TOXIC ALGAE AND OTHER MARINE BIOTA - DETECTION, MITIGATION, PREVENTION AND EFFECTS ON THE FOOD INDUSTRY

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Abstract

Harmful Algal Blooms (HABs) including Cyanobacteria and other toxic marine biota are responsible for similar harmful effects on human health, food safety, ecosystem maintenance, economic losses and liability issues for aquaculture farms as well as the food industry. Detection, monitoring and mitigation are all key factors in decreasing the deleterious effects of these toxic algal blooms. Harmful algal blooms can manifest toxic effects on a number of facets of animal physiology, elicit noxious taste and odor events and cause mass fish as well as animal kills. Such blooms can adversely impact the perception of the efficacy and safety of the food industry, water utilities, the quality of aquaculture and land farming products, as well as cause ripple effects experienced by coastal communities. HABs can adversely impact coastal areas and other areas reliant on local aquatic ecosystems through the loss of revenues experienced by local restaurants, food manufacturers as well as seafood harvesting/processing plants; loss of tourism revenue, decreased property values and a fundamental shift in the lives of those that are reliant upon those industries for their quality of life. This paper discusses Cyanobacteria, macroalgae, HABs, Cyanobacteria toxins, mitigation of HAB populations and their products as well as the ramifications this burgeoning threat to aquatic/ landlocked communities including challenges these toxic algae pose to the field of food science and the economy.

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Dedication

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Introduction

Phytoplankton are major players in the ecological food web that have been shown to cause both marked beneficial as well as detrimental effects on animal, plant, freshwater and marine species alike. Phytoplankton are prevalent in fresh and salt water bodies around the world. Marine environments benefit from the presence of algae species, as these microscopic, photosynthetic organisms provide the foundation for marine food systems; however, toxic species have been linked to massive fish kills and widespread havoc in the food system at all trophic levels (NOAA, 2014).

Although there are roughly 300 known species of phytoplankton known to produce large algal blooms, only a small number, roughly a quarter of those of algae species are toxic (UNESCO, 2016), the effects caused by these toxic algae and bacteria, such as Cyanobacteria have marked effects on human and animal health as well as broad impacts on local and global economies due to adverse ecological impacts, toxicity and taste and odor events. These diverse ramifications demand attention by local and national authorities and can vary in response according to resource availability and economic need (WHO, 1999).

Harmful Algal Blooms (HABs) is the generic term given by scientists to the proliferation of phytoplankton that adversely impact ecosystems (UNESCO, 2016). These HABs can manifest adverse effects on not only natural environmental preservation, but also the human derived benefits that are dependent on these ecosystems. HABs have direct impacts on crucial ecosystems, food science, beverage, restaurant, healthcare and tourism industries (Marques et al., 2010).

The effects of toxic Cyanobacteria on terrestrial freshwater can include the mortality and deleterious effects on the health of domesticated and wild animal species, impacts on human health, adverse effects on vegetable and fruit farming as well as impacts on communities that rely on tourism activities driven by the availability of bodies of water (such as large lakes) (WHO, 1999).

Exposure to the maleficent effects of algal toxins can also be introduced through inhalation and dermal contact with compromised bodies of water during recreational activities. Beach closures and quarantine procedures are often employed by local and national officials to mitigate the damage to the public (CDC, 2013). However, domestic and wild animal species do not typically benefit from these measures.

Algal blooms derive their destructive effects via two mechanisms: production of toxic compounds and the depletion of oxygen from aquatic environments which can suffocate life living in those waters (Anderson, 2004). Aquatic species are adversely impacted through the depletion of oxygen vital to obligate aquatic species as well as through the effects of toxic algal products (WHO, 1999).

Algal blooms are triggered by eutrophication and favorable temperatures. For Cyanobacterial species, an optimal temperature of at least 25^{o}C is required (CEES, 2016). Global warming trends have greatly exacerbated the severity of the occurrences of HABs through greenhouse gas accumulation and ozone depletion (Rossini, 2014). The rates of HABs have increased dramatically as a function of the human industrial revolution, population expansion and agricultural practices (Tables 2-5) (Anderson, 2004). All of these factors have been known to produce nitrogen and/or phosphorous rich runoff products which feed algal blooms. Eutrophication is the artificial enrichment of aquatic environments as a result of natural

and human activities. Eutrophication induces a severe imbalance in the food systems in those bodies of water, eventually inducing anoxia and causing significant damage to those ecosystems (Marques et al, 2010).

There are roughly 300 algal species that are known to cause algal blooms. Roughly 25% of these species produce toxic products which accumulate to manifest deleterious products; however, some species are so toxic that low concentrations of free floating algae can induce illness (WHO, 1999). For example, two species of *Anabeana* and *Aphanizmenon* can produce Anatoxin-(s) and Saxitoxin which have an LD_{50} of only 40 μg/kg and 10-30 μg/kg, respectively (Table 1). Cyanobacteria range in the amino acid β-Methylamino-L-alanine (BMAA) with a size from 0.5 μm to 60μm and often exhibit single cell, colonial, or filamentous morphologies. The most prevalent and concerning are *Microcycstis aeruginosa, Karenia brevis, Gonyostomum semen, Peridinium polonicum* and *Didymosphenia geminate* (Drikas, et al., 2001).

The toxic products/metabolites produced by algae are deleterious to human as well as animal health, and can induce illness and death. These toxins include microcystins, anatoxin-a, saxitoxins, brevetoxins, nodularins, cylindrospermopsin, lipopolysaccharides, lyngbyatoxins, the amino acid β-Methylamino-L-alanine (BMAA) and domoic acid. The ingestion of Cyanobacterial contaminated seafood has been shown to cause amnesiac shellfish poisoning (ASP), diarrheic shellfish poisoning (DSP), ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP) and paralytic shellfish poisoning (PSP) (Zimba, et al., 2001).

The chronic effects of HABs can include genomic and tissue damage (WHO, 1999). Toxic algae exposure has been attributed to a number of health related conditions (Zimba, et al., 2001). A common cancer associated with exposure to algal toxins is liver cancer. The effects on development include all stages of growth including fetal, adolescent and adult development.

Algal toxins can adversely impact metabolic function through suppressed immunological function. The neurological effects include tingling in distal regions such as fingers and toes (WHO, 1999). Noted effects have been seen through inhalation exposure, causing respiratory complications which can manifest through symptoms such as coughing, wheezing and fever (Hester & Harrison, 2011). HABs also have adverse effects on reproductive capacities through individual fitness and reproductive success (WHO, 1999).

There is a strong correlation between taste and odor events in marine environments which contain the analytes 2-methylsorborneol (MIB), geosmin and to a lesser extent β-cyclocitral that are produced by Cyanobacteria (Drikas, et al., 2001). There is a strong correlation between taste and odor events in both fresh and saltwater which reflect the presence of HABs, however such taste and odor events do not always manifest during HABs (Drikas, et al., 2001).

There are a number of methods for detecting harmful algae and their products. The most common methods currently employed for the detection of toxic Cyanobacterial species/metabolites include High Performance Liquid Chromatography (HPLC), Liquid Chromatography–Mass Spectrometry (LC/MS) and Gas Chromatography/Mass Spectroscopy (GC/MS), immunoassays such as the Enzyme Linked Immunoassay (ELISA) and microscopy (WRF, 2015). Additional technologies are currently being developed and tested that may aid in the detection of toxic algal species and their products, including the implementation of micro-sensors and x-rays. Still, these methods can be costly both in terms of time and resources. As a result improvement of cost effective technologies should be investigated so as to make detection methods more available to consumers.

Treatment and elimination of Cyanobacteria and their toxic products can include traditional filtration, chlorination and/or ozonation (Drikas, et al., 2001). Treatment of water

ecosystems in natural environments/larger volumes can employ a singular or multi-faceted approach. This can involve the addition of algaecides, agitates, the novel introduction of plankton ingesting species and the use of certain aquatic plants which have been shown to remove toxic algal species and their products (Department of Primary Industries of Australia, 2015).

Land animals that have been adversely affected by HABs include bovine, porcine, avian, canine and ovine species (WHO, 1999). Aquatic species that have been adversely effected by HABs include shellfish, whales, catfish, salmon, trout and sea turtles (among others) (Miller, et al., 2010). Algal toxins also affect the horticulture industry. Crops exposed to contaminated water can also demonstrate increased damage and mortality rates. Deleterious effects can be seen on root growth, shoot growth and leaf growth of crops irrigated with contaminated water (Pflugmacher, et al., 2007). Potential lysing of bacterial contents onto the surface of fruits/vegetables can also occur as a result of dermal applications (Pflugmacher, et al., 2007).

The economic effect of Cyanobacterial blooms in the United States is $75 million annually (Larkin & Adams, 2007). Countries most effected by HABs are those that have large coastal areas, but even landlocked countries can be affected through Cyanobacterial contamination of freshwater bodies (Larkin & Adams, 2007). Red tides are the most infamous Cyanobacterial bloom occurrences, as they were among the most ominous looking algal blooms documented by early civilization (NOAA, 2014). Cyanobacteria have been attributed to biblical plagues and bad omens (Weller, 2013).

HABs have direct impacts on local economies, including restaurants, merchants, the hospitality industry, recreational fishing, water sports and tourism. Restaurants are impacted though decreased availability of seafood, farm raised fish and compromised final food and

beverage offerings. The hospitality industry has experienced loses stemming from toxic water sources as well as associated taste and odor events that make certain geographical locations at the very least undesirable and at worst uninhabitable. In the same way, recreational fishing and other water sports are also severely hampered by phytoplankton blooms. These factors combine to negatively affect local merchants through a reduction in the number of tourists to popular vacation destinations. All of these factors directly compromise the livelihoods of residents in those areas (Rodríguez, et al., 2011).

Cyanobacterial blooms have marked impacts on the food and beverage industries. The toxicity and malodorous signatures of such blooms can have direct effects on the food and beverage industries alike. Complications in water processing directly impact manufacturers of bottled water, soda and juice (Smith & Hui, 2008). Ingestion of tissue from contaminated land and sea animals impact seafood and meat producers as well as processors (Marques et al., 2010).

