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Toxicology of Freshwater Cyanobacteria

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Abstract

A number of chemical contaminants in drinking water have been shown to cause adverse health effects in humans after prolonged exposure. Cyanobacteria are one of the most potent and diverse groups of photosynthetic prokaryotes. One key component of cyanobacterial success in the environment is the production of potent toxins as secondary metabolites, which have been responsible for numerous adverse health impacts in humans. Anthropogenic activities have led to the increase of eutrophication in freshwater bodies' worldwide, causing cyanobacterial blooms to become more frequent. The present article will discuss about harmful cyanobacteria and their toxicology with special references to microcystin, nodularin and cylindrospermopsin.

Key words

Cyanobacteria; cyanotoxins; harmful algal blooms; cylindrospermopsin; microcystin

Introduction

Cyanobacteria comprise a diverse group of aquatic photosynthetic prokaryotes [1, 2]. Being a successful group of organisms in the environment, under optimal growth conditions they can rapidly multiply in aquatic systems and some species can produce harmful algal blooms (HAB) [3-6]. Under such conditions they are well known for their ability to produce potent toxins which have been responsible for numerous livestock and human poisonings [7-12]. Toxigenic cyanobacteria are increasingly being perceived as a potential health hazard, particularly in waters used for drinking and recreation.

Cyanotoxins can be categorized according to two main criteria; their modes of toxicity to multicellular animals, animal cells or cell systems and their chemical composition and structure [7,10, 13-18]. According to their modes of toxicity cyanotoxins can be branched into hepatotoxins, neurotoxins, cytotoxins, irritants and gastrointestinal toxins. Among cyanotoxins, microcystins (MCs), cylindrospermopsins (CYNs) and nodulations (NODs) are considered as most prevalent and potent cyanotoxins in freshwaters that can cause both acute and chronic illnesses in animals and humans [13, 19-22]. Saxitoxins and related analogs, collectively known as paralytic shellfish poisons (PSPs), are another group of potent neurotoxins which inhibit neuro-transmission by blocking sodium channels [23-27]. Over 20 different variants of PSPs have been identified and classified according to structural groups. While PSPs are associated with marine and tropical freshwaters, the recent spread of *Cylindrospermopsis raciborskii* into temperate regions suggests that PSPs may soon gain a cosmopolitan distribution [28]. The dermatoxic lyngbyatoxins-A, -B, and –C, the PSPs [29, 30], and the dermatoxic alkaloid aplysiatoxin have caused acute dermatitis, poisonings, and animal deaths in several tropical

regions [31, 32]. They act through phospholipid-dependent protein kinase C activation, causing smooth muscle contraction; the symptoms include skin, eye, and respiratory irritation [30]. In addition, lyngbyatoxin-A has also been shown to be a tumor promoter [33].

The attempt of the current article is to review the fast growing literature on freshwater contamination by toxic cyanobacteria. Hence the article will describe toxic cyanobacteria, their secondary metabolites and toxicology of cyanotoxins. Readers are encouraged to consider additional helpful reviews of cyanobacteria and their toxins for further information on cyanobacteria and cyanobacterial toxins [7, 9, 13, 14, 18, 21, 63, 69, 77, 83].

Cyanobacteria and their secondary metabolites

Cyanobacteria are oxygenic photosynthetic gram negative prokaryotes, previously referred to as blue-green algae, due to their capability for plant-like photosynthesis and their role as primary producers among the phytoplankton community [34-36]. However, lack of intracellular membrane bound organelles or a nucleus [37-39], lack of sexual reproduction [40], peptidoglycan cell wall structures and protein synthesis machinery of cyanobacteria are similar to those of other prokaryotes, and therefore they are classified as bacteria [41,42]. The cyanobacteria are a remarkably widespread, highly adaptable and successful group, colonizing freshwater, marine and terrestrial ecosystems (in soil, on rocks and even in the atmosphere) including extreme habitats such as freezing environments in Antarctica, hyper-saline localities, arid deserts and hot springs [43-45]. The basic prokaryotic cellular organization of cyanobacteria is characterized by a thick gelatinous cell wall composed of a peptidoglycan layer surrounded by a mucoid sheath which acts as a protective layer. They lack membrane-bound organelles and well-organized nuclei but do possess thylakoids, gas vacuoles and 70S ribosomes scattered in the

cytoplasm [37, 38]. Cyanobacteria show a remarkable degree of morphological diversity [35]. They may be unicellular or filamentous and variation within these morphological types may also occur [46].

A physiological feature that gives several cyanobacteria species a competitive edge in aquatic ecosystems is their ability to migrate vertically in the water column. Gas-filled vesicles found within vacuoles inside the cells facilitate this vertical movement and accounts to their buoyancy enabling cyanobacteria to occupy an optimum position within the water body [47].

Mass occurrences (blooms) of cyanobacteria under optimal environmental conditions are common in aquatic environments worldwide [48]. A bloom can comprise one or several species, each of which can include both toxic and nontoxic strains. Hence, the toxicity of a bloom is dependent both on the relative proportions of toxic strains present and the toxin production rates of the toxic strains [48]. Such blooms are known as harmful algal blooms (HABs) which can cause livestock and human poisonings [8].

Cyanobacteria have the potential to produce an elaborate array of secondary metabolites with unusual structures and potent bioactivity. They have attracted the attention of scientific communities and general public due to two main reasons: (a) the acute toxicity of the cyanotoxins to animals and humans and (b) the potential therapeutic uses of several secondary metabolites [11, 49, 50]. The secondary metabolites from cyanobacteria include a range of bioactive compounds showing animal toxicity [13] as well as antibacterial [51,52], anticoagulant [53], antifungal [54], anti-inflammatory [55], antiplasmodial [56,57], antituberculosis [55], antiviral [58], anticancer [59], immunosuppressive [60], algicidal [61] and cytotoxic activities [55].

Cyanotoxins

Cyanobacteria are well known for their ability to produce potent toxins which have been responsible for numerous animal and human poisonings [8, 10, 11, 14, 62 - 65]. It is not known why cyanobacteria produce cyanotoxins and only little is documented about why their production varies. However, it is generally believed that these compounds increase the competitive ability of cyanobacteria and acts as a defensive strategy against predators and grazing by zooplankton [66]. Additionally, only certain cyanobacterial species produce cyanotoxins. Even toxigenic cyanobacterial species do not always produce cyanotoxins constitutively and the production of certain cyanotoxins is both species- and strain-specific [67]. Furthermore, toxicity may also vary between clones of the same isolates [13, 68]. Some cyanotoxins can be produced by more than one cyanobacterial species and likewise, the same species is able to produce more than one cyanotoxin [69].

