposure were agricultural activity (percent of land devoted to agriculture) and cattle and swine densities.

Negative binomial regression was modeled to predict the geographic distribution of liver cancer. A partially adjusted model, controlled for age and gender, displayed no correlation between surrogate markers of potential cyanobacterial exposure and liver cancer. Hepatitis B viral infection (rate ratio (*RR*) = 1.16, $p < 0.0014$), recent immigrant status (*RR* = 1.89, $p < 0.0001$), and urban residence (*RR* = 1.25, $p < 0.001$) significantly associated with liver cancer. The highest alcohol abuse tertile (*RR* = 0.84, $p < 0.0321$) exemplified an unusually low rate. No association was observed between increased *RR* and Hepatitis C viral infection. Surrogate markers of Canadian freshwater lakes lacked association with liver carcinogenesis. The conclusions drawn by Labine et al. (2015) contradicted the results from scholars in China, Serbia, and the United States.

Chen et al. (2009) observed daily chronic MC exposure and subsequent health effects in fisherman at Lake Chaohu. For past decades, widespread eutrophication has dominated the lake. Between July 15 and July 24, 2005, 35 fishermen (14 males and 21 females) were arbitrarily chosen, agreeing to cooperate in the study. The study population consisted of fishermen who lived on Lake Chaohu for more than 10 years (27) and between 5 and 10 years (8). Their age, body height, body weight, medical history, and residence was obtained. The fishermen donated blood samples for serum biochemical and

immunological indicators. Synchron Clinical System LX20 (Beckman-Coulter Diagnosis, Fullerton, CA) measured alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, blood urea nitrogen, cholineasterase activity, creatinine, direct bilirubin, globulin, glucose, indirect bilirubin, lactase dehydrogenase, total bilirubin, total bile acids, total cholesterol, total protein, triglyceride, and uric acid. Blood serum samples were tested for Hepatitis Bs antigen, Hepatitis Bs antibody, Hepatitis Be antigen, Hepatitis Be antibody, Hepatitis Bc antigen, and Hepatitis Bc antibody. MCs in fisherman serum samples were detected by LC-MS. Results showed close positive relationships between serum MC concentrations and liver enzymes. Hepatocellular damage was induced by ingesting MC contaminated aquatic foods and drinking water (Chen et al., 2009).

In a cross-sectional study, Li et al. (2015) linked chronic MC exposure and childhood liver damage in the Three Georges Reservoir Region, China. Potential study participants included 1, 441 randomly chosen school children. The 1, 322 eligible subjects aged 7-15 years were classified as low exposure, high exposure, and unexposed. The low exposure category comprised children who consumed aquatic food and water for > 5 years from an occasional cyanobacterial bloom lake (lake 1). The high exposure group had ingested the same aquatic food and water sources for > 5 years but obtained them from a lake with regular cyanoblooms over the previous five years (lake 2). The unexposed group included children who rarely eaten fish or duck and drank well water for > 5 years from lake 1 or lake 2.

Students completed a questionnaire on risk factors related to liver damage and donated blood samples for serum liver enzyme and serum MC analysis. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and γ-glutamyl transferase were tested. Detection of two or more consecutive abnormal serum enzyme levels was indicative of liver damage. ELISA was used to quantify MC-LR concentrations in aquatic food and drinking water sources. Multinomial logistic regression analysis explored the relationship between liver damage in children and serum MC concentrations.

Liver damage was related to Hepatitis B viral infection, hepatotoxic medication usage, and MC exposure. An estimated daily MC-LR intake of 2.03 μg/l was calculated among the high exposure group, surpassing the recommended tolerable daily intake of 0.40 μg/l for children (Chorus & Bartram, 1999). Detectable algal toxin levels supported the hypothesis that childhood liver damage is linked to MC exposure. A potential health outcome of MCs in freshwater lakes supplying aquatic food and drinking water was portrayed. The findings might encourage improved rapid bioassays for detecting MC congeners in dependable water systems (Li et al., 2015).

Summary

The emergence of CHABs worldwide has resulted from the propensity of cyanobacteria to multiply in water. Algae blooms loom quite large, disturbing aquatic ecosystems, economics, and human health. The liberation of cyanobacterial toxins in aquatic sources is damaging to drinking water supplies and recreational sites. CHABs have increasingly received public health attention within the last 2 decades.

The health impacts of MCs are grounded by the SEM. The model factors in the interconnection between nature and society. Placing HABs in a broader human-environmental coupled system permits a comprehensive evaluation of social and ecological determinants of health. Understanding these social and ecosystem dynamics is key to water bloom research and mitigation. A socioecological approach inspires stakeholders to formulate rationales for addressing cyanobacterial bloom reduction.

The literature review recounted the knowledge of MC epidemiology. Relevant epidemiological studies were discussed. High MC concentration intake during water-recreation activities was found to stir respiratory effects in participants. Lakes enduring algal blooms supported the inhalation route of MCs. A case report characterized the progression of respiratory illness to liver toxicity shortly after swallowing bloom inundated water.

Epidemiology scholars consistently identified MCs in drinking water sources as one potential risk factor for PLC. Zhou et al. (2002) noted a correlation between CRC and MC contaminated drinking water. Chen et al. (2009) reported a close positive association between chronic MC exposure and HCC in fishermen. Li et al. (2015) related childhood liver disease to chronic MC exposure. The scholars reinforced the importance of MC exposure in environmental health.

The literature review had some limitations. A major limitation of the literature review was the lack of casual relationships between MCs and health outcomes. Irregularity of publications was another limitation of the synthesis. A third limitation involved few investigations within the United States. The limitations may suggest

insufficient quantitative data, little devotion, and/or limited expertise in cyanotoxin-related health effects. More research is desired to supplement the existing literature to offer a more convincing argument that MCs are environmental risk factors of hepatic disease. The supposition justifies a dissertation examining MC exposure and liver disease mortality.

Chapter 3 describes the research design, research questions and hypotheses, sampling strategy, target population, instrumentation, and methodology of the study. It concludes with a discourse on the importance of validity, reliability, and ethical dilemmas.

Chapter 3: Research Method

Introduction

CHABs are rapid growths of cyanobacteria in surface waters. Some blooms can produce cyanobacterial toxins, poisonous to animals, local ecology, and humans (USEPA, 2017). MCs are considered the most commonly occurring cyanotoxins in the environment (Vesterkvist et al., 2012). The purpose of the study was to determine if average total MCs predicted age-adjusted CLD/cirrhosis death rates in the United States.

A retrospective, U.S. ecological study design was applied to evaluate the hypothesis that MC exposure was an environmental risk factor for liver disease mortality. A longitudinal ecological substudy was conducted in FL to support the potential association between average total MCs and. liver disease mortality. Multiple linear regressions were run in Statistical Package for the Social Sciences (SPSS) Version 25 to answer the research questions. Chapter 3 includes a description of the research questions, data analysis plan, validity threats, and ethical procedures.