CHAPTER 1: THE BROAD REACHING IMPACTS OF EXPOSURE TO ALGAL TOXINS

Toxic products can be associated with a wide variety of microbial and algal species including Cyanobacteria, *Hasrophytes*, *Chlorophytes*, *Euglenophytes*, *Raphidophytes, Dinoflaggelates, Cryptophytes,* macroalgae and diatoms (WHO, 1999). Cyanobacteria, commonly known as blue-green algae, are in-fact, bacteria. They are prevalent in fresh and salt water bodies around the world. The effects of toxic Cyanobacteria on terrestrial freshwater can

include illness and mortality in humans caused by exposure to toxic algal metabolites. This has a direct impact on healthcare costs and local economies, as well as the compromise of consumer confidence in popular recreational venues associated with coastal/near aquatic destinations (NOAA, 2014).

The same algal/Cyanobacterial toxins can have impacts on livestock and other land animals, causing increased disease and mortality rates which then translate to compromise product quality (Marques et al., 2010). Such cyanotoxins can also illicit a decreased consumer confidence in the production of goods near affected areas, as well as possible translation to the products that those animals produce such as milk and eggs (Orr et al., 2001). The effects of toxic algae in marine waters can include many of the same ramifications. This includes increased disease and mortality rates of seafood species. This can also translate to compromised product quality, decreased consumer confidence in the production of goods near affected coastal areas and possible translation to the products that those animals produce such caviar/roe. (Marques et al., 2010).

The horticulture industry is not immune from the deleterious effects of algal toxins. Crops watered using a contaminated source can exhibit higher mortality rates, as well as stunted or irregular growth. Deleterious effects have been observed and documented as it effects root growth, shoot growth and leaf growth after exposure to contaminated water (Pflugmacher et al., 2007). Potential lysing of bacterial contents onto the surface of fruits/vegetables can also occur as a result of dermal applications, leading to an increased likelihood of cyanotoxin exposure to both humans and farms grazing animals.

CHAPTER 2: THE MORPHOLOGY OF TOXIC ALGAL SPECIES

Toxic algae exhibit a number of morphologies. Members of the order *Chroococcales* consist of free floating, isopolar single cells. *Chamaesiphonales* exist as heteropolar single free floating cells. The order *Pleocapsales* exhibit more complex filamentous, pseudoparenchymatous structures (Figure 1). These algae range in diameter from 0.5 µm to 60µm (Figure 2) (Weston, 2013).

Figure 1: Microscopic View of a Filamentous Algal Species (Weston, 2013)

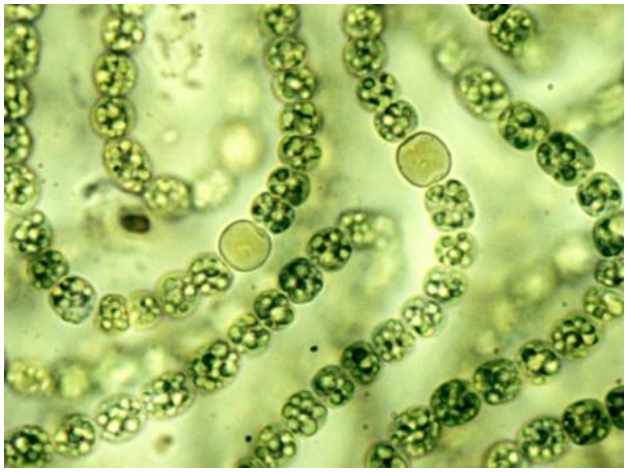
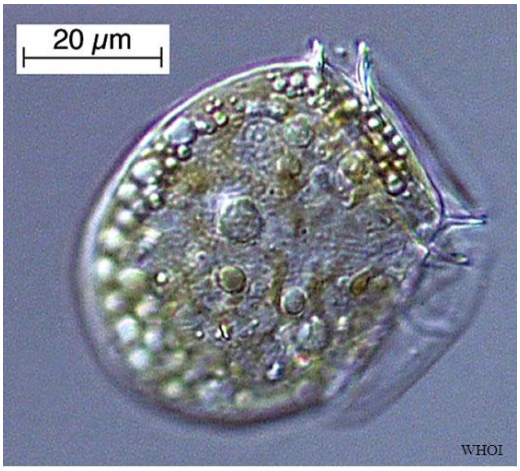


Figure 2: Microscopic View of a Pathogenic Dinoflagellate (Weston, 2013)



The orders of *Stigonematales*, *Oscillatoriales* and *Nostocales* exist as multicellular, filamentous, trichal forms. Heterocysts can be produced by both *Stigonematales* and *Nostocales.*

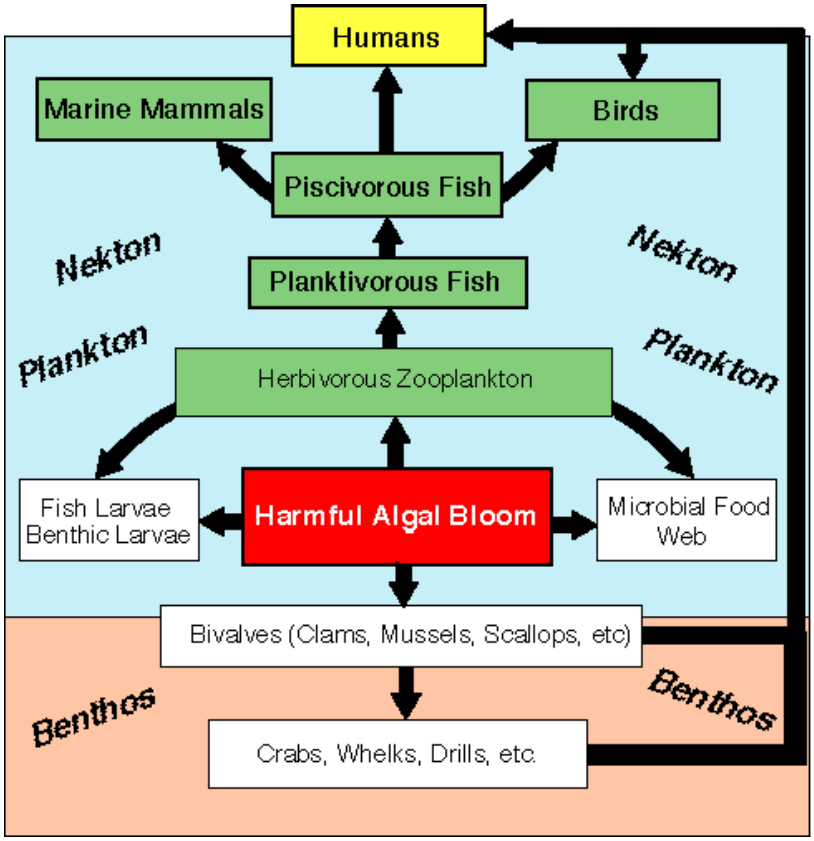
CHAPTER 3: HARMFUL ALGAL BLOOMS (HABS)

Algal blooms are typically seasonal and the result of warmer weather in combination with eutrophication. For Cyanobacterial species, an optimal temperature of at least 25^{o}C is required (CEES, 2016). Eutrophication relies on the increasing levels of nitrogen and phosphorous in ponds and lakes. These nutrients are often introduced in excess to source waters by agricultural runoff and increased urbanization (WHO, 1999). Common household detergents and industrial byproducts can contain excess phosphorous and nitrogen, and when they are added to the water table through the wastewater system, this in turn can feed these algal blooms. The

greenhouse effect that is generated by the use of fossil fuels has also facilitated environmental warming trends (which have fostered algal growth) (Marques et al, 2010). Marques et al. asserted in 2010 that there has been a marked increase in atmospheric greenhouse gas emissions. This includes an increase in carbon dioxide concentrations by greater than 30%. Pre industrial revolution levels have been hypothesized at 280 ppm, whereas current levels are 387 ppm (Marques et al, 2010).

Although higher algal toxin content is typically association with the formation of highly visible green or red surface scum production of aggregated algal cells, elevated concentrations of microcystins have been noted at levels as high as 200–400mg/liter in free floating species that may not produce such tell-tale visual cues as surface scum/biomats. Additionally, the rapid rate at which some species can replicate and produce toxins while in the form of biomats can burgeon a thousand fold in terms of both numbers and toxicity in mere hours (WHO, 1999).

Figure 3: HAB Food Web Impacts (NOAA, 2014)



Eutrophication of bodies of water can induce a major imbalance in the food webs of in those bodies of water. Due to aggregated masses of top floating algae, light is precluded/limited to other photosynthetic organisms. This causes a rapid depletion of dissolved oxygen, making the environment unable to sustain aquatic life. The accumulation of the toxins can build in the tissues of bivalves, crabs, fish and benthic larvae. These organisms then get eaten by other organisms higher up on the food chain, and those toxic products become amplified through the natural food web. This in turn decimates the life in as well as around impacted bodies of water. Although the Lake Atitlan Health Cyanobacteria Resource Center observes that a majority of the research done to date has been directed toward salt water Cyanobacteria, the data shows that about 60% of samples collected globally contain Cyanobacteria (WHO, 1999). The authors then

assert that algae found in marine environments (i.e. dinoflaggelates, diatoms, as well as haptophytes) are substantially less likely to induce illness than fresh water bodies exhibiting scums on their surfaces. This is due to the inability of constantly moving marine water systems prohibiting the aggregation of toxic species, whereas stagnant water bodies are ideal for aggregation and proliferation of microorganisms which create these HABs (WHO, 1999).

The most infamous case of red tides is the burgeoning of *K. brevis*. The phenomena of red tides date back to biblical times. NOAA scientists have hypothesized that this phenomena was in fact caused by an algal bloom (possibly that of *K. brevis*). Such blooms have been associated with occurrences of neurotoxic shellfish poisoning (NSP) (NOAA, 2014). This phenomenon also coincides with serious impacts on commercial shellfisheries and tourism for coastal communities. This phenomenon has caused significant economic losses in the range of tens of millions of US dollars per year in the Florida area alone in recent years (NOAA, 2014).