Cyanotoxins may be either membrane-bound or free within cyanobacterial cells. Typically, cyanotoxins are released passively during cell senescence, lysis, and death rather than by continuous excretion [48]. Studies in vitro showed that during early log phase, cyanotoxins remain inside the cells and are released to the environment in the late-log or stationary phase due to an increased rate of cell death [70-73]. This is an important consideration for water purification centers since removal of healthy cyanobacterial cells intact from the raw water supply may reduce the need for additional adsorptive (activated carbon) or oxidative (ozone or chlorine) methods to remove toxins. However, the release of cyanotoxins is also enhanced by various water treatment practices, such as use of algaecides. For example, treatment of a bloom

with copper sulfate may lead to complete lysis of the bloom within three days and release of all the toxins into the surrounding water [74-76].

Cyanobacterial lipopolysaccharides (LPS) are frequently cited in the cyanobacteria literature as toxins responsible for a variety of health effects in humans, from skin rashes to gastrointestinal, respiratory and allergic reactions. As no cyanobacterial lipid A structures have been described, a review by Stewart et al [77] recommended that cyanobacteriologists should not continue to attribute the diverse range of clinical symptoms to cyanobacterial LPS without objective toxicological evidence. Additionally, after conducting a pilot study with 39 volunteers, these investigators presented the view that case reports and epidemiologic studies do not present convincing findings of mass outbreaks of acute cutaneous responses to planktonic freshwater cyanobacteria and consider the cause as hypersensitivity reactions.

In natural ecosystems, different cyanotoxins exhibit quite different chemical stabilities and biological activities. In general, released cyanotoxins undergo photochemical and bacterial degradation. In addition, a significant fraction of released cyanotoxin becomes unavailable for exposure by adsorption to soil surfaces, depending upon environmental factors, soil properties, and total organic content of the soil [78]. For example, in natural water and in the dark, MC may persist for months or years and is hydrolyzed in waters with elevated or low pH and high temperatures (40 °C) [79]. Further, MC photochemical breakdown rate is increased in the presence of water soluble cell pigments [80] and humic substances [81]. CYN is relatively stable in the dark, with slow breakdown occurring at elevated temperature (50 °C). In sunlight and in the presence of cell pigments, breakdown of CYN occurs quite rapidly. Pure CYN is relatively stable in sunlight [82]. Data on the distribution of CYN on a global scale are still scarce, though

occurrence of CYN has been documented for all continents either through monitoring of field samples and/or by isolation of CYN-producing cyanobacteria.

Based on their mode of action on mammals, they are categorized as hepatotoxins, neurotoxins, dermatotoxins, etc. [2, 14, 19, 21, 83] (Table 1). Two classes of hepatotoxic compounds produced by cyanobacteria are the cyclic peptides MC and NOD, which are highly selective liver protein phosphatase inhibitors [84], and CYN, a cyclic guanidine alkaloid toxin [48, 85,86]. These toxins mainly damage the hepatocytes of the liver [87]. Our main focus will be on the MC, NOD and CYN families and their toxicology.

a) Microcystin (MC) and nodularin (NOD)

Microcystins (MCs) are the most prevalent and potent cyanobacterial hepatotoxins produced by many strains of different cyanobacterial genera; among them *Microcystis* is the most notable genus (Table 1) [48, 88-95]. To date about 90 different isoforms [69, 96] of MC have been chemically identified, with the main differences between variants related to the substitution, hydroxylation, epimerization and demethylation of amino acids [48,96]. Collectively, MCs are monocyclic hepatopeptides (MW 995.17) containing several unusual amino acid residues and differ structurally, primarily in two positions of their backbone. The common structure is cyclo (Adda-D-Glu-Mdha-D-Ala-L-X-D-Asp/D-MeAsp-L-Z), where ‘X’ and ‘Z’ are variable L-amino acids, ‘Adda’ is 3-amino-9-methoxy-2,6,8-trimethyl-10 phenyl-4,6-decadienoicacid, ‘D-MeAsp’ is 3-methylaspartic acid and ‘Mdha’ is N-methyldehydroalanine [97]. Among MCs, the most common and toxic variant microcystin-LR (MC-LR) contains amino acids leucine (L) and arginine (R) at positions 2 and 4, respectively (Figure 1) [69]. MC is synthesized non-ribosomally via an enzyme complex comprising peptide synthetases (PS), polyketide synthases

(PKS), mixed peptide/ polyketide synthases and tailoring enzymes [97,98], which are encoded by a *mcy* gene cluster containing 10 genes (*mcyA- mcyJ*) and spanning nearly 55 kb [99, 100]. *Mcy* gene clusters have been identified and characterized from *Microcystis* [101, 102], *Planktothrix* [98] and *Anabaena* [99]. Non-ribosomal peptide synthetases (NRPSs) possess a highly conserved modular structure, with modules consisting of catalytic domains responsible for adenylation, thioester formation, and condensation of specific amino acids [103]. The arrangement of these domains within the multifunctional enzymes determines the number and order of the amino acid constituents of the peptide product [104]. In *Microcystis aeruginosa* PCC7806, the *mcy* gene cluster is arranged in two divergently transcribed operons, *mcyA-C* and *mcyD-J*. The smaller operon, *mcyA-C* encodes three NRPSs (Mcy A-C), while the larger *mcyD-J*, encodes a modular PKS (McyD), two hybrid enzymes with NRPS and PKS modules (McyE and McyG, tailoring (Mcy J, F and I) and transport (Mcy H) of the toxin (97).

MCs are water soluble and upon ingestion, uptake into liver cells takes place via the bile acid carrier transport system [105-108]. They are potent inhibitors of serine/threonine protein phosphatases type 1 and 2A (PP1 and PP2A) [107]. This inhibition leads to hyperphosphorylation of proteins associated with the cytoskeleton of hepatocytes, leading to cytoskeletal disorganization that is characterized by cellular disruption, lipid peroxidation, loss of membrane integrity, DNA damage, apoptosis, necrosis, intrahepatic bleeding, and eventually death by hemorrhagic shock [88,105-110]. Further, MCs are tumor-promoting substances when combined with compounds that are able to initiate carcinogenesis [87, 111]. They are known to induce hepatocarcinoma (111, 112) and colorectal cancer (113).

