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The current scenario and future aspects of Cyanotoxins: A Review Study

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Abstract

Water bodies that are laden with rich nutrient sources are considered to be a medium for the profuse growth of algae and blooms. They induce the production of harmful metabolites called cyanotoxins that are highly toxic to animals and human beings. Cyanobacteria are light-dependent living organisms that are responsible for producing the highest level of oxygen on earth. Cyanotoxins are considered to be the natural pollutants produced by some cyanobacterial species that occur worldwide. In terms of their role in the ecosystem, biosynthesis, and application, a proper understanding of research and development is needed. This review article summarizes a basic overview of cyanobacterial toxins, entanglement routes, and holistic approaches to mitigate cyanotoxins for public health and a healthy environment. It is expected that the present review will give a useful and novel reference in terms of sustainable approaches for future pilot-based applications to deal with cyanotoxin poisoning.

1. Introduction

The algal blooms produced by cyanobacterial species also called blue-green algae severely disrupt the functioning of freshwater and marine surroundings. It also devastates terrestrial lives too because of eutrophication and climatic alteration. Cyanobacteria are oxygenic photoautotrophic organisms that are determined clearly on lakes, streams, ponds, and different aquatic bodies [1]. A suitable environment allows them to swiftly multiply and inflict blooms formation. Factors that are responsible for their growth are the intensity of light, total sunlight period, nutrient availability (in particular phosphorus and nitrogen sources), pH, water column stability, and growth in precipitation events [2]. Cyanobacteria produce special metabolites that help for their survival and maintain living conditions to overcome external stresses. These are basically low molecular weight metabolites of different chemical groups such as carbohydrates, polyketides, peptides, terpenes, alkaloids, and phenolics having specific bioactive properties. Many of them are toxic in nature and can invade the normal physiological activities of animals and human beings [3]. Cyanotoxins are basically secondary metabolites secreted by some specific groups of blue-green algae, also considered gram-negative photosynthetic bacteria that are highly niche-specific

in the aquatic and terrestrial environment and can be fatal to human beings [4]. Some speculation by researchers is that elevated water temperature favors the growth of bloom-forming cyanobacterial strains, such as Microcystis sp. [5]. Few species of Anabaena, Nostoc, Oscillatoria, Microcystis, Planktothrix, and Synechococcus also produce toxic secondary metabolites called cyanotoxins. The blooms of blue-green algae (BGA) are highly oxygen demanded which creates an anoxic situation, thus affecting the organisms of the aquatic environment [6]. There are four classes of cyanotoxins i.e. microcystins, cyclindrospermopsin, anatoxin-a, and saxitoxins. Nutrients that are frequently associated with cultural eutrophication are nitrogen and phosphorous (N &P) in freshwater have been identified as key drivers of toxin production. However, cyanobacteria in the selected hypereutrophic zone do not produce cyanotoxins. Sulfur and iron have also been associated with the occurrence of toxins [7].

2. Types of Cyanotoxins

2.1 Microcystins (MCs)

The most investigated group of cyanobacterial toxins are microcystins due to their widespread occurrence in freshwater, estuaries, and coastal environment all over the world. MCs are produced by some species of cyanobacteria and the genera include (Microcystis, Anabaena, Nostoc, Limnothrix, Phormidium, etc) [8]. MCs are divided into three sub classes and the highly reported variant is microcystin-LR, which is regarded as the most toxic form of MCs as shown in Fig 1A [9]. The shape of MCs is cyclic peptides that incorporate two varying amino acids and aromatic 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA) which makes their molecular loads around 1,000 Da [10]. *Microcystis aeruginosa* is the first cyanobacterial species that has been reported to produce microcystin-LR. Till now, there are more than 80 known variants of cyanobacterial species reported that synthesize MCs during bloom formation. With several hypotheses proposed, including their mode of nutrient metabolism, colony formation, iron acquisition, allelopathic interaction, and cell death, their role in the environment is currently under discussion [9]. They are hepatotoxic and their exertion involves the hindrance of 1 and 2A hepatocytes which eventually leads to liver damage. It also acts as a potential tumor promoter [11].