Research Design and Rationale

Epidemiology scholars study population disease risk factors. They generate hypotheses and implement study designs based on research questions. There are two broad categories of epidemiological studies: observational and experimental. The former involves observation to examine associations between exposure and outcome variables (Munnangi & Boktor, 2017). The latter controls for the exposed and unexposed groups of an intervention (Munnangi & Boktor, 2017).

One kind of observational study is the ecological design. It offers supporting evidence for a probable causal relationship (Morgenstern, 1995). The unit of study is either the community or population, rather than the individual. Data sources can provide information on risk factors and human disease. Aggregated population health data for comparison may be defined by geographic location, migrant status, time, or occupation and social class (Coggon, Rose, & Barker, 2003). The ecological design is cost-effective, and it measures an ecologic effect, demonstrates widespread utility, and involves simplistic analysis and presentation (Wakefield, 2008). It was employed to ascertain if average total MCs predicted U.S. age-adjusted CLD/cirrhosis death rates .

A longitudinal study is a second type of observational study. In a longitudinal study, scholars measure variables repeatedly over time (Caruana, Roman, Hernàndez-Sànchez, & Solli, 2015). This study design is useful when making associations between risk factors and disease onset. Longitudinal research is challenging in that it entails generally increased financial and temporal demands. Scholars should ensure identical collection methods across study locations while maintaining consistency (Caruana et al., 2015). An ecological longitudinal substudy was performed to compare the associations between average total MCs and age-adjusted CLD/cirrhosis death rates in the FL counties of Brevard and Manatee.

Data Collection

I incorporated primary and secondary data collection methods to address the research questions. Primary data were collected for use as exposure measurement in the substudy and for the predictive model of total MCs. It was necessary to conduct a mixture

of field and laboratory work to obtain data on MCs and nutrients (phosphates, nitrites) at the county-level. Secondary data were gathered from various governmental entities. The BRFSS was the source of prevalence data on alcohol consumption and diabetes. Total MCs were gathered from the USEPA NLA and CDC WONDER provided environmental data on daily sunlight, average daily maximum temperature, and underlying cause of CLD/cirrhosis mortality data.

Secondary Exposure Data

Secondary exposure data were downloaded from the 2007 USEPA NLA. The file contained total cylindrospermopsins, MCs/nodularins, and saxitoxins data. In the U.S., retrospective ecological study, I used total MCs data as the exposure. Nondetectable total MCs (< 0.10 μg/l) were excluded in the analysis. Counties with more than one total MCs observation were averaged. There were 36 states in the dataset, and total MCs for each state were combined.

Secondary Outcome Data

Secondary outcome data were acquired using the CDC WONDER public health information system. Age-adjusted CLD/cirrhosis death rates were obtained for the 2003-2007 period. Records of age-adjusted CLD and cirrhosis death rates (K70, K73-K74) were observed with the International Classification of Disease, Tenth Revision (ICD-10) 113 Cause List. Selected demographics included all ages, genders, origins, and races.

Environmental Data

Data on daily sunlight and daily maximum air temperature were retrieved from CDC WONDER. Dates selected included January 1, 2007 to December 31, 2007. The

time frame was chosen to reflect the year (2007) in which total MCs were measured in the United States. All environmental results were grouped by state.

Crude Prevalence Data

Crude prevalence data on alcohol consumption and diabetes were obtained by the BRFSS. The crude prevalence data spanned between 2005 and 2007. Crude prevalence data were averaged for each of the 36 states in the dataset.

Additional Information

Pilot Study

A pilot study was conducted from April to May 2019 to streamline the methods of exposure measurement in a longitudinal ecological substudy. Two lakes, Lake Washington (Melbourne, FL) and Lake Manatee (Bradenton, FL), represented the sampling locations. Surface water samples were collected in accordance with the direct grab technique of the FDEP (FDEP, 2018). Samples were transported on ice to Eastern Florida State College in Melbourne, FL. Composite samples were prepared by aliquoting equal volumes (100 ml) of grab samples into 473 ml glass amber bottles. Samples were extracted with the Abraxis QuikLyse ^{TM} system, analyzed by the Abraxis Microcystins/Nodularins (ADDA) ELISA kit (Microtiter Plate) PN 520011, and run on a Bio-Rad Model 550 microplate reader at 450 nanometers (nm). ELISA results were entered into Microsoft Excel. A standard curve was generated to determine the concentration of total MCs. A LaMotte water monitoring kit water quality test kit was not available to measure nitrates and phosphates in composites.

Substudy

A longitudinal ecological substudy was performed between June and August 2019 to compare the relationship between total MCs and age-adjusted CLD/cirrhosis death rates in Brevard County and Manatee County, FL.

Populations

The populations studied included Brevard County and Manatee County, FL.

Brevard

Brevard County, FL, is the 10th most populous county with 579,130 people (Data United States of America, n.d.a). The county experienced a 1.94% population increase during the 2015-2016 calendar year (Data United States of America, n.d.a). Brevard County is 75% White, 9.9% Hispanic, and 9.7% Black (Data United States of America, n.d.a). The remaining 5.4% of the population includes Asian, Islander, Native, and Other (Data United States of America, n.d.a). The population included people who lived in Brevard County between 2004-2018.

Manatee

The comparison population included people who lived in Manatee County between 2004-2018. Manatee County, FL, is 15th in terms of population and is occupied by 375,888 persons (Data United States of America, n.d.b). A 3.45% population increase was reported between 2015 and 2016 (Data United States of America, n.d.b). Manatee County is predominately White at 71.5%, followed by Hispanic (16.1%) and Black (8.74%; Data United States of America, n.d.b). Asian, Islander, Native, and Other

constitute the remaining 3.66% (Data United States of America, n.d.). The comparison population included people who lived in Manatee County between 2004-2018.

County-Level Health Data

County-level health data on potential confounders (alcohol consumption and diabetes) were collected from the FDOH. Due to limited prevalence data, mortality data (2004-2018) for these variables were collected. The county-level health data for each year served as one data point in the multiple linear regression analysis.

Sampling Locations

Lake Washington

Lake Washington, which is part of the St. Johns River system, is the largest freshwater lake in Brevard County, FL, spanning 4,362 acres (Eriksen, 1955). It is one of the few lakes in Florida used for drinking water. Lake Washington is a Class I waterbody. The Jon A. Buckley Surface Water Treatment Plant located nearby distributes potable water to 150,000 residents (Waymer, 2012). Lake Washington is home to recreational activities such as boating and fishing.