Nodularin (NOD) (MW 619), produced by brackish and freshwater species of the genus *Nodularia* (most commonly *Nodularia spumigena*), is a cyclic pentapeptide, similar to MC-LR, also possessing a characteristic Adda, D-glutamic acid (D-Glu), N-methyldehydrobutyrine (MeDhb), D-erythro-β-methylaspartic acid (D-MeAsp), and L-arginine (L-Arg) amino acid (Figure 2), but with increased water solubility [114, 115]. Chemically, NODs differ from MCs by the absence of two core amino acids and have N-methyldehydrobutyrine (Mdhb) instead of N-methyldehydroalanine (Mdha) [114]. It is also a potent hepatotoxin and carcinogen as in MC and associated with eukaryotic protein phosphatase (PP) catalytic subunit types 1 and 2A [116]. The toxin inhibits the activity of PP2A to a greater extent than PP1 [116]. NOD biosynthesis occurs via a non-ribosomal mechanism involving large multi-enzyme complexes [117, 118]. These large modular proteins catalyze the activation, modification and condensation of specific amino acid or small chain carboxylic acid substrates. Non-ribosomal peptide synthetases (NRPS) and PKS genes have been associated with the production of NOD in strains of *Nodularia* [118, 119]. These NRPS and PKS enzymes are encoded by *nda* gene cluster which spans nearly 48 kb and consists of nine ORFs (*nda*A-I) transcribed from a bidirectional regulatory promoter region [118]. Seven naturally occurring isoforms of NOD have been reported to date [16, 120, 121]. The toxin has been documented to have effects on numerous invertebrates and fish, but no records on human effects (122). Toxic *N. spumigena* at higher doses has led to death of domestic and native animals by massive liver haemorrhage and in sub-acute doses, acts as a liver tumor initiator and promoter (122-124).

b) Cylindrospermopsins (CYNs)

Cylindrospermopsin (CYN) is recognized as one of the most globally important freshwater algal toxins. Data on the distribution of CYN on a global scale are still scarce, though occurrence of CYN has been documented for all continents, either through monitoring of field samples and/or by isolation of CYN-producing cyanobacteria. CYN is associated with six Nostocales species [125- 130], mainly *Cylindrospermopsis raciborskii*, two Oscillatorian species (*Lyngbya wollei* and *Oscillatoria* sp. strain PCC 6506) [131,132] and one Stigonematales species (*Umezakia natans*) [133]. The first report of CYN intoxication in humans was reported after the outbreak of hepatoenteritis in Palm island Australia in 1979, causing illness to 148 people, mostly children, which required hospitalization [134,135]. CYN (MW 415 Da) is a highly water soluble tricyclic polyketide-alkaloid ($C_{15}H_{21}N_5O_7S$) hepatotoxin containing a sulfate ester of guanidine moiety combined with a hydroxymethyl uracil (Figure 3) [48, 125, 136-138]. The toxin is zwitterionic in nature; a dipolar ion with localized positive (guanide group) and negative (sulfate group) charges [136] and stable under varying heat, light, and pH conditions. Therefore, the toxin can persist for long periods in turbid and unmoving water and cannot be removed by boiling [82]. CYN structural variants include 7-deoxycylindrospermopsin, an analog isolated from *C. raciborskii* and *Raphidiopsis curvata* in which the hydroxyl group on the uracil bridge (C-7) has been removed (Figure 3) [128, 139,], and 7-epicylindrospermopsin (Figure 3), an analog with a 7*S*-hydroxyl group that was isolated from *Aphanizomenon ovalisporum* [126,140]. Recently, the presence of CYN and deoxy-CYN were also recorded from the cyanobacterium *Raphidiopsis mediterranea* FSS1-150/1 of a eutrophic reservoir in Queensland, Australia [141. CYN shows

hepatotoxic, nephrotoxic, and cytotoxic effects and is a potential carcinogen by inhibition of glutathione, cytochrome P450and protein synthesis [142-144]. Mihali et al (2008) sequenced and characterized the CYN biosynthesis gene cluster for the first time in *C. raciborskii* AWT205 strain using 'gene walking' technology [145]. Further, it has been reported to be present in *C. raciborskii* [146], *R. curvata* [147], *Aphanizomenon* strain 10E6 [148] and *Oscillatoria* PCC6506 [149]. The *cyr* biosynthesis gene cluster spans 43 kb and has 15 open reading frames which encode all the functions required for the biosynthesis, regulation, and export of the toxin. At both ends of the gene cluster are a further 35 kb that contain putative hyp accessory genes, which include molecular chaperones involved in the maturation of hydrogenases (145). Like other cyanotoxins, CYN is synthesized by a complex series of NRPS, PKs and mixed NRPS/PKs systems [145, 150]. Each module is made up of a set of three domains, two of which are catalytic and one which acts as a carrier, that together are responsible for the central chain-building reactions of polyketide or polypeptide biosynthesis [151]. The biosynthesis of CYN is initiated by an amidinotransferase and completed by NRPS-PKS-type enzymes in combination with tailoring enzymes [152]. Shalven – Alon et al (2002) reported in *A. ovalisporum* CYN biosynthesis involve three putative genes; aoaA, aoaB and aoaC that encode an amidino transferase, a hybrid NRPS/PKS and a PKS, respectively [153]. CYN extinction coefficient is 9800 and specific rotation is $+17.0^0$ [154].

A review by Kinnear [155] integrates the most current information on CYN accumulation, including notes on the global distribution of CYN producers, and a précis of CYN's ecological and human effects. Studies on the bioaccumulation of CYN are systematically reviewed, together with an analysis of patterns of accumulation, notes on detection, monitoring

and risk assessments, and key gaps in the existing research. Bioaccumulation of CYN can occur even with exposure to trace quantities of the toxin, suggesting that caution must be exercised, particularly when developing risk assessment guidelines for aquatic ecosystem health.

Exposure routes

Some species of cyanobacteria produce toxins that affect animals and humans. The most frequent and serious health effects are caused by drinking water containing the toxins (cyanobacteria), by ingestion during recreational water contact and inhalation of aerosolized particles. Other sources include algal food supplements. The World Health Organization (WHO) has provided guideline levels of 1 and 20 μg/L of MC in drinking and recreational water, respectively, in order to protect public health [156]. According to Zhang et al [157] the WHO guidelines for recreational exposure to cyanobacteria uses a three-tier approach based on cyanobacterial density and chl-a level and for protection of health, due to the irritative or allergenic effects of cyanobacterial toxins, a guideline level of 20,000 cyanobacterial cells/ml (corresponding to 10 mg chl-a/L under conditions of cyanobacterial dominance) has been derived. In 2006 in Lyon, France, the International Agency for Research on Cancer (IARC), performed an assessment of the carcinogenesis of MC-LR and concluded that MC-LR is a possible carcinogen for humans, classifying it as a group 2B carcinogen [158]. However due to the quality of the available epidemiological data, the IARC (2006) concluded that it was not possible to associate the excess risk of hepatocellular carcinoma and of colorectal cancer specifically with exposure to cyanotoxin, MC.

Cyanotoxin exposure routes for humans

Contamination of natural waters by toxic cyanobacteria is a growing problem worldwide, resulting in serious health hazards to all living being. The major routes of human exposure to cyanotoxins are: chronic and accidental ingestion of contaminated drinking water; inhalation or contact with the nasal mucous membrane, and dermal contact with toxins during recreational activities such as swimming, canoeing or bathing; consumption of contaminated vegetables and fruits irrigated with water containing cyanotoxins; consumption of aquatic organisms from contaminated waters; oral intake of cyanobacterial dietary supplements; and the specific intravenous route caused by dialysis. Humans are affected with a range of symptoms including skin irritation, eye irritation, rashes, and blisters around the mouth and nose, stomach cramps, vomiting, nausea, diarrhoea, fever, sore throat, headache, muscle and joint pain, blisters of the mouth, liver damage and the end result could be either acute, a life threatening chronic disease or even the death.

a.) Recreational activities

The most common exposures to cyanobacteria and their toxins are believed to occur during recreational activities via oral, dermal, and inhalation routes. Oral exposure may occur from accidental or deliberate ingestion of contaminated water. Dermal exposure may occur by direct contact of exposed parts of the body to water containing cyanobacteria cells. Inhalation may occur through the aspiration of water containing cyanobacteria cells and their toxins. Wind-driven currents may cause buoyant cyanobacterial blooms to amass on shorelines. These accumulations of cyanobacteria cells are orders of magnitude larger than blooms in open waters,

thus presenting a greater risk to human and animal health. Cyanobacteria cells can also accumulate in bathing suits, particularly diving suits. When the algal cells that have accumulated break, the wearer's skin is exposed to the toxins thereby resulting in the manifestation of exposure symptoms despite the absence of direct contact between the wearer's skin and the contaminated water.