The Environmental Protection Agency (EPA) placed algal toxins on its Drinking Water Contaminant Candidate list in 1998 (Karner et al., 2001). The EPA funded a study that was published in 2001 that concluded that microcystins were present in 80% of the source water systems in the US and 4.3% of these samples were at levels above those set by the World Health Organization (WHO). The same study concluded that the levels in virtually all finished water produced by utilities were reduced to ones that were acceptable (WRF, 2015). The WHO has set a provisional limit of 1 µg/l for microcystins (Hoeger et al, 2005). Conventional water treatment methodologies (including filtration and chlorination) have been shown to lead to a 1-3 log reduction in microcystin content when algal toxin levels in raw waters have exceeded levels of 1,000 ng/L (Karner et al., 2001).

CHAPTER 4: TOXICOLOGY OF ALGAL TOXIN EXPOSURE

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Exposure to algal toxins can result in a number of adverse effects on human health, the most notable and prevalent malady is caused by microcystins. Microcystins have been shown to promote tumors, and in turn manifest a carcinogenic effect on the liver (WHO, 1999). It has been noted that toxic algal produce toxins that are not detectable through taste or smell, and their pathogenicity is resistant to a number of forms of processing, including exposure to extreme conditions such as acidity or heat (Freitas et al., 2014). It has been shown that traditional cooking/food preparation methods meant to remove pathogenic microorganisms are ineffective at attenuating this threat. The suspected mechanism for this malady is through the inhibition of the protein phosphatase enzymes (Hoeger et al., 2005). Conventional water treatment methodologies can mitigate these toxins, however they do not completely eradicate their presence.

Although there are nearly 300 species of algae and bacteria that have been correlated with blooms, only about a quarter of the species are capable of manufacturing toxic metabolic products (WHO, 1999). Some products are so toxic that that even in trace amounts, they can induces illness. *Microcycstis aeruginosa, Karenia brevis, Gonyostomum semen, Peridinium polonicum* and *Didymosphenia geminate* are the most prevalent of the species of concern (Table 1). The LD50 for a mouse of Microcystin congers can range from 45 to 1000 µg/kg. The LD50 for a mouse exposed to Nodularin is only 30-50 µg/kg. Anatoxin-a exhibits an LD50 of 40-250 µg/kg. Saxitoxin is one of the most potent, given an LD50 of 10-30 µg/kg. However, the Lake Atitlan Health Cyanobacteria Resource Center notes that the freshwater species *Dinophyceae,* as well as the *Prymnesiophyceae* and an *Ichthyotoxic* species have all been shown to manifest toxicity in brackish waters (WHO, 1999).

Table 1: Cyanobacteria and Associated Toxicity (WHO, 1999).

Cyanobacterial Taxa	Cyanotoxin produced	Lethal dose (LD 50) µg/kg	Deleterious effects
Anabanea *Hapalospihon Mycrocyctis,* *Nostoc,* *Ocillatoria* *Platrothix*	**Blocks protein phosphatase pathways** Ca 60 Microcystin congers Microcystin L-R Microcystin Y-R Microcystin R-R	 45-1000 25-125 70 300-600	 Inhibits protein phosphatase inhibition causing liver hemorrhage. Long term exposure can cause cancer or liver failure.
Nodulars spumigerna sp.	Nodularin	30-50	
Anabanea *Aphanizemeon* *Cyclopsidrospemopsis* *Oscillatori* Found in only two species of *Anabeana,* *Aphanizmenon* *Anaebena,* *Cyclindrospermopsis raciborskii* *Lygnbya*	**Neurotoxins** Anatoxin-a Anatoxin-a(s) Saxitoxins Alkaloids	 250 40 10-30	 Disables post-synaptic depolarization of the nervous system Inhibits sodium channel function and inhibits acetylcholinesterase function
Cyclindrospermopsis raciborskii	**Cyanotoxins** Cyclospermopsin	 2100 in 1 day Vs. 200 in 5-6 days	Disables protein synthesis in the body and cumulative effects can be fatal

Toxic products can be associated with a wide variety of microorganisms including Cyanobacteria, *Hasrophytes, Chlorophytes, Euglenophytes, Daphidophytes, Dinoflaggelates Cryptophytes* as well as macroalgae and diatoms (WHO, 1999). All are prevalent in fresh water ecosystems although their presence can range geographically. There are currently 84 known cyanotoxins (WHO, 1999). The most prevalent and concerning cyanotoxins are microcystins, anatoxin-a, saxitoxin, cylindrospermopsin, brevetoxins, nodularin, lipopolysaccharides, BMAA, lyngbyatoxins and domoic acid.

Algal toxins typically have one of three chemical hallmarks integrated into their structures: cyclic peptides, alkaloids, or lipopolysaccharides (Zanchett & Oliveira-Filho, 2013). Saxitoxins, anatoxin-a, lyngbyatoxin-a and lipopolysaccharides all have alkaloid structures. Both microcystins and nodularins possess cyclic peptide components (Zanchett & Oliveira-Filho, 2013). The only cyanotoxin that falls into the category of a polyketide is aplysiatoxin (WHO, 1999).

Although most algal toxins remain in the algal cell during the cell's lifetime, they can then be released into their environment. These toxins can take days, weeks, or months to degrade (Drikas, et al., 2001). Cyanobacterial toxins are further categorized into three main types: hepatoxins, neurotoxins and dermatoxins.

Hepatoxins manifest their effects on the liver. Microcystins, a class of hepatoxins, are one of the primary algal toxins responsible for liver tumor production and subsequent carcinogenesis (this includes in the microcystin-LR and microcystin-LA molecules) acceptable (WRF, 2015). The toxin microcystin derives its name from the Cyanobacteria *Microcystis aeruginosa*. There are approximately 60 variations of microcystins. Given a neutral pH, microcystins can retain their toxicity even after boiling (Karner et al., 2001).

Neurotoxins have a marked effect the nervous system. These algal toxin classes include anatoxins and saxitoxins. Deleterious impacts on health can include suffocation, tingling in distal regions such as fingers and toes (WHO, 1999). According to the Lake Atitlan Health Cyanobacteria Resource Center, Anatoxin a(s) is the only known organophosphate cholinesterase inhibitor found in nature, and is one of the most neurotoxic substances found on earth. The "s" in the name is indicative of the excessive salivation it induces, as well as a number of other maladies including gastrointestinal distress, tremors and death within minutes of exposure (WHO, 1999).

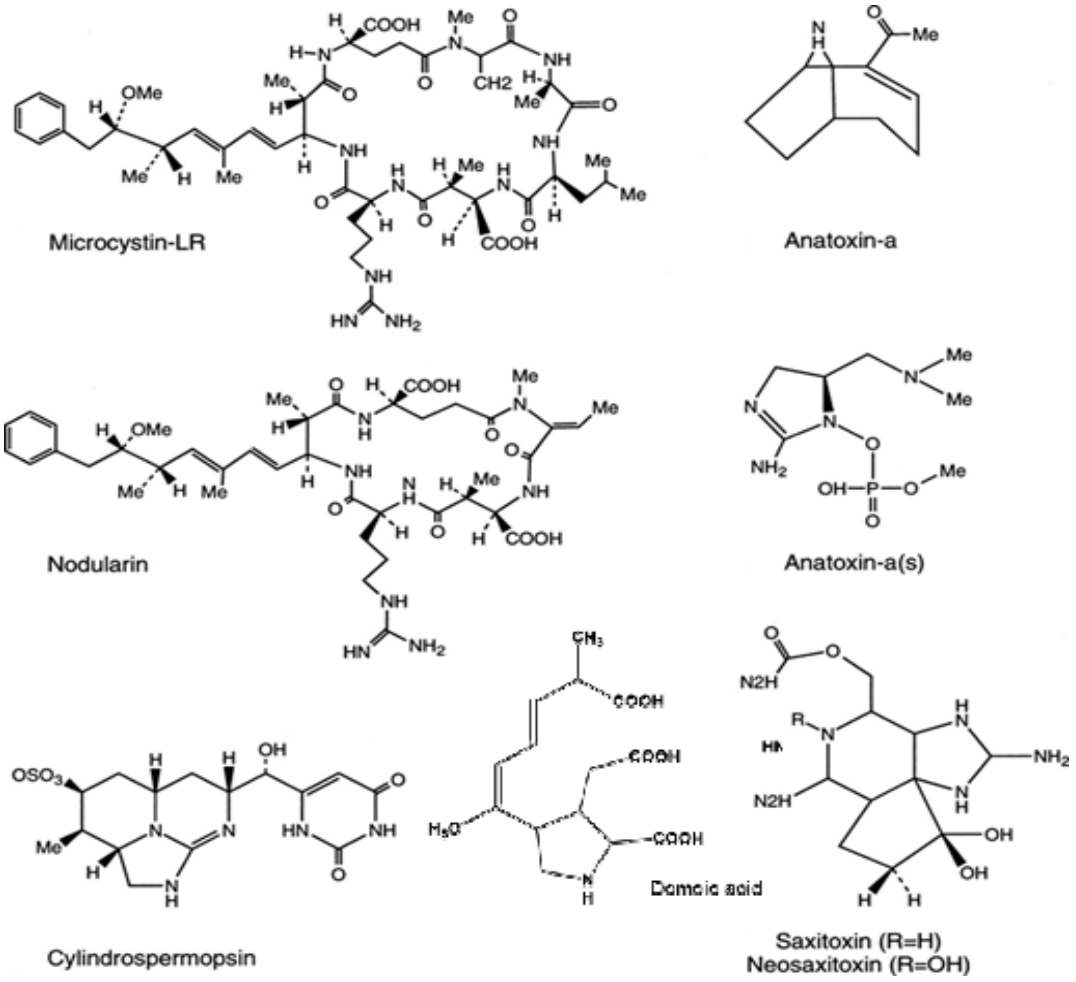
Lastly, dermatoxins irritate and can even necrotize the skin or dermis. This group includes aplysiatoxins and lyngbyatoxins (WRF, 2015). Algal toxins have also been known to be allergens (Drikas et al., 2001). Dermatoxins have deleterious effects on dermal tissue. Exposure to certain cyanotoxins can have mild to severe effects on skin that can range in severity from mild skin irritation to the manifestation of severe rashes. Known species of dermatoxins include the *Nodularin, Anabaena, Gloeotrichia, Aphanizomenon* and *Oscillatoria* species (WHO, 1999).