Lake Manatee

Lake Manatee is a humanmade reservoir located in Manatee County, FL. The Manatee River meets Lake Manatee at the east side and flows west into Tampa Bay. The Lake Manatee Water Treatment Plan provides drinking water to more than 350,000 persons of Bradenton, Palmetto, municipalities on the barrier islands, and parts of Sarasota County (General Electric, 2019). Lake Manatee State Park on the south end of the reservoir is adequate for boating and swimming (Owens, 2013).

Sampling Procedures

Surface Water Sampling

Twenty surface water samples were collected monthly between June and August 2019 from different sites in Lake Washington and Lake Manatee (Table A1). The standard operating procedure for the direct grab technique was followed (Florida Department of Environmental Protection [FDEP], 2018). Bottles were rinsed and submerged beneath the surface to fill. The date, time of collection, geographical coordinates, environmental conditions, and method of access were documented.

Composite Sampling

Composite sampling was used to identify sampling units. The sample design is not a computed statistic (USEPA, 2002). Composite sampling works by collecting discrete samples in sets and aliquoting equal volumes from subsamples to create a homogenous sample (USEPA, 2002). The scheme is frequently used jointly with other sampling designs, particularly when the objective is to generate an estimated mean of analyte concentration (USEPA, 2002).

Preparation of Composites

Thirty composite samples (400 ml/composite) were prepared monthly from sets of four grab samples (100 ml/grab sample) over the sampling period. A composite was made by measuring 100 ml of sample in a graduated cylinder and decanting the volume into a 473 ml glass amber bottle. The step was repeated three more times to achieve a maximum volume of 400 ml. Samples were mixed, labeled, and stored in a refrigerator at 4 C.

Water Monitoring Kit

A LaMotte water monitoring kit for nitrate and phosphate in water using a Low Range Comparator was tested on composites. Sample color was matched to a color standard. Readings were recorded as parts per million (ppm).

Nitrate

A Nitrate-N & Phosphate Comparator bar was inserted into a Low Range Comparator. One test tube was filled with 10 ml of composite sample water and placed in the comparator. Another test tube was filled with 2.5 ml of composite sample water. The sample was diluted with 2.5 mL of Mixed Acid Reagent. The sample was capped, mixed, and incubated at room temperature for 2 minutes. One-tenth of a gram (g) of Nitrate Reducing Agent was measured with a spoon and added to the composite sample water. The sample was capped and inverted 60 times in one minute. The composite was incubated for 10 minutes at room temperature. After, the composite sample water was mixed, and the cap was removed. The test tube was inserted into the Low Range Comparator. The comparator was tilted so the light shined through the sample. The color of the reaction was matched to the color standards. The result was converted to nitrate by multiplying the reading by a factor of 4.4. The final value was recorded as ppm nitrate. The procedure was completed for each composite sample from Lake Washington and Lake Manatee.

Phosphate

A Low Range Comparator was set with a Nitrate-N & Phosphate Comparator bar. A test tube was filled with 10 ml of composite sample water and placed in the

comparator. A second test tube was filled with 5 mL of composite sample water. Five hundred microliters (µl), or 0.5 ml, of Phosphate Acid Reagent was added to the sample, capped, and mixed. Five hundredth of a g of Phosphate Reducing Agent was measured with a spoon and added to the composite. The sample was capped and mixed until dissolved. The composite sample water incubated at room temperature for 5 minutes. The sample cap was removed and placed in the Low Range Comparator. The comparator was tilted towards the light and the sample color was matched to the color standards. The reading was recorded as ppm orthophosphate. The procedure was done for each composite sample from Lake Washington and Lake Manatee.

Microcystin Extraction

The Abraxis QuikLyse ^{TM} system was used as the sample preparation method to extract MCs in raw water. Reagents were stabilized to room temperature before use. One ml aliquots were transferred to glass vials. One hundred µl of Reagent A was added to samples, capped, and shaken for 2 minutes. Samples incubated for 8 minutes at room temperature. Ten µl of Reagent B was then added to samples, capped, and shaken for 2 minutes. Samples incubated for 8 additional minutes at room temperature prior to immunoanalysis.

Enzyme-linked immunosorbent assay

ELISA is an immunological assay that screens for freshwater blue-green algal toxins (Weller, 2013). In a longitudinal ecological substudy, I used an indirect competitive ELISA to quantify the levels of total MCs in surface water samples.

Microcystins-ADDA ELISA

Toxin extracts were analyzed by Abraxis Microcystins/Nodularins (ADDA) ELISA kit (Microtiter Plate) PN 520011. The test was an indirect competitive ELISA for the congener-independent detection of MCs and nodularins. Fifty µl of standard solutions, control, and samples were loaded in duplicate on a 96-well microtiter plate. In addition, 50 µl of antibody solution was added to sample wells, mixed for 30 seconds, and incubated for 90 minutes at room temperature. After the incubation, wells were filled successively with 250 µl of wash buffer. One hundred µl of enzyme conjugate solution was then added to sample wells, mixed for 30 seconds, and incubated for 30 minutes. A volume of 250 µl of wash buffer was added a second time to individual wells sequentially. One hundred µl of substrate solution was dispensed into wells to bind to the enzyme. The plate was mixed for 30 seconds and incubated in the dark for 20 minutes. Following the last incubation of the assay, a volume of 50 µl of stop solution was pipetted to halt the reaction in the wells. Samples were read at 450 nm with a Bio-Rad Model 550 microplate reader (Figure 5).

Figure 5. Bio-Rad 550 microplate reader with 96-well microtiter plate (Photographed by Rajesh Melaram)

Total Microcystins Evaluation

A manual evaluation of total MCs was completed over the longitudinal substudy. Absorbance values were keyed into Microsoft Excel spreadsheets. Duplicate standard readings generated mean absorbance values. The $\%B/B_0$ for each standard was determined by dividing the mean absorbance value for each standard by the zero standard (Standard 0) absorbance. Graphing the decadic logarithm of known standard MCs concentrations against $\%B/B_0$ resulted in a standard curve. Total MCs of standards, controls, and samples were calculated using the equation of the line.

GreenWater Laboratories

GreenWater Laboratories/CyanoLab was established in 2001. It is a private full-service company dedicated to algal identification and cyanobacteria toxin research. GreenWater Laboratories/CyanoLab is equipped with a team of cyanobacteria experts who provide analytic testing, analyses, monitoring and research to clienteles. I used the

laboratory to verify the method of MC determination (Table A2). The CyanoLab Division used a direct competitive ELISA to detect MCs and nodularins (GreenWater Laboratories, 2018).