Weirich et al [65] presented a case report of healthy teenage boys in Dane County, Wisconsin who were exposed to cyanotoxins in July, 2002 by swimming in a pond contaminated with cyanobacterial scum. All became ill with mild to severe symptoms of nausea and diarrhea. The most severe symptoms occurred in boys who had reportedly swallowed water. Approximately 48 h after exposure, one of the boys suffered a seizure and died of heart failure. Tests of stool and stomach contents were positive for the toxigenic species *Anabaena flos-aquae* and for anatoxin-a. The toxicology of the most common HAB toxins is well known and most algal toxins are acutely effective at very low doses (e.g., parts per billion). Children and young adults are most likely to be affected by cyanotoxins, due to a combination of smaller size, developmental stage, and tendency to engage in risky behaviors [65].

b.) Ingestion of cyanotoxin-contaminated drinking water

Groundwater is heavily used all over the world to provide primary sources of domestic drinking water supplies, and contaminated groundwater enhances risk to public health. Cyano toxins have been detected in municipal drinking water in both industrialized and economically emerging countries including Argentina, Australia, Bangladesh, Canada, Czech Republic, China, Finland, France, Germany, Latvia, Poland, Thailand, Turkey, Spain, Switzerland, USA [159]. However,

regular testing of finished drinking water for algal toxins is generally not practiced in most of these countries including Sri Lanka. Even though cyanobacterial blooms have become a serious problem for water resources in the United States, no federal regulatory guidelines for cyanobacteria or their toxins in drinking or recreational waters exist to date [157]. In the absence of federal criteria for cyanotoxins in recreational water, the Oregon Health Authority (OHA) developed guideline values for the four most common cyanotoxins in Oregon's fresh waters (anatoxin-a, CYN, MCs, and saxitoxins). OHA developed three guideline values for each of the cyanotoxins found in Oregon. Each of the guideline values is for a specific use of cyanobacteria-affected water: drinking water, human recreational exposure and dog recreational exposure. Oregon's cyanotoxin guideline values summarized by Farrer et al intended to be applied to acute or short-term exposures. Additionally the review article had made a detailed comparison of the developed OHA Guideline Values with other nations and states [160].

Kinnear [155] integrates the most current information on CYN accumulation, including notes on the global distribution of CYN producers, and a précis of CYN's ecological and human effects. Studies on the bioaccumulation of CYN are systematically reviewed, together with an analysis of patterns of accumulation. As described, the emerging research indicates that bioaccumulation of CYN can occur even at trace quantities of exposure to the toxin: suggesting that caution must be exercised, particularly when developing risk assessment guidelines for aquatic ecosystem health. Ferrão-Filho et al observed a high variability among the different bioaccumulation studies which made them inter-comparison difficult [161].

c.) Exposure through inhalation

Algal cells and waterborne toxins can be aerosolized by a bubble-bursting process via wind-driven, white-capped waves [157]. Aerosol samples, taken during recreational activities on bloom impacted lakes, have been found with detectable levels of MCs [157]. Although the levels of aerosolized toxin were generally low, laboratory investigations have found that treatment of mice by the intranasal route to MC-LR, the most toxic known variant of MC, was an effective method for toxin exposure [157].

d.) Dietary intake via consumption of cyanotoxins in contaminated foods and algal dietary supplements

The consumption of fish flesh (muscle) is usually considered safe but there are fish species and fish organs, especially the liver, and stomach/intestinal contents which may contain considerable amounts of cyanotoxins. Generally, hepatotoxic MCs and NODs are more common than the neurotoxins in aquatic animals including mussels, clams, crab larvae, prawns, crayfish and zooplankton, causing hepatotoxic effects in the fish and the accumulation of toxins in their organs. Consumption of contaminated shellfish and fish with cyanotoxins can lead to impacts on the liver and the nervous system. Ibelings and Chorus [162] outlined how humans get exposed to cyanobacterial toxins through consumption of food (e.g. fish, crayfish, prawns, mussels) harvested from water bodies that supported cyanobacterial blooms.

Microcystin is known to accumulate in fish and other aquatic biota, however the prevalence of MC in fish tissue and the human health risks posed by MC exposure through fish consumption remain poorly resolved. Poste et al show that MC is pervasive in water and fish from several tropical (Ugandan) and temperate (North American) lakes, including lakes that

support some of the largest freshwater fisheries in the world [163]. They found that fish consumption can be an important and sometimes dominant route of MC exposure for humans, and can cause consumers to exceed recommended total daily intake guidelines for MC. Detection of MCs in tissues has been well-documented [164], yet the species of the organism and high variability between organisms of the same species with regard to uptake of MCs complicate calculations for safety guidelines. Limited knowledge on the rate of metabolism and rate of formation of the conjugated MC-LR products is available and the potential human health risk of MC intoxication due to food web transfer of MCs also remains uncertain, although MCs and their metabolite products have been detected in some organisms of different trophic levels [164]. The human health impact of these metabolized products being excreted and released into the environment is also uncertain. As such risk assessments for specific settings should investigate the partitioning of exposure between drinking-water and food rather than presuming a general allocation of 80% of total cyanotoxin exposure to drinking water, which is presently the standard procedure. Consumption of vegetables, irrigated with contaminated waters, could also represent an additional source of human exposure to cyanotoxin**,** where certain vegetables can retain MCs present in irrigation water infested by blooms or scums [165].