CHAPTER 5: OVERVIEW OF CYANOTOXINS

Some cyanotoxins are more prevalent than others because their makeup and relative toxicity of each compound is species dependent (WHO, 1999). Exposure to these toxins can manifest a range of symptoms from mild skin irritation, to more severe ailments such as genomic

and tissue damage, as well as adverse effects on every stage of life including fetal damage and adolescent and adult development as well as metabolic processes. Although some species of animals are more susceptible to different toxins, any toxin can have a deleterious effect on individual fitness and reproductive success. The cyanotoxins that HABs produce can include one or a number of the following toxic substances: anatoxin-a, brevetoxins, domoic acid, saxitoxins, nodularins, cylindrospermopsin, lyngbyatoxins, BMAA and lipopolysaccharides. Cyanotoxins typically exhibit one of three chemical configurations: cyclic polypeptides, alkaloids or lipopolysaccharides. Figure 4 illustrates the configurations of several common cyanotoxins.

Figure 4: Chemical Structures of Common Toxins Associated with HABs (Kaebernick & Neilan, 2001)



Anatoxin-A has the dubious nickname of Very Fast Death Factor (VFDF) (Tufariello et al., 1984). It is an acetylcholine inhibitor, which when present can induce respiratory paralysis. It has been detected in North America, Europe, Africa, New Zealand and Asia.

Microcystins are one of the most prevalent of the cyanotoxins. There are many variants in existence; this includes MC-LR, MC-RR, MC-YR and MC-LW. There have been 90 congers discovered to date in plant tissue which range in deleterious effects from cell death to damage to the mitochondrial membrane (Romero-Oliva et al., 2014). Accumulation of microcystin

congeners in different aquatic plants and crops were documented in a case study from lake Amatitlán, Guatemala (Romero-Oliva et al., 2014). It is believed that the molecule interferes with the process of mitosis and causes an alteration of cell shape. Microcystins can induce severe liver damage, tumor promotion and even death through necrosis of the liver. This tissue damage can occur anywhere within a few hours to a few days (WHO, 1999).

Exposure to the toxic metabolic product domoic acid can induce a rather serious series of health effects. In humans it can induce amnesiac shellfish poisoning, which entails nausea, vomiting and other gastrointestinal symptoms of distress as well as neurotoxic effects ranging in scope from short term memory loss to seizures, to brain damage (NOAA, 2014). In marine mammals, such as dolphins and the California sea lion, it can induce seizures and even miscarriage (NOAA, 2014). It derives it toxicity from its ability to induce degenerative imbalances in calcium levels. Neuronal damage occurs through domoic acids ability to activate pathways such as those of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kinase receptors. This has direct impacts on the brains hippocampus; thus, the observed effect of short-term memory loss (Pulido, 2008). It should be noted that it may also cause kidney damage – even at levels considered safe for human consumption, a new study in mice has revealed damage to the kidneys can occur at a hundred times lower than the concentration allowed under FDA regulations (Pulido, 2008).

Saxitoxins are the most notorious of the compounds that induce Paralytic Shellfish Poisoning (PSP). This toxin is typically produced by the *Anabaena, Alexandrium, Pyrodinum* and *Gymnopseridium* sp. Neurotoxins are collectively referred to as saxitoxins. The pure form of this molecule is known as pure anatoxin (STX). This toxin is attributed to major closures of commercial clam, mussel and scallop farming/ harvesting units in areas such as Oregon,

Washington, New England as well as international locations such as Thailand, the Philippines, Japan and South America (Landsberg, 2002).

Nodularins tend to be found in Cyanobacteria living in estuaries where salt and fresh waters begin to mix. They are produced by *Nodularia spumigena*, which causes some of the largest algal blooms in the world (WHO, 1999). These blooms typically occur in late summer and pose a serious threat to human and animal health. Nodularins are a hepatoxin, therefore are very toxic to liver tissue (WHO, 1999).

The unarmored dinoflagellate species *Karenia brevis* is responsible for the production of brevetoxin. This cyanotoxin derives its deleterious nature from its ability to bind to sodium channels (Watkins, 2008). This causes damage to the nervous system resulting in gastrointestinal distress and slurred speech (Watkins, 2008). Exposure can occur through both inhalation and ingestion. Exposure can cause intensive bronchial spasms and damage. Consumption of shellfish contaminated with brevetoxin induces Neurotoxic Shellfish Poisoning (NSP) (CDC, 2013).

Cylindrospermopsin is a compound derived from the toxic algae *Cylindrospermopsis raciborskii* (Ohtani et al., 1992). Exposure to this toxin can include liver and kidney failure, after several days; however exposure to the unadulterated toxin can induce liver, kidney and lung injury. Additional complications to the intestine and adrenal glands have also been demonstrated. This can be attributed to the toxins ability to adversely impact protein synthesis (WHO, 1999).

Lipopolysaccharides are an integral part of gram negative bacterial cell membranes. They are composed of an O antigen bound to lipid A. These molecules serve as a safeguard against phagocytosis. Because they are also present in the cell membranes of Cyanobacteria,

when those bacteria lyse, their lipopolysaccharide membrane components are released into their environment. These molecules have been shown by some scientists to cause skin irritation, although the research is still ongoing to definitively categorize it as a dermatoxin (Rietschel et al., 1994).

Lyngbyatoxins are a defensive chemical secretion produced by the Cyanobacteria species *Moorea producens* (this organism was formerly known as *Lyngbya majuscula).* These Cyanobacteria produce lyngbyatoxins to deter fish from ingesting that algal species. It can induce a range of symptoms from dermal irritation (also known as seaweed dermatitis) due to the blister inducing vesicant properties; therefore, it is classified as a dermatoxin (Cardellina et al., 1979). It is also a known carcinogen (Fujiki et al., 1981).

β-Methylamino-L-alanine (BMAA) is currently being investigated for its potential as a neurotoxin. Exposure can lead to both acute and chronic neurodegenerative conditions. Exposure is hypothesized to exacerbate individuals suffering from Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (Cox et al., 2005).

As noted above, the toxic effects of HABs can impact a range of health functions for humans, land and aquatic animals alike. Cyanotoxins such as anatoxin-a, brevetoxins, domoic acid, saxitoxins, nodularins, cylindrospermopsin, lyngbyatoxins, BMAA and lipopolysaccharides can cause widespread systemic damage to tissue. They have been shown to alter the development of organisms, adversely impact immunological and neurological functions and reproductive ability, cause respirational distress as well as the portion of tumors that can cause cancer. Tables 2-5 document human illnesses that have resulted from exposure to contaminated HAB water bodies.

Table 2: Human Illness Events Associated with Exposure to Cyanobacteria in Recreational Water from 1930-1980 (WHO, 1999)

1931: **USA:**	A massive *Microcystis* bloom in the Ohio and Potomac rivers caused illness of 5000–8000 people whose drinking-water was taken from these rivers. Drinking-water treatment by precipitation, filtration and chlorination was not sufficient to remove the toxins (Tisdale, 1931).
1959: **Canada:**	In spite of a kill of livestock and warnings against recreational use, people still swam in a lake infested with Cyanobacteria. Thirteen persons became ill (headaches, nausea, muscular pains, painful diarrhea). In the excreta of one patient—a medical doctor who had accidentally ingested water—numerous cells of *Microcystis* spp. and some trichomes of *Anabaena circinalis* could be identified (Dillenberg & Dehnel, 1960).
1968: **USA:**	Numerous cases of gastrointestinal illness after exposure to mass developments of Cyanobacteria were compiled by Schwimmer & Schwimmer (1968).
1975: **USA:**	Endotoxic shock of 23 dialysis patients in Washington, DC, was attributed to a Cyanobacterial bloom in a drinking-water reservoir (Hindman et al., 1975).
1979: **Australia:**	Combating a bloom of *Cylindrospermopsis raciborskii* in a drinking-water reservoir on Palm Island with copper sulfate led to liberation of toxins from the cells into the water and resulted in serious illness (with hospitalization) of 141 people supplied from this reservoir (Falconer et al., 1994).

Table 3: Human Illness Events Associated with Exposure to Cyanobacteria in Recreational Water from 1980-1990 (WHO, 1999)

1981: **Australia:**	In the city of Armidale, liver enzyme activities (a sign of exposure to toxic agents) were found to be elevated in the blood of the population supplied from surface water polluted by *Microcystis* spp. (Falconer et al., 1983).
1985: **USA:**	Carmichael (1994) compiled case studies on nausea, vomiting, diarrhoea, fever and eye, ear and throat infections after exposure to mass developments of Cyanobacteria.
1988: **Brazil:**	Following the flooding of the Itaparica Dam in Bahia State, some 2000 cases of gastroenteritis were reported over a 42-day period, of which 88 resulted in death. Investigation of potential causes of this epidemic eliminated pathogens and identified a very high population of toxic Cyanobacteria in the drinking-water supply in the affected areas (Teixeira et al., 1993).
1989: **England:**	Ten out of 20 soldiers became ill after swimming and canoe training in water with a heavy bloom of *Microcystis* spp.; two developed severe pneumonia attributed to the inhalation of a *Microcystis* toxin and needed hospitalization and intensive care (Turner et al., 1990). Swimming skills and the amount of water ingested appear to have been related to the degree of illness.

Table 4: Human Illness Events Associated with Exposure to Cyanobacteria in Recreational Water from 1990-1999 (WHO, 1999)

1993: **China:**	The incidence of liver cancer was related to water sources and was significantly higher for populations using Cyanobacteria-infested surface waters than for those drinking groundwater (Ueno Y, 1996)
1994: **Sweden:**	Illegal use of untreated river water in a sugar factory led to an accidental cross connection with the drinking-water supply for an uncertain number of hours. The river water was densely populated by *Planktothrix agardhii* and samples taken a few days before and a few days after the incident showed these Cyanobacteria to contain microcystins. In total, 121 of 304 inhabitants of the village (as well as some dogs and cats) became ill with vomiting, diarrhea, muscular cramps and nausea (Anadotter et al., 2001).
1995: **Australia:**	Epidemiological evidence of adverse health effects after recreational water contact from a prospective study involving 852 participants showed elevated incidence of diarrhea, vomiting, flu symptoms, skin rashes, mouth ulcers, fevers and eye or ear irritations within 2–7 days after exposure (Pilotto et al., 1997). Symptoms increased significantly with duration of water contact and density of Cyanobacterial cells, but were not related to the content of known cyanotoxins.
1991: **Australia:**	Largest algal bloom to date. Over 100km of water was contaminated with *Anabaena circinalis* in the Darling River (Bowling 1991).
1996: **Brazil:**	In total, 131 dialysis patients were exposed to microcystins from the water used for dialysis; 56 died. At least 44 of these victims showed the typical symptoms associated with microcystin, now referred to as "Caruaru Syndrome", and liver microcystin content corresponded to that of laboratory animals having received a lethal dose of microcystin (Jochimsen et al., 1998).