Nutrient Analysis

Colorimetric assays (Phosphate Assay Kit ab65622 and Griess Reagent Kit ab234044), that were performed separately, measured phosphates and nitrites in a random selection of 30 samples from Lake Washington and Lake Manatee. Standard curves were constructed for every assay performed. Assay procedures occurred in 1.5 ml microcentrifuge tubes and transferred into 1 ml cuvettes. Thus, all reaction components were increased by a factor of 5. A Spectronic 200 spectrophotometer analyzed samples containing phosphates (650 nm) and nitrites (540 nm). Nutrient concentrations were calculated by dividing the amount of phosphate or nitrite in the sample from the standard curve by the original sample volume.

Data Analysis Plan

Variables

Region, state, average daily maximum temperature, daily sunlight, crude alcohol consumption prevalence, crude diabetes prevalence, average total MCs, age-adjusted CLD/cirrhosis death rates, age-adjusted alcohol consumption death rate, age-adjusted diabetes death rate, nitrite concentration, and phosphate concentration were variables in the study. Their names, type, and measurement levels are depicted in Table 1.

Table 1

Variable Names and Levels of Measurement

Variable Names	Levels of Measurement
Region	Nominal
State	Nominal
Average Daily Max Temperature	Interval
Daily Sunlight	Interval
Crude Alcohol Consumption Prevalence	Interval
Crude Diabetes Prevalence	Interval
Total Microcystins	Interval
Age-adjusted Alcohol Consumption Death Rate	Interval
Age-adjusted Diabetes Death Rate	Interval
Age-adjusted Chronic Liver Disease and Cirrhosis Death Rate	Interval
Nitrite Concentration	Interval
Phosphate Concentration	Interval

The data dictionary of the retrospective, U.S. ecological study is displayed in Table 2.

Table 2

Data Dictionary

Variable Name	Label	Type (Width)	Value Codes	Missing Code
State	State	Numeric (14)	1 = “Alabama” 2 = “Arizona” 3 = “Arkansas” 4 = “California” 5 = “Colorado” 6 = “Connecticut” 7 = “Delaware” 8 = “Florida” 9 = “Georgia” 10 = “Idaho” 11 = “Illinois” 12 = “Indiana” 13 = “Iowa” 14 = “Kansas” 15 = “Kentucky” 16 = “Louisiana” 17 = “Maine” 18 = “Maryland” 19 = “Massachusetts” 20 = “Michigan” 21 = “Minnesota” 22 = “Mississippi” 23 = “Missouri” 24 = “Montana” 25 = “Nevada” 26 = “New Jersey” 27 = “New York” 28 = “North Carolina” 29 = “Oklahoma” 30 = “Oregon” 31 = “Pennsylvania” 32 = “Rhode Island” 33 = “South Dakota” 34 = “Tennessee” 35 = “Texas” 36 = “Virginia 37 = “Washington”	None

Region	Region	Numeric (2)	1 = “South” 2 = “Northeast” 3 = “Midwest” 4 = “West”	None
AverageDailyMaxTemperatureF	Average Daily Max Temperature (F)	Numeric (5)	None	None
DailySunlightKJm2	Daily Sunlight (KJ/m2)	Numeric (28)	None	None
TotalMicrocystinsµgL	Total Microcystins µg/L	Numeric (25)	None	None
AverageAlcoholConsumptionBingeDrinkingCrudePrevalence20052007	Average Alcohol Consumption (Binge Drinking Crude Prevalence 2005-2007)	Numeric (18)	None	None
AverageDiabetesCrudePrevalence20052007	Average Diabetes Crude Prevalence (2005-2007)	Numeric (18)	None	None
AgeAdjustedCLDCirrhosisper100000020032007	Age-Adjusted CLD/Cirrhosis per 100,000 2003-2007	Numeric (18)	None	None

Multiple Regression

Multiple regression is a continuation of simple linear regression. The statistical test attempts to explain the association between two or more independent variables and one dependent variable. Multiple regression is a predictive analysis that illustrates the relative contribution of predictors on the overall fit of the model (Laerd Statistics, 2018).

Assumptions

Eight assumptions must be verified for valid multiple regression results:

1. The dependent variable is measured on a continuous scale,
2. Two or more independent variables are measured as categorical or continuous,
3. Each observation is independent of the others,
4. There is a linear relationship between every independent variable and the dependent variable and collectively between the predictors and outcome,
5. The data reveals homoscedasticitiy,
6. No multicollinearity is shown in the data,
7. High leverage points and significant outliers should not be present, and
8. Residuals are approximately normally distributed (Laerd Statistics, 2018).

Statistical Analysis

Descriptive and inferential statistics were completed in SPSS Version 25. Mean and standard deviation of predictors were computed. Average total MCs in the retrospective, U.S. ecological study were log transformed. In the longitudinal ecological substudy, average total MCs in Manatee County, FL were log transformed. Average total

MCs in Brevard County, FL and average aggregated total MCs were normally distributed and remained untransformed.

Simultaneous and stepwise multiple linear regressions analyzed the association between average total MCs and U.S. age-adjusted CLD/cirrhosis death rates. In the U.S., retrospective ecological study, average daily maximum temperature, daily sunlight, alcohol consumption, and diabetes were controlled for in the model. Alcohol consumption and diabetes were controlled for in the model of the substudy. Nutrient and toxin concentrations were listed in order from lowest to highest. Simultaneous multiple linear regression examined the association between nitrites, phosphates, and total MCs in Lake Washington and Lake Manatee.

Research Questions

RQ1: What is the association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes?

$H_0$1: There is no association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes.

H_a1: There is an association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes.

RQ2: What is the association between average total MCs and age-adjusted CLD/ cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes?

$H_0$2: There is no association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes.

H_a2: There is an association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes.

RQ3: What is the association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee?

$H_0$3: There is no association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee.

H_a3: There is an association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee.

Threats to Validity

External Validity

External validity is the degree to which study findings are contextualized to a large audience (Andrade, 2018). A study is generalizable if a sample of the population is randomly drawn and represents the population. Results may not always generalize to other groups. Thus, studies with poor external validity exclude vulnerable populations or inherent sociodemographic restrictions have poor external validity (Andrade, 2018).

Another threat of external validity is confounding. Confounding effects are eliminated before or after data collection (Pourhoseingholi, Baghestani, &Vahedi, 2012). Oftentimes, the researcher is unable to explore all characteristics related to exposure and outcome. Generalizability is diminished if certain factors are uncontrolled for in statistical models (Laerd Dissertation, 2012). According to Pourhoseingholi, Baghestani, and Vahedi (2012), regression models can handle many variables in a single analysis. Controlling for select covariates may enhance generalizability.