Potential risks from exposure to the toxins in contaminated health food products that contain blue green algae (BGA) have been largely ignored [166]. Among the neurotoxins, anatoxin-a is one of the most common worldwide, being reported in countries such as Canada [167], USA [168], Scotland [169], Germany [170] as well as in Africa [171]. This dispersion stresses the need to evaluate its ecotoxicity and human intoxication risk. The possibility of accumulation of anatoxin in fish was tested with the common carp—Cyprinus carpio that

belongs to the largest and most successful family of primary freshwater fish in Eurasian waters as well as Northern American and African [172]. Results indicated that the bioaccumulation factor (BAF), the ratio of chemical concentrations in the organism to that in the water or food, was higher for the lowest toxin concentration because carp probably were exposed for a longer period (4 d) than at 107 cells ml^{-1} (24–27 h). The differences in the BAF between the two treatments, pointed out that cyanobacterial blooms of lower cell density may represent higher risk for toxin accumulation. Due to high instability of anatoxin-a in the water [173,174] and to the lack of human casualties caused by this toxin, it has been considered of less concern than other cyanotoxins. Nevertheless, this work draws the attention for anatoxin-a and its risks, considering the possibility of human exposures to this toxin through contaminated fish ingestion as well as the accumulation of anatoxin-a in aquatic ecosystems.

e.) Exposure from water used in medical treatments

The first documented lethality to humans from cyanobacterial hepatotoxins, occurred via the intravenous route, in a dialysis clinic in the city of Caruaru, Brazil, during 1996 [9]. An outbreak of acute liver failure occurred at the clinic and 116 (89%) of 131 patients experienced visual disturbances, nausea, and vomiting after routine hemodialysis treatment. Subsequently, 100 patients developed acute liver failure, and of these 76 died and 52 of the deaths were attributed to a common syndrome now known as Caruaru syndrome. Examination of phytoplankton from the dialysis clinic's water source, analyses of the clinic's water treatment system, plus serum and liver tissue of clinic patients led to the identification of two groups of cyanobacterial toxins, the hepatotoxic cyclic peptide MCs and the hepatotoxic alkaloid CYN. Comparison of victims' symptoms and pathology using animal studies of these two cyanotoxins lead to the conclusion

that the major contributing factor to death of the dialyses patients was intravenous exposure to MCs, specifically microcystin-YR, -LR, and -AR. From liver concentrations and exposure volumes, it was estimated that 19.5 μg/L MC was in the water used for dialysis treatments. This is 19.5 times the level set as a guideline for safe drinking water supplies by the WHO.

Human causalities due to cyanotoxin exposure

Cyanobacteria have been linked to illness in various regions throughout the world, including all five continents ranging from North and South America, Africa, Australia, Europe, Scandinavia and China. There are no reliable figures for the number of people affected worldwide. Cyanobacterial toxins in lakes, ponds, and dugouts in various parts of the world have long been known to cause poisoning and one of the earliest records was from China dating back more than 1000 years [9]. Miller and Trisdale [175] described a drought-related event in Charleston, SC in the USA that occurred in 1930 in which pollution of public drinking water supplies with the presence of algae was associated with a widespread epidemic of intestinal disorders among 8,000 to 10,000 individuals.

The documented and scientifically substantiated human deaths due to cyanobacterial toxins are limited and most listed article was the cyanotoxin exposure during dialysis which was discussed under the exposure from water used in medical treatments (e.g., medical dialysis). In many instances people exposed through drinking-water and recreational-water has required intensive hospital care and Hitzfeld et al [176] describes the human casualties by cyanotoxin due to participating in recreational activities.

FHAB-related illnesses associated with drinking water consumption have affected a larger number of individuals, and similar to recreational exposures, children are often affected [65]. First report was the well investigated case of over the past, where more than 100 children and 10 adults on Palm Island, northern Queensland, Australia, became ill with gastroenteritis after the water supply for the island had been treated with copper sulfate to control odor and taste issues. However, ecological studies had confirmed the presence of blooms of CYN producing *C. raciborskii* in the drinking water reservoir [134,135,177] which was the cause for the illness.

While outbreaks of acute FHAB-related illness due to contaminated drinking water are rare, long-term consumption of drinking water containing low levels of FHAB toxins, particularly MCs, has been linked to chronic diseases [178]. This is a concern for children since they are most sensitive to FHAB toxins and have a longer time of exposure to develop chronic diseases [65,179]. A study of 1322 children aged 7–15 years in the Three Gorges Reservoir Region, China, showed that children who received their drinking water from sources containing MC and other FHAB toxins had higher levels of liver enzymes in blood than children who receive drinking water with low or no FHAB toxins [180]. FHAB toxins are but one potential cause of liver problems. A high rate of primary liver cancer in certain areas of China and Eastern Europe has been linked to the combined effects of long-term (i.e., over a lifetime) consumption of MCs in drinking water [180], aflatoxin in stored grains [181], and high rates of hepatitis B infection [182].

Tumor initiation and growth in animal studies have formed the basis for epidemiologic investigations of the association of MC exposure with epidemic liver and colorectal cancers in China and countries in Eastern Europe [180-183]. Lun et al found a higher incidence of

colorectal cancer in citizens of Haining City of Zhejiang Province, China, who obtained their drinking water from river and pond compared to those obtaining drinking water from wells or tap water [183]. It was suggested that MCs could be involved because concentrations of MC were significantly higher in the pond and river. Similarly, in Serbia, Svircev et al [181] and Pantelic et al [184] detected upto 650 µg/L MC-LR in reservoirs that were used for drinking water and 2.5 µg/L in the tap water, well over the WHO-recommended MAC for drinking water. The authors also observed associations between high incidences of death due to primary liver cancer in regions where FHABs were intensified. In the United States, a study by Fleming et al [185] showed higher incidence of primary liver cancer in people within the service area of a municipal drinking water plant in Florida drawing from surface waters afflicted by FHABs, compared to people living nearby. However, it was not significant compared to random sampling of Florida residences or background levels of liver cancer in Florida.

Despite the potential health risks of cyanobacterial toxins, shown by animal studies, a limited number of epidemiological studies have been reported in humans. A statistically significant association between cyanobacterial blooms and non-alcoholic liver disease in the contiguous United States had been observed at the population level [157]. Remote sensing-based water monitoring provides a useful tool for assessing health hazards, but additional studies are needed to establish a specific association between cyanobacterial blooms and liver disease.

A recent detailed study showed that dialysis patients who had detectable MC-LR (median = 0.33µg/L) in their blood over an 8-week period expressed indicators of hepatic cellular injury including elevated serum concentrations of aspartate amino-transferase(AST), alanineamino transferase(ALT), and gamma-glutamyl transferase (GGT) [186]. Early and consistent increase

in GGT and alkaline phosphatase (ALP) in some patients was consistent with cholestasis and biliary dysfunction. A review by Paskerová et al [187] discusses about the intracellular detoxification enzymes and their suitability as biomarkers to determine the exposure to MCs and cyanobacteria by organisms.

Attempts have been made to determine what levels of exposure would be “safe” for MCs and other toxins [65]. A no-observed-adverse-effect-level (NOAEL) of 40 μg/kg body weight (b.w.) has been determined for MCs based on toxicological data from mice orally administered with MC-LR [210]. The NOAEL for MC-LR has been adopted for all MCs and NOD because (a) MC- LR is the most potent and most often detected MC worldwide, (b) has the most information available, and (c) there is a lack of sufficient toxicological data on NOD to establish a specific NOAEL.