Table 5: Human Illness Events Associated with Exposure to Cyanobacteria in Recreational Water from 2000-2015

2000: **New Zealand:**	First documented instances of toxic freshwater cyanobacteria documented in the Mataura River, leading to canine deaths (Hamill, 2001).
2003: **USA**:	Blooms of *Pseudo-nitzschia* forced the closure of shellfish harvesting in Puget Sound, Washington area citing domoic acid poisoning risk (Trainer et al., 2007).
2005: **USA**:	Alexanrdrium bloom crippled the shellfish industry from Maine to Martha's Vineyard (Carlowicz, 2005).
2009-2010**: USA**:	11 freshwater HAB blooms were reported in New York, Ohio, and Washington state (CDC, 2014).
2009-2012: **Brazil**:	Dinophysis AND Pseudo-nitzschia species diarrhetic shellfish poisoning were detected, which can cause amnesic shellfish poisoning (Tibiriçá et al., 2015)
2009-2010: **USA:**	11 freshwater HAB blooms were reported in New York, Ohio, and Washington State (CDC, 2014).
2012: **France:**	Alexandrium species halted shellfish harvesting, citing paralytic shellfish poising concerns (Chapelle et al., 2015).
2014**: USA:**	Excess nitrogen runoff triggered a toxic algal blooms, causing the Toledo, Ohio drinking water supply to be shut down (Pelley, 2016).
2015**: USA:**	*Pseudo-nitzschia* bloom halts clam, crab, sardine and anchovies farming in the states of Alaska, Washington, Oregon and California. This bloom resulted in a number of marine mammal and bird mortalities due to domoic acid exposure. This was the largest and most long lasting bloom event on the west coast in the last 15 years (NOAA, 2015).

CHAPTER 6: TASTE AND ODOR EVENTS ASSOCIATED WITH ALGAL BLOOMS

Algal blooms can have a discernible effect on the taste and odor of water (WRF, 2015). This can reflect not only metabolic products produced by Cyanobacteria, but also the distinctive smell of decay due to the poisoning of marine species, such as fish kills due to the ingestion of contaminated waters. Therefore, there are often two distinct taste and odor phenomena paired with toxic algal blooms. The rotten fish odor can be pungent, and the metabolic products of the algae themselves can manifest themselves as earthy or musty odors (WRF, 2015).

These odors are often attributed to the molecules geosmin, β-cyclocitral and 2-methylsorborneol (MIB). However, the correlation between taste and odor events and algal toxin presence is not necessarily a direct cause-and-effect relationship (Drikas et al., 2001). As the Water Research Foundation noted, that there have been inconclusive results when attempting to directly correlate taste and odor events with definitive HAB events, as non-toxic species can cause similar problems (WRF, 2015). The researchers do note however that species that produce these toxins also produce taste and odor compounds, so the phenomena can be used as a primitive diagnostic tool to trigger increased monitoring during those events (WRF, 2015).

β-cyclocitral is said to be the least odoriferous compound as compared to geosmin and 2-Methylisorborenol-(MIB) (Lopez et al., 2008), however, all are odoriferous and can impact the flavor profile of raw and finished water as well as deterring recreational activities such fishing, swimming, boating and waterfront-driven tourism. According to the World Health Organization, the algal species that are known to produce geosmin include *Microcystis, Anabaena, Oscillatoria, Phormidium, Aphanizomenon, Lyngbya, Schizothrix* and *Symploca* (Falconer et al., 1983). All are known to produce toxins, save for *Symploca* sp. (Lopez et al., 2008).

A paper published by Lopez et al. in 2008, asserts that in an analysis of catfish aquaculture, an industry valued in 2008 at over $450 million annually in the US, noted that the second most detrimental factor in the sales in that market are directly proportional to the earthy and musty compounds produced by Cyanobacteria. It is estimated that geosmin and 2-methylsorborneol (MIB) accounted for about 80% of economic losses in this aquaculture industry, creating an overall cost increase in catfish market prices that ranged from 3-17% at the wholesale level (Lopez et al., 2008). Interestingly, that same source also noted that the only states that have implemented a formal program to monitor taste and odor problems associated with algal blooms are those of Kansas, California and Arizona.

CHAPTER 7: METHODS FOR DETECTING ALGAL TOXINS

The earliest popular method for the detection of cyanotoxin was developed in the 1960s through the employment of bioassay technology (WHO, 1999). Purified toxin was injected into mice subjects in order to determine the health risks associated with exposure to drinking water supplies; however, this method was only employed for about two decades after which time it was deemed to not only be resource prohibitive, but unethical as well. Since that time the number of technologies that have been expanded to facilitate detection of toxic Cyanobacterial products has burgeoned (WHO, 1999).

Presently there are a number of methods are used to identify and quantify cyanotoxin content. Analysis via high-performance liquid chromatography (HPLC), gas chromatography–

mass spectrometry (GC/MS), liquid chromatography–mass spectrometry (LC/MS, microscopy, Radioactive Protein Phosphate Assay (rPPA), Enzyme Linked Immunosorbent Assay (ELISA), as well as Polymerase Chain Reaction (PCR) technologies can all be used. Each method obviously has its own quantitation limits. Recently the use of micro-sensors as well as x-ray technologies have also been investigated (van der Schot et al., 2015).

ELISA testing has proven to be the most effective and popular of the bioassays currently available. It is ideal for microcystin quantification due to its ability to capture both intact and partially intact molecules (Hoeger et al., 2005). The ELISA test specific for the detection of algal hepatoxins was first developed in 1989. It is now largely commercially available and has been a popular assay for microcystin quantification. The assay can detect the microcystin variants microcystin-LR, RR and YR, as well as nodularin (Karner et al., 2001). The limit of detection for cyanotoxins via ELISA can be as low as 0.1 ppb (Preece et al., 2015).

Liquid chromatography has proven to be slightly less efficient, as there are numerous microcystin variants, as such, an analysis is difficult as their relative absorbances can vary, and there can be peak interference from other compounds in the solution (Hoeger et al., 2005). One report documented a robust LC/MS/MS method developed for the detection of saxitoxin, anatoxin and cyclopermopsin, all in a single analytical run (Larkin & Adams 2007). The limit of detection for cyanotoxins via LC/MS can be as low as 0.5 ppb (Hardey et al., 2015).

Radiolabeling algal toxins can yield analysis though the Radioactive Protein Phosphate Assay (rPPA). This assay can be disadvantageous as it relies on the radiolabeled molecule remaining intact, and can consequently cause an underestimate of the quantity of dissolved microcystins (Hoeger et al., 2005). Polymerase Chain Reaction (PCR) has also proven to be a promising technology as it is a rapid method for real-time detection of algal toxins in field

situations, but it is a relatively nascent technology whose scope and application is improving rapidly (WRF, 2015). This technology can detect as few as 3.52 gene copies when detecting microcystins (Te et al., 2015).

It should be noted, however, that the primary factor to consider in algal toxin detection is availability (i.e. economy and accessibility) of such resources to the public. If the technology is not accessible, then it can do the public little good. Thus improving technologies to make detection cost effective, less time consuming and more field friendly should be investigated.

CHAPTER 8: OPTIMAL WATER TREATMENT METHODS FOR ELIMINATING ALGAL TOXINS TO PRODUCE FINISHED WATER

Algal toxin removal is a complicated and potentially expensive endeavor. It has been poignantly noted that the removal of these toxic alga can prove to be problematic due to their small size (Karner et al., 2001). Cyanobacteria can range in size from 0.5 to 40 μm in diameter (Maier et al., 2000). Current common water treatment practices are not specifically designed to remove these hazards. In fact, they have been shown to exacerbate the problem by inducing cell lysis and the excretion of the toxins (Hoeger et al., 2002). However, more modern treatment methods (such as ozonation) have been employed with relative success, and later these methods will be described in greater detail (Hoeger et al., 2002).

The most commonly used methods for water treatment in the United States involve a combination of sedimentation, flocculation, filtration and chlorination (Drikas et al., 2001).

Sedimentation is meant to remove larger matters such as leaves, sticks and small rocks that are taken in with source river water. Flocculation is the process of adding certain metals to the resulting water to settle out the finer particulate matter. Filtration then allows the flocculated water to pass through layers of sand and carbon to remove other organic compounds (Drikas et al., 2001). Chlorination serves as a disinfectant step.

Sedimentation, although beneficial, derives its limits on algae removal due to algal cellular size. In one study sedimentation reduced the number of *M. aeruginosa* cells by approximately 50% (Drikas et al., 2001). Another study demonstrated that a reduction in microcystin level of 50% can be seen at the pretreatment level (Karner et al., 2001).

For sedimentation to be truly effective, periodic flocculation is required. Flocculation is a key step in algae removal (Drikas et al., 2001). Flocculation involves the addition of certain chemicals, such as aluminum- and iron-based compounds to destabilize the surface charge of particulate matter in the water. The binding of the aluminum/iron to the particulate matter creates what is known as floc. A study performed by Drikas et al. 2001 showed that flocculation did not increase the amount of dissolved microcystin concentration in the test waters. They did note that this conclusion may be slightly inaccurate because no studies have shown how cultured cells and wild type cells may bind differently to aluminum/iron species (Drikas et al., 2001).

Flotation can be used as an alternative to sedimentation. Its mechanism is the opposite of sedimentation. When pressurized water is intermittently introduced, this can lead to the release of tiny bubbles, which can thereby attach to the floc particles (Drikas et al., 2001). This can cause the floc particles to float to the top of the tank, as which point they can be skimmed off of the surface (Drikas et al., 2001).