Experimenter bias is probable in studies because of personal biases. Hypotheses are formulated and tested in quantitative research. The scholar can express subjective beliefs during the process. An inclination for a topic or having prior experience and knowledge of the variables could affect the study outcomes. Repeatability by another scholar using the same methods may produce different results. Such differences would affect the original study liable to experimenter bias (Laerd Dissertation, 2012).

Ethical Procedures

Ethical procedures are integral in in academics. I was ethical when I collected secondary health data. Ethics is about preserving moral character, making sound decisions, and respecting personal liberties. Any research concerning health data can go awry. Problems may arise because of disregard, ethical insensitivity, or technical errors.

A thorough review from the Institutional Review Board (IRB) was required to certify the dissertation complied with U.S. federal regulations and University ethical standards. I completed Form A to receive tailored guidance. Then, I completed Form B to acknowledge public accessibility of secondary data. The IRB granted approval of my

research methodology (04-23-19-0659783). Failure to adhere to research ethics and compliance can disqualify the research and degree (Walden University, 2018).

Summary

Research methodology is essential to a quantitative study. It lays the groundwork for answering research questions. The methodology presented in Chapter 3 delineates a systematic plan of assessment. It described the study designs, data collection methods, and statistical procedures. An evaluation of validity threats and ethical procedures were discussed. Chapter 4 will highlight the pilot study and major results of the dissertation study and substudy.

Chapter 4: Results

Introduction

The association between average total MCs and U.S. age-adjusted CLD/cirrhosis death rates was studied. In a U.S., retrospective ecological study, I used secondary exposure and health outcome data from the USEPA and FDOH. In a longitudinal ecological substudy, I compared probable MC exposure-related liver mortality rates in Brevard and Manatee counties, FL. The relationship between nitrites, phosphates, and total MCs was explored to construct a model of MC prediction. Multiple linear regression analyses were completed with the SPSS Version 25 software platform. Chapter 4 includes the research questions and descriptive and inferential statistics.

Research Questions

RQ1: What is the association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes?

$H_0$1: There is no association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes.

H_a1: There is an association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States, controlling for daily sunlight, average daily maximum temperature, alcohol consumption, and diabetes.

RQ2: What is the association between average total MCs and age-adjusted CLD/ cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes?

$H_0$2: There is no association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes.

H_a2: There is an association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard and Manatee counties, FL, controlling for alcohol consumption and diabetes.

RQ3: What is the association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee?

$H_0$3: There is no association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee.

H_a3: There is an association between nitrite concentration, phosphate concentration, and total MCs in Lake Washington and Lake Manatee.

Pilot Study

A pilot study was performed to evaluate the water quality conditions and total MCs concentrations of Lake Washington and Lake Manatee. Environmental factors, pH and temperature, were measured in the lakes (Table 3). A water quality test kit was not available on site to measure nitrates and phosphates. The pH and temperature values were similar in Lake Washington and Lake Manatee. Lake Washington had a pH range of 7.8 to 8.4 and water temperature of 86 F. Lake Manatee had a pH range of 7.2 to 7.8 and

water temperature of 82 F. Overall, water quality indicators were slightly higher in Lake Washington.

Table 3

Environmental Factors

Lake	Date	Collection Sites	Water Clarity	pH	Ambient Temperature (F)	Water Temperature (F)
LW	04/28/19	Canal, Dock	Murky	7.8-8.4	81	86
LM	05/01/19	Boat Ramp, Shore	Murky	7.2-7.8	88	82

Note. *LM = Lake Manatee, LW = Lake Washington

ELISA results of total MCs were attained. A standard curve was prepared to determine unknown total MCs concentrations in water samples. It resulted in a R^2 of 0.9856 (Figure 6). Corrected absorbance readings, $\%B/B_0$, and total MCs of Lake Washington and Lake Manatee composites were calculated (Table 4). Total MCs were greatest in Lake Washington composite 1 (LWC1) at 0.34 µg/l. The lowest MC levels were detected in Lake Manatee composite 1 (LMC1).

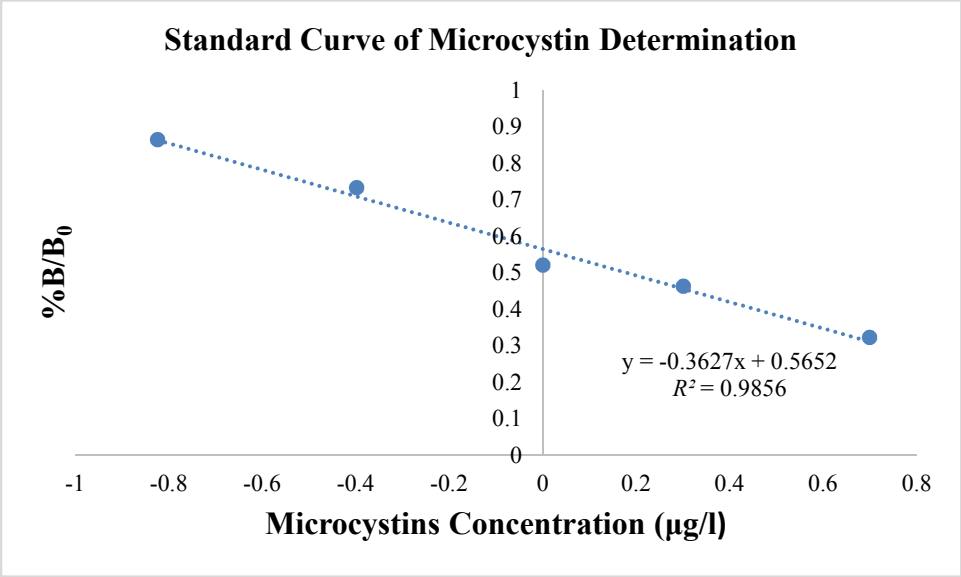

Figure 6. A standard curve of microcystin determination.

Table 4

Enzyme-Linked Immunosorbent Assay Parameters

Sample ID	Average Absorbance (450 nm)	Average %B/B$_0$	Total Microcystins (µg/l)
LWC1	1.468	0.734	0.34
LWC2	1.527	0.976	0.07
LMC1	1.595	1.02	0.05
LMC2	1.4685	0.939	0.09

Note. *LM = Lake Manatee, LW = Lake Washington

Data Collection

The 2007 USEPA NLA dataset was used to collect the exposure data on total MCs in the retrospective, U.S. ecological study. In the longitudinal ecological substudy , surface water samples were collected from June to August 2019 in Lake Washington and Lake Manatee to measure total MCs concentrations.