Identifying short-term overt effects due to blue green algal (BGA) consumption is important. For example, one reported effect of low-level exposure to MCs is gastrointestinal (GI) disturbance [19, 188,189], and GI disturbance is apparently a fairly common experience of BGA consumers. MedWatch has received several reports of nausea, vomiting, and diarrhea associated with BGA consumption [190]. Although GI disturbance is acknowledged by the BGA industry as a potential consequence of BGA consumption, it was attributed to "detoxification" of the body.

Cyanobacteria have been linked to several types of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer’s disease, which occur through exposure to toxins produced by cyanobacteria [191]. Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. Both familial and spontaneous forms of ALS

have been described; multiple genetic traits have been associated with the former (192) while various hypotheses have been formulated for the latter (193). Notably, the β-N-methylamino-L-alanine (L-BMAA) toxin has been postulated to be involved in the occurrence of sporadic ALS. L-BMAA is also found to be associated with proteins in cyanobacteria [194, 195, 196] and in brain tissue of patients with ALS [194, 197, 198]. It has recently been proposed that L-BMAA may be mis- incorporated into proteins and thus may lead to protein aggregation, a hallmark of neurodegenerative diseases [199, 200] inducing a chronic exposure to low levels of L-BMAA [199]. L-BMAA was found to be produced by a wide range of cyanobacteria [194 - 197, 201-203]; recently, it was shown that diatoms, the most common group of algae, could also produce it [204]. However, the level of free or bound L-BMAA detected in cyanobacteria is controversial and the high concentrations reported in the first studies were challenged by several recent studies. L-BMAA could be transferred from cyanobacteria or diatoms via zooplankton to organisms at higher trophic levels [205]. Cox and collaborators have highlighted the bio magnification (increasing accumulation of bioactive, often deleterious, molecules through successively higher trophic levels of a food chain) of L-BMAA in trophic chain (195, 196, 206, 207), explaining the large amounts detected in flying foxes from Guam [195]. Owing to eutrophication and, to a lesser extent, climate changes [208, 209] cyanobacterial blooms seem to be increasing in freshwater ecosystems worldwide.

France is not exempt from this phenomenon as different genera of cyanobacteria are found on its territory [210, 213]. Therefore, exposure of French patients with ALS to cyanobacteria, and thereby to cyanotoxins as L-BMAA [214], is a reasonable hypothesis that could potentially explain some ALS cases. The review by Chiu et al [215] clearly demonstrates

the neurotoxicity of BMAA and its ability to affect neural tissues. Additionally BMAA is also being identified as a potentially significant compound in the etiology of neurodegenerative disease.

Drinking contaminated water can cause both acute and chronic illness based on level of contamination in water as well as the duration of exposure to particular toxin. Of much higher concern are low-level chronic exposures. Bored and dug wells can be difficult to disinfect because the shallow depth and inadequate protection can allow contaminants to enter the well. Many people in the United States receive their water from private ground water wells and regulations that protect public drinking water systems do not apply to privately owned wells. As a result, owners of private wells are responsible for ensuring that their water is safe from contaminants.

A recent study from Sri Lanka highlights the situation in a developing country. Among drinking water sources, well waters are the most common in rural areas in Sri Lanka. Chronic kidney disease (CKD), also known as chronic renal failure, is characterized by progressive destruction of renal mass with irreversible sclerosis and loss of nephrons over a period of months to years, depending on the underlying etiology [216]. Diabetic nephropathy, hypertension, and reno-vascular diseases are the most common causes of CKD in developed countries whereas it is glomerular diseases in tropical countries [241- 221]. In the majority of patients with CKD, the underlying cause remains unknown and is therefore termed as CKDu. The quality of the drinking water in relation to chronic kidney disease of uncertain etiology (CKDu) is of increasing interest to both the scientific community and to the general public since wells are the main sources of potable water in the affected regions and both chemical and biological contaminants are of

common concern. The disease is also an emerging major health problem in Sri Lanka. Many researches were undertaken to identify the prevalence and etiology of the CKDu in Sri Lanka such as long term exposure to cadmium [222, 223] pesticides [224] and possible naturally occurring fungal toxin with carcinogenic and nephrotoxic properties in consumer foods; Ochratoxin A [225], aluminium, fluorine [226] heavy metals; iron and lead [227] and arsenic [228]. However, these findings were later challenged by other studies and the cause still remains unresolved. Therefore, it was important to assess the water quality of Sri Lankan wells with regards to both biological and chemical contaminants. Among the potential biological contaminants that might be present in well water, cyanotoxins have a high potential to cause adverse health effects in exposed humans.

Liyange et al (229) performed a study to evaluate water samples obtained from wells used by CKD and CKDu patients living in the Girandurukotte area, which is a high CKDu endemic area that belongs to the dry zone of Sri Lanka. The samples were tested for the presence of toxic species of cyanobacteria and for the MC, CYN, and NOD cyanotoxins for water quality and possible human intoxications with respect to morphology and molecular methods in comparison to water samples collected from other parts of Sri Lanka as controls. In addition to water quality testing a questionnaire analysis was carried out with 330 subjects (CKD n=33, CKDu n= 244, and healthy individuals n=53). Significant correlations with CKDu ($p<0.05$) were found for eleven factors (Table 2).

Among them, well water source for drinking was ($p=0.00$) notable. Potential MC- and CYN-producing cyanobacteria were more prevalent in samples obtained from wells used by CKDu patients than from wells used by healthy controls or from CKD patients (Table 3). Out of

74 CKDu well water samples, 10 samples did not have any cyanobacterial growth. Among 64 samples, species belonging to 13 genera were found as MC producers and three genera as CYN producers. Out of 11 well water samples from CKD patients, seven genera were found as MC producers in most of the samples while in two samples *Anabaena* and *Lyngbya* were found as CYN producers. Compared to Girandurukotte patients' well water samples, cyanobacterial diversity was found to be less in healthy individuals' well waters which were collected from other parts of Sri Lanka. Overall, out of 25 well water samples, one sample did not have any cyanobacterial growth. Among 24 samples, species belonging to seven genera were MC producers while *Lyngbya* species were found as the only CYN producer in one sample. Among potential toxin producers, presence of *Phormidium* spp in CKDu patients' well waters were found to be significant (P=0.004) compared to other two populations. The presence of toxigenic cyanobacteria in these water samples was confirmed for MC, CYN, and NOD producing organisms by molecular analyses for the *myc*E gene, CYN-specific NRPS and PK genes, and the *nda* NOD synthetase gene, respectively. Among these, presence of CYN producers (p= 0.049) and NOD producing *Nodularia* species (p= 0.0029) were found to be significant (Table 4). The results of this study indicate that the presence of cyanobacteria with toxin generating ability in well water sources increases the risk for chronic renal disease for people living in the dry zone of Sri Lanka [229].

Perspectives

- Governing bodies of all countries should realize that it is their duty to provide clean drinking water to people as it is a basic need of a human whether the person is rich or

poor. As such they should implement and practice the policies identified by the WHO on this regard.