Filtration is the next step in the water treatment process. Filtration by itself is an ineffective method for algae removal due to the small diameter of the algal cells; thus, it should not be used alone to remove algae from water. Cyanobacteria can be as small as 0.5 in diameter (Maier et al., 2000). However, in combination with flocculation, filtration can be an important tool in the arsenal of the water treatment plant. Slowly all of the remaining particulate matter or sludge gathers on the sand filter, and the clarified water can be run through a bed of activated carbon to remove particulate matter as well as organic compounds that can cause odor and health issues (Drikas et al., 2001). In one study, the cyanobacterium *M. aeruginosa* was noted to be captured in the sludge from this part of the treatment process (Drikas et al., 2001). Using filtration to remove algae does carry a risk; however, in the previous study, the algae then began to degrade after two days, thereby releasing microcystins (WRF, 2015). This illustrated the importance of periodic filter backwash. Backwashing is the process of running clean water in reverse though the filter bed so as to remove the accumulation of settled material.

The final step in the traditional water treatment process is chlorination. Chlorine gas is introduced into the water to act as a disinfectant. The addition of chlorine gas creates chlorite and chlorine dioxide species, which then inhibit the growth of microorganisms (Karner et al., 2001). The use of chlorine and fluorine (used to maintain optimum tooth enamel strength) in water are controversial as they both have advantages and disadvantages when introduced into the water supply (Karner et al., 2001). Chlorine can act as a disinfectant, inhibiting the growth of pathogenic organisms; however, it can be toxic at certain levels. The National Institute for Occupational Safety and Health has set a limit of exposure of 10ppm for chlorine (CDC, 1994). The benefits typically outweigh the risks of using this agent, and it has been employed in the water treatment process for decades (Karner et al., 2001).

A more modern and effective method for finished water treatment can include ozonation. Ozone gas is pumped through water and that ozone causes the algal cells to die and a majority of the algal toxins to degrade (Hoeger, et al., 2002). It is notable, however, that although ozone has been shown to provide protections from microcystins and anatoxin-a, it is not effective in the degradation of saxitoxins (WRF, 2015). Regardless, ozonation remains the most effective technology to combat algal toxins in finished water.

One study conducted evaluated the effectiveness of the two most popular methods for the removal of these toxins from drinking water: filtration followed by flocculation and ozonation followed by filtration through sand and then activated carbon (Hoeger et al., 2002). Their research indicated that the latter was the most effective method of treatment, as the ozonation process not only killed the Cyanobacteria but oxidized the resulting lysed toxins. Subsequent filtration steps capture any ozone created in the ozonation process and can serve as a biofilm to metabolize other undesirable organic compounds.

Ozone, chlorine and potassium permanganate have all been known to be effective in the degradation of both intra- as well as extra- cellular microcystin and anatoxin-a removal (Karner et al., 2001). Treatment using ozonation, in combination with filtration first through sand and then activated carbon, has been shown to be the most effective way to remove microcystins. This combination of treatment methods yielded up to a 97% reduction in microcystins (Hoeger et al., 2002).

CHAPTER 9: OPTIMAL WATER TREATMENT METHODS FOR ELIMINATING ALGAL TOXINS FROM WATER BODIES

The presence of cyanotoxins in raw water sources pose a serious risk to the health of humans and animals alike (WHO, 1999). A recent bloom in the Lake Erie triggered the shutdown of the Toledo, Ohio drinking water supply (Table 5) (Pelley, 2016). This resulted in a mandatory ban of tap water ingestion by residence for 3 days (Ho & Michalak, 2015). Officials documented microcystin levels of up to 2.5 µg/L, which was over twice the WHO limit of 1 µg/L (Pelley, 2016).

In the past, algal blooms have been treated with the addition of copper sulfate into the source waters; however this causes the algal cells to lyse and they subsequently release their toxins into those same waters, which exacerbates the problem (WRF, 2015). Over time, algaecides have improved, agitates have been employed, induction of artificial destratification has been utilized to reduce the nutrient availability in water bodies (Heo & Kim, 2004). The use of aquatic plants, such as *E. crassipes* and *Typha* sp., have also shown promise in combating toxic algal and bacterial species (Hudnell, 2008). These powerful/promising tools can be used individually or in combination to decimate the populations of these toxic algae and bacteria (Hudnell, 2008).

The use of algaecides has been the most common method of algal bloom mitigation (Marques et al., 2010). Although its use can dramatically alter the ecosystem of the body of water though the accumulation of these toxic products. As with any agent that targets selective groups of microorganisms, there is obviously a risk of selecting for algae that are naturally resistant to the algaecides. This can exacerbate taste and odor problems associated with their

metabolic products geosmin and *2-Methylisoborneol* (Marques et al., 2010); however often the benefits often can outweigh the risks. There is also the risk of killing non-targeted populations of aquatic plants and animals, as well as sediment dwelling microorganisms due to the accumulation of the copper based algaecides in the lake sediment (Department of Primary Industries of Australia, 2015).

Other methods of algal bloom mitigation can include artificial destratification through agitation of the body of water. Through the use of pumps and water mixers, water from different depths of the affected body of water can be circulated, disrupting floating algal colony biomats. These devices have met with mixed success rates (Hudnell, 2008). Through the disruption of the surface layer, algal cells can be transported into the depths of the water column, limiting their light exposure and increasing mortality rate of algal cells (Heo & Kim, 2004).

The use of surfactants and chemical flocculation can be employed for additional means of control. Clay is the most common and cost effective flocculent, and it has been shown to create minimal unintended adverse environmental consequences (Park et al., 2013). As agricultural processes have progressed, so have farming strategies to avoid unnecessary eutrophication of bodies of water (WHO, 1999). Farmers and scientists recommend the fertilization of fields during the fall and winter months. This can help curtail the nutrient availability for toxic algae during the spring and summer months as by that time any excess nutrients are introduced into the local watershed (WHO, 1999).

The use of aquatic plants for a bioremediation of algal toxins has been explored in recent decades (Park et al., 2013). It has been noted that certain species, such as *E. crassipes* and *Typha* sp., are very efficient at removing cyanotoxins from water. *E. crassipes* has demonstrated an accumulating capacity of 166±11 mg/kg of microcystins in dry

weight, followed by *Typha* sp., which can accumulate 18±0.03 mg/kg of dry weight of microcystins. (Romero-Oliva et al., 2014). Additionally the use of protozoan grazers such as *C. polykrikoides* have been used for algal population control, however this kind of mechanism for removal can take roughly 6-14 days (Park et al., 2013).

CHAPTER 10: A MODEL SYSTEM FOR HAB DETECTION - THE NATION OF KOREA

Korea has developed HAB detection and response measures, and has continued to improve and develop these systems since 1980; nationwide programs were officially implemented in 1995 (Park et al., 2013). That was the year the government officially created the HABs Emergency Center. Officials remotely monitor chlorophyll and turbidity levels at various locations, such as susceptible aqua farming operations. The National Fisheries Research & Development Institute (NFRDI) now also monitors multiple facets that can correlate with HABs. This includes cell density, chlorophyll concentrations, water pH, salinity, temperature, nutrient composition and dissolved oxygen data obtained at least one time per week during bloom events. They readily make this information available to the public via nearly real-time updates of its homepage (Park et al., 2013).

If toxic algae are detected, supplemental oxygen can be fed into aqua farm waters to combat the hypoxia stress that fish encounter during algal blooms. The fish are then transferred to non-contaminated water so clay dispersion can be employed in affected water bodies. Since

employing clay dispersion methods of HAB mitigation techniques, the losses resulting from HABs has dropped more than 80% (Park et al., 2013).

CHAPTER 11: LAND ANIMALS THAT HAVE BEEN ADVERSELY AFFECTED BY HABS

Cyanobacterial blooms have a multi-fold effect on the production livestock and other animals within a given ecosystem. Tainted source waters can be imbibed by livestock, inducing serious illness and even death (Karner et al., 2001). The first documented animal deaths associated with algal toxins occurred in Australia in 1878 after livestock ingested contaminated water. Land animal poisonings and intoxication via cyanotoxins have been documented for a number of additional species including sheep, birds, pigs, horses, rodents and amphibians (WHO, 1999). Canine deaths have occurred after swimming in or drinking from contaminated water bodies (Karner et al., 2001).

In addition to the accumulation of cyanotoxins in the tissues of exposed animals, it has even been hypothesized that given sufficient exposure, those toxins may be transferred to milk and eggs from exposed animals (Orr et al., 2001). One study was conducted to examine the transference of microcystin exposed cattle into their milk (Orr et al., 2001). Their findings indicated that at exposure levels of up to 15mg of Microcystin LR (MC-LR), there was no real health risk associated with the microcystin levels present in the milk; however, the scientists also admitted that HPLC analysis of milk had certain limitations in that there were interference peaks

present at key retention times (those of the internal standard and others), possibly lowering the sensitivity of that method for cyanotoxin quantification. The same scientists also noted that after a three week exposure to environmentally realistic cell concentrations of *M. aeruginosa*, Microcystin LR (MC-LR) concentration was 430 times greater than accurate quantitation would allow (Orr et al., 2001).

Land farmers and public agencies have a responsibility to keep abreast on this emerging HAB threat. Water sources used by livestock should be routinely tested during the spring through the early autumn months to preserve the health of livestock (Orr et al., 2001). Although monitoring for toxic algae does have a cost associated, maintaining healthy animals leads to a higher quality of products for those farmers and therefore lends to a more trusted brand/company/manufacturer perception.

CHAPTER 12: AQUATIC ANIMALS THAT HAVE BEEN ADVERSELY AFFECTED BY HABS

A paper published in by Marques et al., asserted that climate change is now a reality for both land and water based ecosystems. They caution that we have very limited information about the macro and micro effects of this novel global phenomena as well as its effects on seafood efficacy from ‘‘farm to fork” will prove to manifest many problems in all stages of the food manufacturing chain, including complications in not only the processing, production, transport and trade of these goods (Marques et al., 2010). Among the aquatic species, shellfish

(such as oysters and mussels) (Rodríguez et al, 2011), whales, catfish, salmon, trout, shrimp, sea turtles, sea otters (Miller et al., 2010) and dolphins (Fire et al., 2011) are among the species that have been documented to have been adversely effected by HABs. Roughly 8% of the world's population is reliant on seafood for nutrition and employment (Marques et al., 2010); therefore, the ramifications of HABs cannot be understated nor should they be overlooked.