Descriptive Statistics

The U.S., retrospective ecological study comprised 36 conterminous states. Average total MCs in the year 2007 was 0.93 µg/l. Between 2003 and 2007, mean age-adjusted CLD/cirrhosis death rates resulted in 8.91 deaths per 100,000 people. Log-transformed mean total MCs returned a concentration of 0.26 µg/l. The average daily maximum temperature and amount of daily sunlight across the United States was 64.29 F and 16395.28 kilojoules per meters squared (kJ/m^2), respectively. The average percent of individuals who engaged in binge drinking during the 2003-2007 period was 14.53%. Over the same 5-year mark, the average percent of individuals who said they had diabetes from a doctor was 7.99%.

Inferential Statistics

Simultaneous multiple linear regressions predicted U.S. age-adjusted CLD/cirrhosis death rates from average total MCs, environmental climate factors, and potential liver disease risk factors. Multiple correlation between predictors and age-adjusted CLD/cirrhosis death rates was strong ($R = 0.814$). All seven variables (average daily maximum temperature, daily sunlight, average total MCs, average crude alcohol consumption prevalence, average crude diabetes prevalence, state, region) attributed to 66% in age-adjusted CLD/cirrhosis death rates variation. The regression model was statistically significant (F (7, 28) = 7.846, $p < 0.01$, $R^2 = 0.662$; Table 5).

Using stepwise regression, I tested explanatory variables of age-adjusted CLD/cirrhosis death rates. Average total MCs and daily sunlight strongly predicted age-adjusted CLD/cirrhosis death rates ($R = 0.786$). Average total MCs and daily sunlight

explained 5.7% variance in age-adjusted CLD/cirrhosis death rates. For every one-unit change increase in average total MCs, age-adjusted CLD/cirrhosis death rates increased 4.198 standard deviations. Age-adjusted CLD/death rates increased 0.001 standard deviations per one unit increase in daily sunlight. The two-variable model was a good fit in predicting U.S. age-adjusted CLD/cirrhosis death rates (F (1, 31) = 4.651, p = 0.039; Table 5).

Table 5

Model Summary

Method	*R*	*R* Square	*F* Change	*df*1	*df*2	Significance *F* Change
Simultaneous	0.814	0.662	7.846	7	28	< 0.01
Stepwise	0.786	0.618	4.651	1	31	0.039

In the simultaneous regression model, state, daily sunlight, and average total MCs were significant predictors of age-adjusted CLD/cirrhosis death rates. When such effects were held constant, region (β = 0.125, t = 0.641, p = 0.527), average daily maximum temperature (β = -4.39, t = -1.524, p = 0.139), average crude alcohol consumption prevalence (β = 0.153, t = 0.981, p = 0.335), and average crude diabetes prevalence (β = 0.363, t = 1.821, p = 0.079) lacked predictive value (Table 6).

Table 6

Coefficients

Variables	Beta	*t*	Significance
State	0.307	2.426	0.022
Region	0.125	0.641	0.527
Average Daily Maximum Temperature	-.439	-1.524	0.139
Daily Sunlight	0.999	4.307	< 0.01
Average Total Microcystins	0.362	2.960	0.006
Average Crude Alcohol Consumption Prevalence	0.153	0.981	0.335
Average Crude Diabetes Prevalence	0.363	1.821	0.079

Substudy

Population Characteristics

Between 2004 and 2018, a comparison of population characteristics displayed similarities in Brevard and Manatee, as seen with median age, gender, and race in Table 7. Age-adjusted chronic disease death rates showed differences across locales. The age-adjusted CLD/cirrhosis death rate was higher in Brevard (16.22) compared to Manatee (11.45) over the 15-year mark (Table 7).

Table 7

Population Characteristics

Attribute	Brevard	Manatee
Population	549,262	332,970
Median Age, years	45.78	45.97
Gender, %	48.93% (Male) 51.07% (Female)	48.39% (Male) 51.61% (Female)
Race, %	84.75% (White) 15.25% (Black & Other)	87.07% (White) 12.93% (Black & Other)
CLD/Cirrhosis, per 100,000	16.22	11.45
Alcohol Consumption, per 100,000	7.96	6.25
Diabetes, per 100,000	18.58	12.74

Note. *Median Age, years was only available between 2009 and 2017.

Algal Bloom Sightings

During the sampling period, June to August 2019, two algal bloom sightings occurred, one in Lake Manatee and the other in Lake Washington. The first sighted algal bloom occurred on June 22, 2019 in a cove on the north side of Lake Manatee. Visible scum floated along the shoreline extending several feet (Figure 7). On July 28, 2019, blue-green algae blanketed the banks of Lake Washington. Blue-green algae streaks extended well into the western lake region (Figure 8).

Figure 7. Algal mats along Lake Manatee (Photographed by Rajesh Melaram)

Figure 8. Blue-green algae streaks in Lake Washington (Photographed by Rajesh Melaram)

Water Quality Factors

Water quality factors in Lake Washington and Lake Manatee were compared during the algae bloom (Table 8). Lake Washington exhibited narrower pH (7.5-7.8) and water temperature ranges (84-86 F) than Lake Manatee. Nutrient levels seemed consistent throughout lake composites despite few showing elevated amounts. Average total MCs in Lake Manatee (0.26 µg/l) barely surpassed those of Lake Washington (0.23 µg/l).

Table 8

Water Quality Factors

Lake	pH	Water Temperature (F)	Nitrites (ppm)	Nitrates (ppm)	Orthophosphates (ppm)	Average Total Microcystins (µg/l)
Lake Washington	7.5-7.8	84-86	0.2	0.88	0.2-1.0	0.26
Lake Manatee	7.2-8.4	86-95	0.2-0.4	0.88-1.76	0.2-0.4	0.23

Microcystin Predictors

Average MC predictors between June and August in Lake Manatee and Lake Washington presented similar trends, as displayed in Table 9. The lowest average nutrient concentrations in nanomoles (nm) appeared in June. August had the highest average detectable nutrient concentrations. Lake Manatee and Lake Washington experienced their lowest observable total MCs in July. Average total MCs were greatest in Lake Manatee.

Table 9

Descriptive Statistics of Microcystin Predictors

Lake	Month	Average Nitrites (nmol)	Average Phosphates (nmol)	Average Total Microcystins (μg/L)
LM	June	1.86	2.62	0.190
LW		1.73	0.298	0.199
LM	July	6.66	2.55	0.148
LW		6.85	2.78	0.141
LM	August	8.10	4.25	0.234
LW		7.79	3.49	0.218

Note. *LM = Lake Manatee, LW = Lake Washington

Findings

Multiple linear regression models for average total MCs, alcohol consumption, diabetes, and age-adjusted CLD/cirrhosis death rates were constructed. Multicollinearity emerged within initial models, causing removal of alcohol consumption. Average total MCs and diabetes moderately predicted age-adjusted CLD/cirrhosis death rates in Brevard County, FL (R = 0.638). They explained 40% variation in age-adjusted CLD/cirrhosis death rates. The stepwise method supported a statistically significant association between average total MCs and age-adjusted CLD/cirrhosis death rates in Brevard County, FL (F (1, 13) = 7.571, p = 0.016, R^2 = 0.368). In Manatee County, FL, a weak positive association between diabetes, average total MCs, and age-adjusted CLD/cirrhosis death rates occurred (R = 0.318). The model indicated statistical insignificance (p = 0.527). A statistically insignificant association resulted amid pooled county-level data (p = 0.290).