- Water treatment plants not only have to reduce cyanobacterial cells, odour and colour during the water treatment process but also toxins produced by cyanobacteria
- Monitoring of freshwater bodies used for recreational activities
- Controlling the cyanobacterial blooms, to minimize or preventing them to accumulate in aquatic organisms.
- Educating the public, about the risks of drinking, bathing or water sports in water likely to contain high densities of cyanobacteria and the contribution they can make to minimize the problem (cyanobacteria are a normal and natural component of the phytoplankton and although the occurrence of blooms has been referred to for many centuries, it is well established that the intensity of blooms has increased as a consequence of worldwide anthropogenic eutrophication
- Modelling approaches can be of great assistance in meeting the challenges of identifying and predicting the impacts of HABs and pathogens on human health (the ability to predict concentrations and toxicity/pathogenicity, identify the appropriate space and time scales over which to measure and model, and integrate model predictions with assessment of human health risk and management strategies).
- Educating the consumers on dietary supplements and other alternative health care products (most assume that these products could not be sold without the absolute assurance of safety. But the dietary supplement industry is largely self-regulated, and assuming that these products are entirely safe may not, in fact, be a safe assumption).

- Research on epidemiology to confirm the relationships between cyanotoxins and chronic disease in humans

In conclusion though much research has been carried out on the toxicology of cyanotoxins on humans the data regarding long term effects on humans is scant. Therefore, more epidemiological research is needed to prove this hypothesis.

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Table 1: Cyanotoxins, principal target organ systems and their producers

Toxin or toxin group	Classification by principal target organ systems	Toxin-producing genera
Microcystins	Hepatotoxins	*Anabaena*, *Anabaenopsis*, *Aphanocapsa*, *Arthrospira*, *Hapalosiphon*, *Microcystis*, *Nostoc*, *Oscillatoria*, *Planktothrix*, *Snowella*, *Woronichinia*, *Phormidium*, *Radiocystis*, *Aphanizomenon*
Nodularins	Hepatotoxins	*Nodularia*
Anatoxin-a, homoanatoxin-a	Neurotoxins	*Anabaena*, *Aphanizomenon*, *Arthrospira*, *Cylindrospermum*, *Microcystis*, *Oscillatoria*, *Phormidium*, *Planktothrix*, *Raphidiopsis*
Anatoxin-a(s)	Neurotoxin	*Anabaena*
Saxitoxins	Neurotoxins	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix*
Cylindrospermopsin	General cytotoxin	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Raphidiopsis*, *Umezakia*
Aplysiatoxin, debromoaplysiatoxin	Dermatotoxins	*Lyngbya*, *Schizothrix*, *Planktothrix*
Lyngbyatoxin A	Possible gastro-intestinal inflammatory toxin	*Lyngbya*

Adapted from; Chorus and Bartram, 1999; Falcorner, 1999; Chorus et al. 2000; Stewart et al., 2006.

Table 2. Exposure to possible risk factors and the significance to the disease

No	Variable	Analysis type	P -- Value
			Control+ CKD/CKDu
1	Gender	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
		Fisher's Exact Test	0.000
2	Age	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
3	Duration of stay at the same area for more than 10 years	Pearson Chi-Square	0.092
		Likelihood Ratio	0.100
		Fisher's Exact Test	0.261
4	Level of education	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
5	Occupation -- Being a paddy farmer for more than 20 years	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
		Fisher's Exact Test	0.000
6	Drinking habitual	Pearson Chi-Square	0.006
		Likelihood Ratio	0.006
		Fisher's Exact Test	0.008
7	Smoking habitual	Pearson Chi-Square	0.001
		Likelihood Ratio	0.001
		Fisher's Exact Test	0.002
8	Family members involved in paddy farming	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
		Fisher's Exact Test	0.000
9	Any snake bite incident	Pearson Chi-Square	0.132
		Likelihood Ratio	0.179
		Fisher's Exact Test	0.146
10	Drinking water source	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
11	Water source for other activities -- Recreation / washing	Pearson Chi-Square	0.000
		Likelihood Ratio	0.000
12	Method of storage of drinking water/ cooking	Pearson Chi-Square	0.017
		Likelihood Ratio	0.013
13	Any other chronic diseases	Pearson Chi-Square	0.130
		Likelihood Ratio	0.139
		Fisher's Exact Test	0.142
14	Family history of kidney disease	Pearson Chi-Square	0.011
		Likelihood Ratio	0.009

		Fisher's Exact Test	0.011

Table 3. Morphologically identified cyanobacterium/ cyanobacteria in well waters collected from CKDu, CKD and healthy population

Serial Number	Population	Cyanobacterium/cyanobacteria in environmental sample	Cyanobacterium/cyanobacteria in cultured sample	Cyanobacterium/cyanobacteria at order level
1	CKDu	*Chroococcus*, **Aphanocapsa*, *Gloeocapsa*, *Aphanothece*, *Gloeothece*, *Synechococcus*	*Chroococcus*, **Aphanocapsa*, *Gloeocapsa*, *Aphanothece*, *Gloeothece*, *Dactylococcopsis*, **Microcystis*	Chroococcales
		**Chroococcidiopsis*, *Dermocarpa*	**Chroococcidiopsis*, *Dermocarpella*, *Dermocarpa*	Pleurocapsales
		† Phormidium*, **Limnothrix*, **†Arthrospira*, **Calothrix*, **†Oscillatoria*, *‡•×Lyngbya*, *Leptolyngbya*,	**†Phormidium*, **†Arthrospira*, ***‡•×Lyngbya*, **†Oscillatoria*, **Limnothrix*, *Trichodesmium*, *Pseudanabaena*, **†‡•Planktothrix*	Oscillatoriales
		Scytonema, **Nostoc*	**†♦**‡Anabaena*, *Scytonema*, **Anabaenopsis*, ***†Raphidiopsis*, *♦♦Nodularia*, *Plectonema*, *Tolypothrix*, *Coleodesmium*, **Nostoc*	Nostocales
		Mastigocladus,**Westiellopsis*, **Hapalosiphon*	*Stigonema*, *Mastigocladus*, *Westiellopsis*, **Hapalosiphon*, *Chlorogloeopsis*	Stigonematales
2	CKD	*Gloeocapsa*	**Aphanocapsa*, *Gloeocapsa*, *Gloeothece*, *Chroococcus*,*Cyanothece*, **Microcystis*,	Chroococcales

			Coelosphaerium	
		**Chroococcidiopsis*	**Chroococcidiopsis*, *Dermocarpa*	Pleurocapsales
		Leptolyngbya, **†Phormidium*	**†Phormidium*, ***‡•×Lyngbya*, *Trichodesmium*	Oscillatoriales
		**Nostoc*, *Scytonema*	**†♦**‡Anabaena*	Nostocales
		**Hapalosiphon*, **Westiellopsis*	**Hapalosiphon*, *Mastigocladus*	Stigonematales
3	Healthy individuals	*Chroococcus*	*Chroococcus*, *Gloeothece*, *Gloeocapsa*, *Aphanothece*, *Dactylococcopsis*, *Cyanothece*, *Synechococcus*	Chroococcales
		**Chroococcidiopsis*,	**Chroococcidiopsis*, *Dermocarpa*	Pleurocapsales
		Leptolyngbya, **†Arthrospira*	**†Arthrospira*	Oscillatoriales
		**Calothrix*	**Calothrix*, *Coleodesmium*	Nostocales
		**Hapalosiphon*, *Chlorogloeopsis*	**Hapalosiphon*	Stigonematales