In 2015, a bloom of *Pseudo-nitzschia* affected the clam, crab, sardine, anchovy along the west coast of the US (Table 5) (NOAA, 2015). This impacted the states of Alaska, Washington, Oregon and California (Milstein, 2015). It was demonstrated to be the largest and most long lasting *Pseudo-nitzschia* bloom event in the last 15 years (NOAA, 2015). The cost to the commercial crab fishing industry alone has been estimated to be $60 million (Wilson, 2015). The cost to the razor clam industry was estimated to be $9 million for one month alone (Milstein, 2015).

The ingestion of Cyanobacterial contaminated seafood has been shown to cause amnesiac shellfish poisoning (ASP), diarrheic shellfish poisoning (DSP), ciguatera fish poisoning (CFP), neurotoxic shellfish poisoning (NSP) and paralytic shellfish poisoning (PSP). All of these symptoms are deleterious to human and animal health, and can induce illness and death to all species exposed. These poisonings are derived from the consumption of cyanotoxin exposed bottom feeding organisms such as clams, oysters, mussels and shrimp (Zimba et al., 2001). The consumption of finfish is also a common method of exposure, so there has also been research published on catfish, salmon, rainbow trout, tilapia and carp (as well as others) (Zimba et al., 2001). HABs can decimate fish populations, impacting the food web at both lower and higher trophic levels (Marques et al., 2010). Marques et al., 2010, hypothesized that the affordability of seafood as a protein and nutrient source in combination with the plethora of health benefits

seafood can impart will begin to see new and emerging challenges as consumer confidence is rattled by increasing incidences of compromised products due to toxic algae (Marques et al., 2010).

There are serious difficulties in processing seafood that may have come into contact with HABs. Cyanotoxins are remarkably heat and acid stable (Freitas et al., 2014). One study conducted by researchers examined the heat stability of microcystins in the processing of clams (*Corbicula fluminea*) (Freitas et al., 2014). Clams were exposed to the Microcystin-LR (MC-LR) toxin producing *Microcystis aeruginosa* at concentrations of $1x10^5$ cells/mL for four days. Researchers then examined the microcystin levels of the clams, which were stored under various conditions (such as frozen and refrigeration), as well as heat treatment methods commonly used in the area of food science/home food preparation (such as boiling, microwaving). This was followed by an analysis of bioavailability of microcystins via proteolytic digestion and subsequent analysis via LC/MS. Notable findings included that during this 4 day period, MC-LR levels decreased in uncooked clam stored at room temperature (25^o C). But MC-LR levels increased in uncooked clams stored at refrigerated (4^o C) and frozen conditions (-20^o C) over storage time. The researchers concluded that short periods (microwaving for periods of .5 and 1 minute, and boiling for 5 and 15 minutes) of cooking easily released MC-LR from the contaminated clams, making them more bioavailable during human consumption than that of raw clam consumption. They also concluded that the water soluble nature of MC-LR creates problems for food processing methods that include boiling can facilitate release of the toxin (Freitas et al., 2014). They added the caveat, however; that although microwave (MW) cooking methods seemed to have a beneficial effect by lessening the bioavailability of MC-LR in clams, this cooking method leads to unappealing taste and texture qualities (Freitas et al., 2014).

Consequently, due to the hardiness of these toxins as well as matrix complications, further research should be conducted in this field as it creates great challenges to the government in terms of the maintenance of public health and the food science industry in terms of product safety and efficacy. Additionally the HACCP (Hazard Analysis Critical Control Points) guidelines that dictate appropriate processing times (as set forth by the US government) and reiterate proper storage, cooking methods and time tables that will emphasize those steps when examining Critical Control Points (CCPs) for safe industrial processing (Freitas et al., 2014).

CHAPTER 13: EFFECTS ON AGRICULTURE

Contaminated water is often used to irrigate crops in underdeveloped countries (Pflugmacher et al., 2007). If contaminated water comes into contact with fruits or vegetables and the algal cells lyse their contents onto the surface of those crops, the algal toxins can be released and pose a serious risk to the consumer, as well as have serious economic ramifications for the producers of those goods (Hoeger et al., 2005). Researchers assert that microcystins will be easily absorbed by sedimentation and integration in clay and soil matrices. When given plant species are exposed to algal toxin contaminated water, effects have been noted on root, shoot and leaves of land and aquatic plants alike (Pflugmacher et al., 2007). Targeted crops for research on this topic include rice, lettuce, seaweed and wheat cultivars.

Due to the toxic and highly soluble nature cyanotoxins transference and exposure through contaminated water should be viewed as a real threat to issues of food protection, crop quality

and the preservation of human as well as economic health (Hoager et al., 2005). It has been proven that cyanotoxins can be absorbed through plant tissues such as the root system, and are translocated through the stem system of exposed crops (Pflugmacher et al., 2007). It has been noted that globally surface water has been utilized to irrigate agricultural crops, and this practice poses risks for the agricultural community as any toxins present in areas irrigated with those waters can pose a risk for potential uptake of those toxins by plants (Pflugmacher et al., 2007). Adverse impacts on plant development can include a decreased photosynthetic ability, elevated antioxidant-response enzyme levels (which are typically produced as a countermeasure to exposure to reactive oxygen species), stunted root and shoot growth, damage to the sucrose transport system and necrosis of plant tissues.

There have been studies concerning MC-LR accumulation in other plant varieties, many focused on growth inhibition via MC-LR contaminated irrigation water. This has been documented in the case of lettuce, broccoli, rape, spinach, alfalfa, wheat and white mustard crops exposed to MC-LR containing water. Aquatic plants can also be very sensitive to microcystins, as they can absorb these cyanotoxins from the water (Pflugmacher et al., 2007).

In rice it has been found that the microcystin MC-LR can not only accumulate in rice grains, but in higher levels it can induce irregularities in growth (Chen et al., 2012). Inhibition of root elongation, lateral and crown root of primordia was also observed. For crown roots, the average length of plants exposed to high concentrations of MC-LR (0.5–4 μg/mL) over ten days demonstrated a 46-96% reduction in the length of crown roots, and a decrease of 47% in the number of crown roots (as treated with 4 μg/mL MC-LR). This has been attributed to anomalous quantities of Reactive Oxygen Species (ROS) and Nitric Oxide (NO) as the plant senses and responds to the stress induced by MC-LR exposure (Chen et al., 2012). China has experienced a

dramatic increase of MC-LR exposure over the last several decades, which has induced blooms that cover nearly 40% of the third largest lake in China, Lake Taihu (Chen et al., 2012). The farmers around this area and their employees rely on rice production as roughly 75% of the total arable land is dedicated to rice production. Additionally as rice is a major staple to the diets in these areas, blooms can cause food shortages, health problems and challenge the way of life for those that rely on the bounty of this agricultural area (Chen et al., 2012). Although Chen et al. did not find levels would exceed the WHO daily intake limits, children and the elderly may prove to be more susceptible to exposure even at lower concentrations.

The effects of wheat irrigated with cyanotoxin containing water have also been examined in a study by Pflugmacher et al (Pflugmacher et al., 2007). Stunted root and shoot growth was observed, as well as necrosis of plant tissues. For root tissues, this could result in a 70% decrease in length, and for shoot length, a 20% decrease could be observed. In this study it was found that wheat plants tended to be stunted and all exposed were smaller and less healthy than the control plants. Chlorophyll a and b levels were seen to be diminished by as much as 64%. Germination rates were reduced by 23% after a mere three days of exposure (Pflugmacher et al., 2007).

A study conducted in Saudi Arabia showed that plants irrigated with microcystin containing water showed that vegetable plants accumulated these cyanotoxins in their leaves and roots at concentrations ranging from 0.07 to 1.2 μg/kg fresh weight (Mohamed & Shehri 2009). The levels of microcystin concentration in the wells that irrigated these crops was determined to be 1.3–1.8 μg/L. These concentrations were directly proportional to the amount of microcystin that was found to be in the various wells with which these crops were irrigated. The amount of toxin in plants was also species dependent, with the highest levels found in tuberous vegetables

and those with large tap roots such as radishes and carrots as cyanotoxins have a propensity to accumulate in the roots of plants. In radishes, microcystin levels of 1.2 µg/g fresh weight were observed. There was an exception to their findings, however when culturing dill, the cyanotoxin concentration was greater in the leaf tissue than in the root tissue. Levels of 0.2 µg/g fresh weight were observed in the leaf tissue and 0.15 µg/g fresh weight were observed in the root tissue. The authors of the study assert that due to the fact that vegetables and herbs are typically eaten without processing, radish roots and dill, parsley, lettuce and cabbage leaves that can certainly contain microcystins levels that will pose a threat to human health. Despite detecting low levels of microcystin concentrations detected in the edible leaves of plants (0.11–0.26 µg/kg fresh weight) and of edible roots (0.36 µg/kg fresh weight) did not exceed the WHO guideline of microcystin exposure levels of 1 gram per liter, those levels can plausibly be higher than WHO guidelines (Mohamed & Shehri 2009). They went on to calculate that given an average consumption of 200-300g per day of contaminated produce, that a 100g serving would contain 11-36 µg/kg of microcystins (this is 5-18 times the recommended limit for drinking water) (Mohamed, & Shehri, 2009).

Another study conducted in Hungary examined the effects of watering sward/grass expanse with water from a local algal bloom (Bácsi et al., 2011). It was found that the grass samples collected all tested positive for various microcystin congeners. This exposure led to 83% decreased levels of chlorophyll content and an 80% decrease in green mass. The scientists also noted that at one point in the experiment root growth had been inhibited 3 times more than that of the shoot inhibition. In terms of relative tolerances to cyanotoxins, *Lolium perenne* or *Festuca rubra* appeared to be robust to the effects of microcystins; the applied microcystin concentrations as treatments of 5.9–56.4 lg 9 L-1 did not affect seed germination in the case of

the two grass species. It was concluded that rye-grass was the least sensitive to microcystins (Bácsi et al., 2011).

Aquatic plant species have been shown to manifest susceptibility to cyanotoxins (Romero-Oliva et al., 2014). Their ability to uptake these toxins has advantages and disadvantages in that they are effective at removing free microcystin content in water, but due to tolerances that are species dependent, there are varying degrees of expression of deleterious effects. It has been noted that in the aquatic plant species *C. demersum, Myriophyllum sp., Hydrilla sp., V. natans, C. vulgaris, E. canadensis,* as well as *P. maackianus* plants species that had been exposed to various concentrations of microcystins (ranging from 0.001 to 5 mg/L) exhibited detrimental effects as demonstrated by oxidative stress, decreased plant size and even plant death (Romero-Oliva et al., 2014). This research also showed that after only 3-5 days, microcystin containing water with species such as *L. minor, M. elantinoides, H. verticillata and C. thalictroides* showed the most promise of toxin removal, resulting in an 89-92% removal of microcystins from water.