Average total MCs in Brevard County, FL established predictive value relative to age-adjusted CLD/cirrhosis death rates (β = 0.592 t = 2.656 p = 0.021; Table 10). In Manatee County, FL, average total MCs lacked predictive value (β = 0.207 t = 0.711 p = 0.491; Table 10). Aggregated average total MCs lacked statistical significance with age-adjusted CLD/cirrhosis death rates. Diabetes exhibited statistical insignificance in all three scenarios (Brevard, Manatee, Brevard and Manatee; Table 10).

Table 10

Age-Adjusted Chronic Liver Disease/Cirrhosis Death Rates by Average Total Microcystins and Diabetes

County	Predictors	Beta	*t*	*p-value*
Brevard	Average Total Microcystins	0.592	2.656	0.021
	Diabetes	0.197	0.884	0.394
Manatee	Average Total Microcystins	0.207	0.711	0.491
	Diabetes	-0.322	-1.107	0.290
Brevard and Manatee	Average Total Microcystins	0.434	1.647	0.126
	Diabetes	-0.118	-0.446	0.663

Note. *LM = Lake Manatee, LW = Lake Washington

Nitrites and phosphates significantly predicted total MCs in Lake Manatee. As nitrites increased one-unit change, total MCs decreased 1.774 standard deviations. Total MCs increased 2.191 standard deviations for every one-unit change in phosphates. Total MCs strongly associated with nitrites and phosphates in Lake Washington (F (2, 13) = 155.277, $p < 0.01$, $R^2 = 0.960$). When controlled for total MCs, phosphate was a significant predictor (β = 0.516, t = 2.187, p = 0.048; Table 11). Aggregated total MCs in the drinking water sources revealed statistical significance with phosphates ($p < 0.01$; Table 11).

Table 11

Inferential Statistics of Microcystin Predictors

Lake	Predictors	Beta	*t*	*p-value*
LM	Nitrites	-1.774	-4.265	< 0.01
	Phosphates	2.191	5.268	< 0.01
LW	Nitrites	0.471	1.996	0.067
	Phosphates	0.516	2.187	0.48
LM and LW	Nitrites	-0.173	-1.764	0.085
	Phosphates	1.154	11.756	< 0.01

Note. *LM = Lake Manatee, LW = Lake Washington

Summary

In Chapter 4, I conveyed the study results. In the U.S., retrospective ecological study, I found a positive significant association between average total MCs, daily sunlight, and age-adjusted CLD/cirrhosis death rates. In the longitudinal ecological substudy, I found a positive significant association between total MCs in Lake Washington and age-adjusted CLD/cirrhosis death rates of Brevard County, FL. No relationship was detected between average total MCs and age-adjusted CLD/cirrhosis death rates in Manatee County, FL. Elevated phosphate concentrations predicted increased total MCs in Lake Washington and Lake Manatee. In Chapter 5, I elaborate on the research findings, discuss the study limitations, and describe implications and potential impacts for positive social change.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

The purpose of the study was to examine the ecological association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States. An ecological design was used because aggregated population health data spanned various geographical locations over time. The study was undertaken due to increases in reported HABs and their potential health risks. Few scholars investigated MCs and liver health outcomes. In retrospective, ecological findings, I found statistically significant associations between average total MCs and age-adjusted CLD/cirrhosis death rates. State of residence and average daily sunlight predicted U.S. age-adjusted CLD/cirrhosis death rates. A substudy produced similar results about MCs being a possible environmental risk factor for age-adjusted CLD/cirrhosis death rates in Brevard County, FL. High MC levels in Lake Manatee and Lake Washington were associated with increased phosphates. In Chapter 5, I interpret the results of the main and substudy, followed by a discussion on research recommendations and implications.

Interpretation

The study findings extend knowledge in environmental epidemiology on MCs and liver disease mortality. I discovered that daily sunlight, state of residence, and average total MCs influence age-adjusted U.S. CLD/cirrhosis death rates. It affirmed Melaram's (2019) national ecological analysis examining climate exposure variables, total MCs, and age-adjusted CLD/cirrhosis death rates. This study included liver disease risk factors while the former lacked them altogether. It concurred with Labine et al. (2015) in which

urban residence associated w contaminated freshwater lakes. Individuals who live near lakes should monitor water quality for possible cyanobacterial toxin contamination.

Prior scholars have identified potential associations between cyanobacterial and MC exposure and liver pathologies (Chen et al., 2009, Fleming et al., 2002, Labine et al., 2015, Li et al., 2015, Svirčec et al., 2014; Ueno et al., 1996; Zhou et al., 2002). Scholars have not correlated average totals MC with liver disease mortality rates at the state level. I implemented an indirect-competitive ELISA to quantify total MCs in surface water samples of two FL freshwater lakes. This method included two antibodies for MC detection. Therefore, I targeted a wider range of MCs to represent the exposure in the substudy.

In the substudy results, I inferred no difference in MC exposure and age-adjusted CLD/cirrhosis death rates in Manatee County, FL but one in Brevard County, FL. The detected association could have been influenced by geographical proximity to exposure sites in Lake Washington and a larger proportion of people suffering from preexisting comorbidities. Lake Manatee is more geographically isolated so persons may experience less exposure towards MCs. The explanation may be supported by fewer persons having cooccurring conditions in Manatee County, FL.

Additionally, the difference in the association between average totals MCs and age-adjusted CLD/cirrhosis death rates might be explained by differences in population. CLD is a leading cause of death in Blacks, whereas the cause is uncertain in Whites (United States Department of Health and Human Services, 2017). In Brevard County, FL, the percentage of Black and Other between 2004 and 2018 was 15.25%, compared to

12.93% in Manatee County. FL. The difference in race among the two locales could have influenced the association between average total MCs and age-adjusted CLD/cirrhosis death rates.

The survey generated a model of MC prediction. In Lake Washington and Lake Manatee, total MCs increased with increased phosphates between May and July. Davis et al. (2010) exemplified a positive effect of phosphate concentrations on *Microcystis* cyanobacterial blooms. Thus, it appears that phosphate levels affect toxin production in bloom-forming lakes. Nitrites lacked predictive capacity for total MCs in Lake Washington. Nitrites exerted a positive association with total MCs in Lake Manatee. Pimentel and Giani (2014) determined a direct relationship between increased MCs and nutrient limitation. Consequently, there is agreement on nutrient levels and MCs in lakes.