* Potential MC producers

** Potential CYN produces

† Potential Anatoxin-a, homoanatoxin-a producers

‡ Potential Saxitoxins producers

• Potential Aplysiatoxin, debromoaplysiatoxin producers

♦Potential Anatoxin-a(s) producers

♦♦ Potential Nodularin producer

× Potential Lyngbyatoxin A producers

Table 4. Exposure to possible cyanotoxin and presence CKDu non CKDu

Variable	No. of subjects with				Odd ratio (95% CI)	P value
	CKDu		Non CKDu			
Exposure to CYN	Y	12	Y	1	7.5789 (0.9252 to 62.0811)	p = 0.0491
	N	38	N	24		
Exposure to MC	Y	3	Y	1	1.5319 (0.1511 to 15.5263)	P = 1.0000
	N	47	N	24		
Exposure to NOD	Y	26	Y	04	5.6875 1.7051 to 18.9710	P = 0.0029
	N	24	N	21		

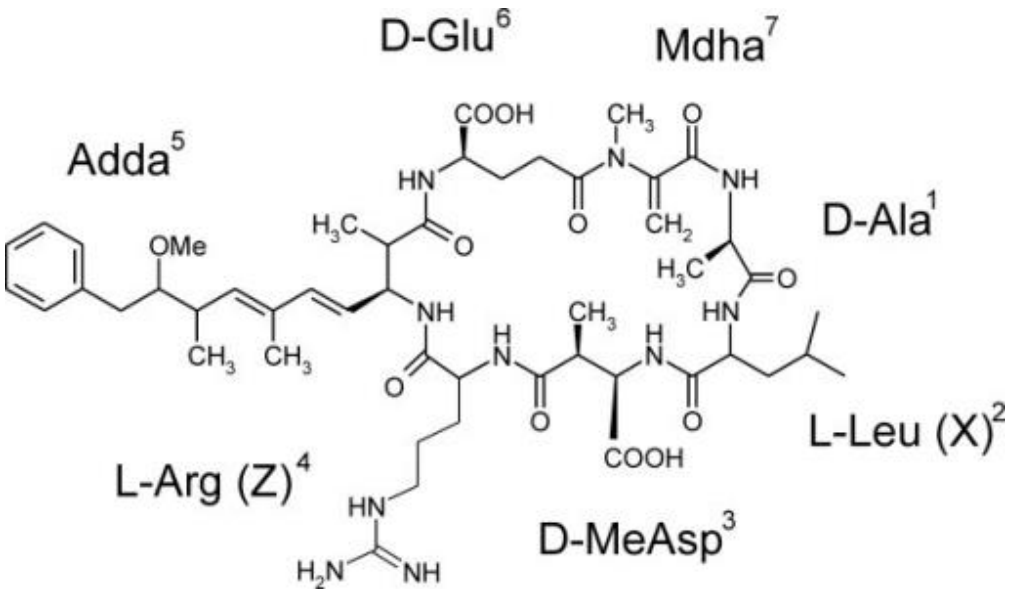


Figure 1. The chemical structure of microcystin LR cyclo-D-Ala1-X2-D-MeAsp3-Z4-Adda5_D-Glu6-Mdha7

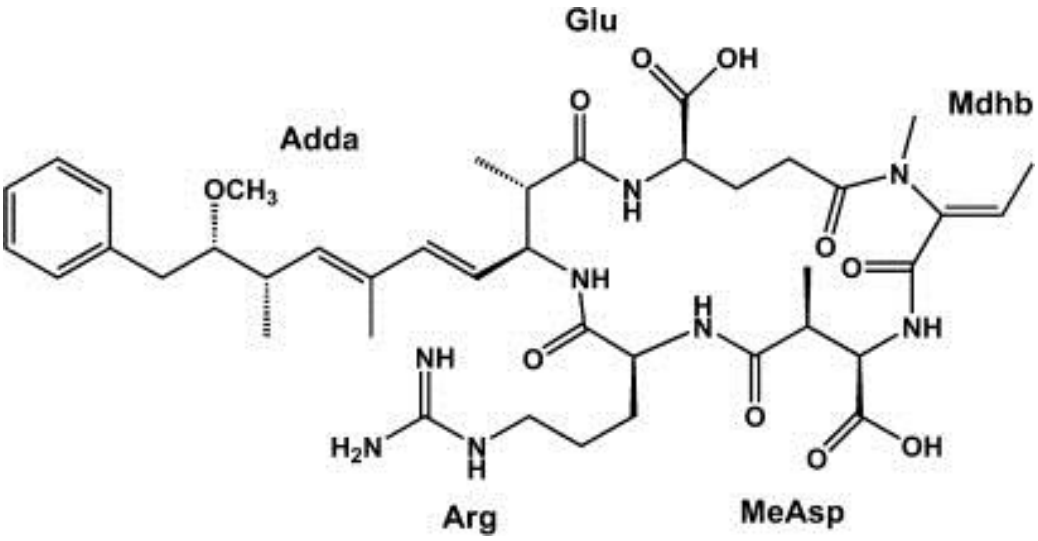
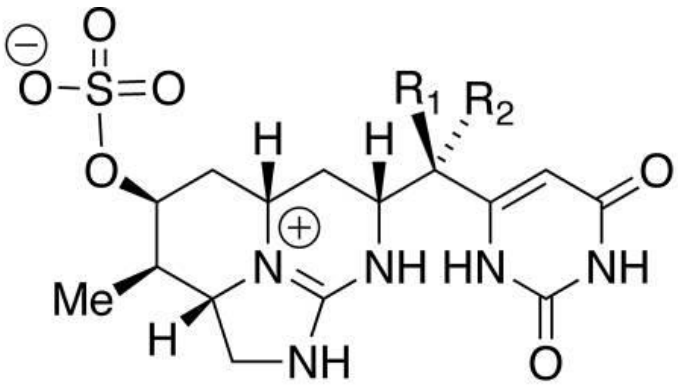


Figure 2. The chemical structure of nodularin: cyclo-(D -MeAsp 1 - L -arginine 2 -Adda 3 - D -glutamate 4 -Mdhb 5)



Compound name	R_1	R_2	Mw
Cylindrospermopsin (7-*R*)	OH	H	415.42
7-Epi-cylindrospermopsin (7-*S*)	H	OH	415.42
7-Deoxy-cylindrospermopsin	H	H	399.42

Figure 3. Chemical Structures of the cylindrospermopsin (CYN) and of its natural analogs