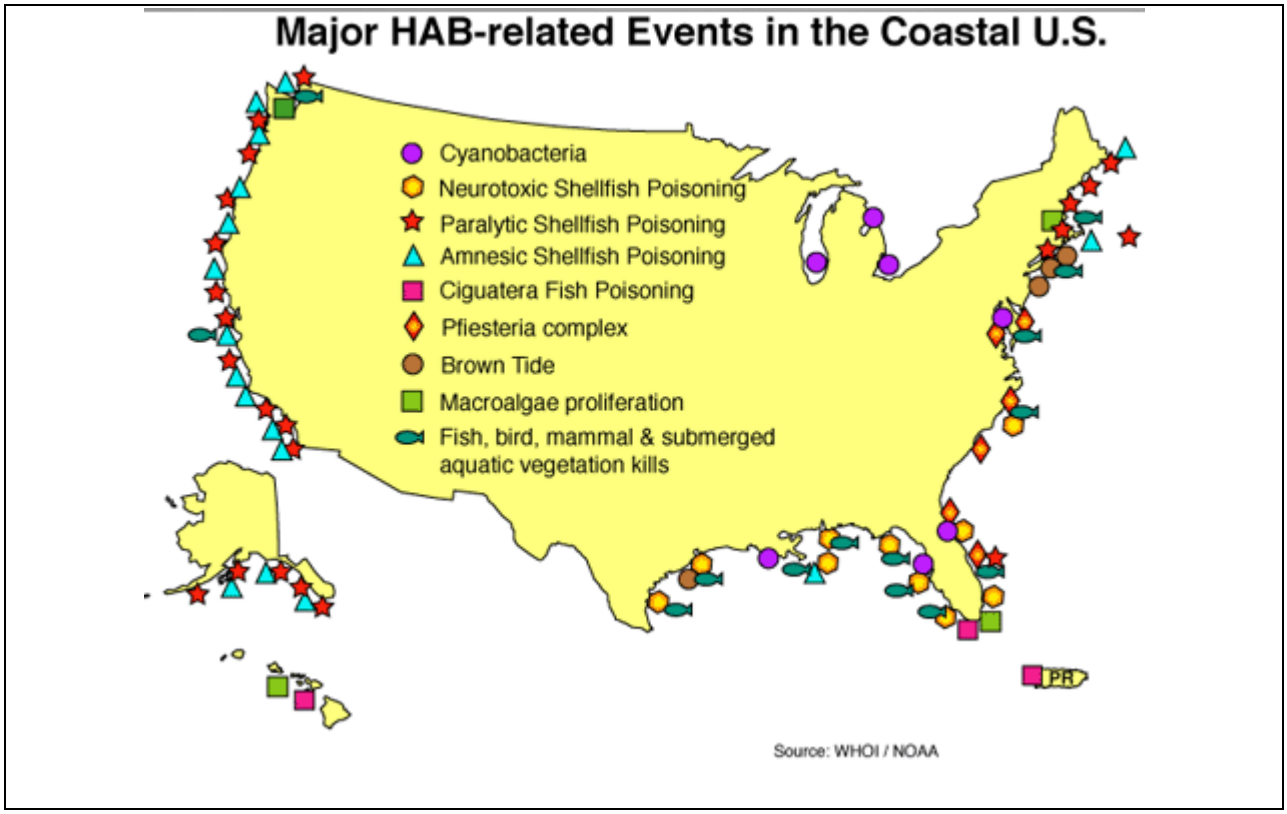
The cyanotoxin uptake of varieties of tomato and pepper species has also been documented (Romero-Oliva et al., 2014). Researchers found that after exposure to microcystins after irrigation with contaminated water, levels of 1.16±0.67 µg/kg DW MC-RR were noted in *S. lycopersicum* , whereas in *C. annuum* concentrations of MC-LR were observed at levels of 8.10±4.67 µg/kg. In terms of MC-LR, *S. lycopersicum* exhibited levels at 0.70 µg/kg DW, whereas in *C. annuum* concentrations of MC-LR were observed at levels of 1.03±0.75 µg/kg DW. The authors assert that given these results, the Tolerable Daily Intake (TDI) for an average sized individual (approximately 60 kg) that assuming an average daily consumption of three fruits per day with a weight of approximately 390g would consume approximately to 0.006-

0.008g of microcystins per day, which is below the recommended intake of and 0.006 µg/kg MCs; thus, under the recommended limit of 0.04 µg/kg per day (Romero-Oliva et al., 2014).

CHAPTER 14: ECONOMIC IMPACTS OF HABS

The economic losses induced by HABs reflects a number of factors that range from decreased property values, loses on income to those families that rely on aquaculture for lifestyle as well as income, lost revenues in tourism, declines in the customer perception of food safety, closure of aquaculture business, closures of tracts of coastline, to community and social stress for those coastal communities (Figure 5) that rely on the resources that have based their way of life on the coastal ecosystems (Rodríguez, et al., 2011). The cost associated with HABs in the United States from 1987 to 2002 alone was tabulated at $22 million a year. One study found that red tides in Florida alone can impact revenues for the restaurant/lodging sector by 29.3-34.6% , whereas a hurricane alone manifests a mere 4.8% loss on average for the restaurant sector alone while the passage of a tropical storm (or hurricane) could only result in the loss of 4.8% for the restaurant sector only (Larkin & Adams, 2007). That same report indicated that NOAA reports that current annual losses associated with toxic algal blooms now total $75 million annually.

Figure 5: Coastal States that have been affected by HABs (NOAA, 2004)



In Korea alone, there have been losses calculated at $121 million over the past three decades. This is reflective of the forced closure of fisheries and shellfish farms, and the decimation of other aquaculture farms. Meanwhile, mussel production for Spain is responsible for about half of European mussel production, behind that of Thailand and China. In Spain, HABs have cost roughly $100 million per year nationwide (Rodríguez, et al., 2011).

There have been a number of closures of commercial fisheries and aqua farms as a result of HABs. In the 1989, the Georges Bank Surf Clam Fishery was forced to close as a result of toxic algal blooms (Chorus & Bartram, 1999). In Washington State, HABs resulted in a $10 million US loss in the fishing sector from 1997 to 2005 (Larkin & Adams, 2007). In Alaska, the Alaskan Shellfish Resources service estimated a total revenue loss of $10 million US due to

fishery and shellfish closures. Costs for the states of Oregon, Washington, Massachusetts, Connecticut, Maine, New York, New Hampshire, New Jersey, New York and North Carolina alone were tabulated to average $2 million a year for monitoring and management costs alone (Anderson et al., 2000). The same source indicates that the overall cost totals $1 billion given the overall impacts over the last several decades. This reflects an estimated 37% for commercial fishery losses, 13% of recreation/tourism losses as well as 4% for management and monitoring of HABs. Table 6 illustrates the pervasive nature of toxic algae in fresh water samples around the globe.

Table 6: Global Frequencies of HABs in Freshwaters – WHO, 1999)

Country	# of Samples Tested	% of Toxic Samples	Type of Toxicity
Australia	231	42	Hepatotoxic Neurotoxic
Australia	31	841	Neurotoxic
Brazil	16	75	Hepatotoxic
Canada, Alberta	24	66	Hepatotoxic Neurotoxic
Canada, Alberta	39	95	Hepatotoxic
Canada, Alberta (3 lakes)	226	741	Hepatotoxic
Canada, Saskatchewan	50	10	Hepatotoxic Neurotoxic
China	26	73	Hepatotoxic
Czech and Slovak Rep.	63	82	Hepatotoxic
Denmark	296	82	Hepatotoxic Neurotoxic
Former German	10	70	Hepatotoxic
Germany	533	721	Hepatotoxic
Germany	393	22	Neurotoxic
Greece	18	--	Hepatotoxic
Finland	215	44	Hepatotoxic Neurotoxic
France, Brittany	22	731	Hepatotoxic
Hungary	50	66	Hepatotoxic
Japan	23	39	Hepatotoxic
Netherlands	10	90	Hepatotoxic
Norway	64	92	Hepatotoxic Neurotoxic

CHAPTER 15: CONCLUSIONS AND SUGGESTIONS FOR THE FUTURE

Due to the increasing incidence in alga blooms over the last several decades, and their potential for detrimental effects on human health, industry and economic health, it is imperative to take further steps to both increase the public awareness of the implications of algal blooms as well as advance research and technology for detecting as well as treating HABs. Additionally, through modification of water purification practices at the municipality level would virtually eradicate these toxins in the finished water the public relies on.

First, a public awareness campaign must be launched in order to emphasize how farmers can mitigate their roles in the eutrophication of the water table. The farming community could be advised to fertilize fields in the colder fall and winter months. This helps to curtail water table nutrient imbalances that feed algal blooms during the warmer spring and summer months when blooms are typically observed. The general public could be informed about more ecofriendly/balanced detergents, as well as potentially shifting/scheduling different yard watering/fertilizing practices so as to limit eutrophication and preserve quality of life for those communities. Modifications of local water treatment plants, although expensive, should also be considered. The eventual switch from chlorination to ozonation would cause a virtual eradication of toxins in finished drinking water derived from contaminated waters.

Increased monitoring programs with locally and nationally defined acceptable exposure limits will also prove to be necessary as the global climate shifts and the phenomena of HABs continue to increase. Additional innovation in the detection and turnaround time for algal toxin testing should also be emphasized. More research should be done on foliar applications using contaminated water, soil irrigated with contaminated water and seed germination grown using

contaminated water sources, as this could potentially have great impacts on human health and the food science industry. Although there are a number of adequate tests for detecting and quantifying certain algal toxins, the ability to develop a cheap, rapid, field friendly test system will ultimately be key to allow localities to react appropriately in order to safeguard human health and quality of life, as well as the delicate ecosystems humans enjoy both for their beauty but the bounty of life and resources they harbor.

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