Limitations

This dissertation had some limitations. A major limitation of the study was the interpretation of the data of the ecological study. The results were confined to populations, not individuals. A single person exposed to MCs cannot surrender to CLD/cirrhosis. A second limitation was the usage of old data in the secondary analysis. The potential ecologic association fails to inform the public about the present-day situation on MCs and liver disease mortality. Thirdly, a model on nutrients and MCs was partial. Including other environmental parameters, such as water temperature and pH, may have resulted in a more complete model. A fourth limitation was the measurement of phosphates in Lake Washington. A concentration of 0.298 nmol was quantified in June.

The low value may have been attributed to sampling error or technical error in the substudy.

Recommendations

The study findings warrant future epidemiological studies on MCs and liver disease mortality. Prospective scholars can employ biochemical assays to extrapolate data on freshwater MCs for exposure measurement. Scholars may incorporate additional population characteristics like education, race, and urbanization in multiple regression analysis when examining the association between MC exposure and liver disease mortality. More research is needed on climatic and environmental variables to better understand their relationship with age-adjusted CLD/cirrhosis death rates. The current state of knowledge on environmental climatic measures and liver disease is limited. The implementation of longitudinal studies in environmental epidemiology may inform public health on MC exposure and CLDs.

Implications

Positive social change can involve promoting environmental awareness in communities. Targeting an older population may entail dissemination through newspaper reports or forming an activist group. This might attract viewers on an environmental health issue within the community. Moreover, I will present this work at local academic institutions on the emerging issue of CHABs. This may facilitate the development of new curriculum in the health sciences. Shared findings might elicit consistent algal bloom warning signage. Sometimes blooms go unreported as residents do not know who to contact if unusual changes occur in the water. Posting consistent warnings signs at

bloom-forming lakes can protect animal and human health. The results could inform routine biomonitoring practices for MCs in drinking water reservoirs. Inefficient monitoring means less MC data. By screening all year-round, public health can access more detailed information on water safety. An interim guidance value on MCs can result at the policy level. To date, no federal regulation on MCs exists in FL. Establishing a regulatory level can prevent human poisoning cases attributed to MC pollution.

Conclusion

Harmful cyanobacteria living in freshwater bodies can intensify, producing toxic substances. Metabolic products such as MCs can wreak havoc on water quality and environmental health. I found a statistically significant association between average total MCs and age-adjusted CLD/cirrhosis death rates in the United States. Daily sunlight and state of residence were significant ecological predictors in the main study. In the substudy, average total MCs significantly predicted age-adjusted CLD/cirrhosis death rates in Brevard County, FL. Despite a statistically insignificant association in Manatee County, FL, scholars could investigate how race may influence the association between average total MCs and age-adjusted CLD/cirrhosis death rates in FL counties. Elevated phosphate concentrations were positively associated with total MCs in Lake Washington and Lake Manatee. In the lakes, nitrites decreased in response to increased total MCs. Researchers should explore nitrite concentrations and totals MCs in bloom-forming lakes. Cross-sectional studies may extend knowledge on cyanotoxins and acute health effects during the potential peak algae bloom season.

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Appendix

TableA1

Water Sampling Data

Sample ID	Date	Time of Collection	Coordinates	Field Notes
LMC1	6/22/2019	2:38 PM	27.4847, -82.3388	Sampled by canoe.
LMC2	6/22/2019	2:52 PM	27.4805, -82.3403	Bloom sighted in
LMC3	6/22/2019	3:19 PM	27.4889, -82.3318	cove.
LMC4	6/22/2019	3:48 PM	27.4849, -82.3285	
LMC5	6/22/2019	4:20 PM	27.4843, -82.3409	
LMC6	7/13/2019	12:18 PM	27.4814, -82.3406	Sampled by shore. No
LMC7	7/13/2019	12:27 PM	27.4816, -82.3405	visible bloom.
LMC8	7/13/2019	12:50 PM	27.4832, -82.3415	
LMC9	7/13/2019	1:30 PM	27.4767, -82.3018	
LMC10	7/13/2019	1:39 PM	27.4769, -82.3018	
LMC11	8/23/2019	12:28 PM	27.4851, -82.3416	Sampled by canoe. No
LMC12	8/23/2019	12:42 PM	27.4880, -82.3460	visible bloom.
LMC13	8/23/2019	12:59 PM	27.4902, -82.3393	
LMC14	8/23/2019	1:13 PM	27.4851, -82.3404	
LMC15	8/23/2019	1:20 PM	27.4842, -82.3428	
LWC1	6/29/2019	11:17 AM	28.1536, -80.7520	Sampled by boat. No
LWC2	6/29/2019	11:31 AM	28.1667, -80.7468	visible bloom.
LWC3	6/29/2019	11:50 AM	28.1221, -80.7467	
LWC4	6/29/2019	12:11 PM	28.1491, -80.7353	
LWC5	6/29/2019	12:50 PM	28.1471 ,-80.7390	
LWC6	7/31/2019	11:04 AM	28.1614, -80.7361	Sampled by boat.
LWC7	7/31/2019	11:19 AM	28.1420, -80.7543	Widespread
LWC8	7/31/2019	11:28 AM	28.1191, -80.7467	cyanobacterial bloom.
LWC9	7/31/2019	11:39 AM	28.1451, -80.7424	
LWC10	7/31/2019	11:49 AM	28.1475, -80.7344	
LWC11	8/31/2019	9:57 AM	28.1474, -80.7344	Sampled by shore.
LWC12	8/31/2019	10:07 AM	28.1473, -80.7345	No visible bloom.
LWC13	8/31/2019	10:13 AM	28.1471, -80.7340	
LWC14	8/31/2019	10:21 AM	28.1477, -80.7338	
LWC15	8/31/2019	10:26 AM	28.1479, -80.7338	

Table A2

GreenWater Laboratories Data

LM Sample ID	Average Assay Value ng/ml)	LW Sample ID	Average Assay Value ng/ml)
LMC1	0.005	LWC1	0.125
LMC2	0	LWC2	0.03
LMC3	0.02	LWC3	0.1
LMC4	0.03	LWC4	0.08
LMC5	0.015	LWC5	0.06
LMC6	0.035	LWC6	0.085
LMC7	0.08	LWC7	0.12
LMC8	0.045	LWC8	0.065
LMC9	0.04	LWC9	0.085
LMC10	0.005	LWC10	0.08
LMC11	0.045	LWC11	0.075
LMC12	0.075	LWC12	0.03
LMC13	Missing	LWC13	0.07
LMC14	0.045	LWC14	Missing
LMC15	0.055	LWC15	0.19