2.2 Nodularins (NODs)

It is a cyclic pentapeptide, and its structure is quite similar to MCs. Fig 1B [12], is showing composition and the structure of NODs. The structure of NODs consists of Larginine, N-methyldehydrobutyrine, D-glutamic acid, and D-erythro-β-methylaspartic acid [12]. Among 10 analogs, NOD-R is the most commonly found cyanotoxin. It is considered a potent cyanotoxin that is responsible for the death of animals and human beings, however, due to a lack of data, it is not considered a carcinogen [13]. NODs were first isolated from cyanobacteria called Nodularia and were widely found in coastal and freshwater [4].The newly identified NODs were isolated from cyanobacteria *Iningainema pulvinus* (Scytonemataceae) in Australia. Because of their similar structure to MCs, it is expected to have similar toxicity mechanisms so their identification methods also relay the same [14].

2.3 Cylindrospermopsin (CYN)

Cylindrospermopsin (CYN) belongs to the alkaloid family. It is a tricyclic guanidine moiety combined with hydroxymethyl uracil having a molecular weight of 415 Da as shown in Fig 2A. The toxin CYN has two forms called: 7-epi-CYN and 7- deoxy-CYN [15]. It was first discovered in 1992 in blue-green algae called Raphidiopsis raciborskii. However, CYN was later confirmed to be produced

by Nostocales and several other freshwater filamentous cyanobacteria belonging to the order Osscillatorials, Aphanizomenon, Lyngbya, Rhadiopsis, Planktothrix, etc… [15].Under certain pH, condition cylindrospermopsin acts as a zwitterion that makes it polar [16].The biological function of CYN is a question of concern, with several studies. This suggests that other photoautotrophic microalgae participate in the addition of inorganic phosphates. Since it is a relatively stable compound under various environmental factors such as ultraviolet and visible light, flexible pH, and temperature, CYN degradation can be inhibited under anaerobic conditions [17].

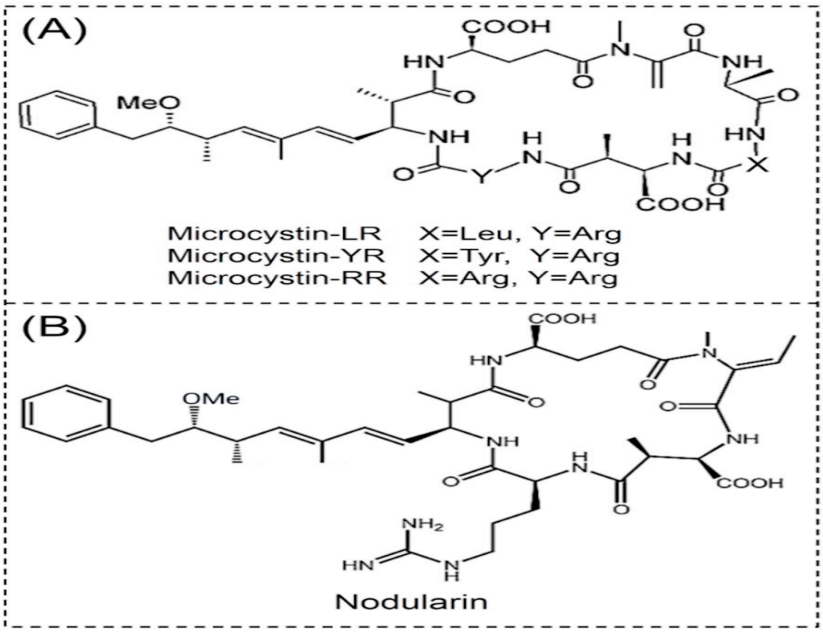

Figure 1. (A) Microcystin-LR, YR and RR, (B) Nodularin.

2.4. Saxitoxin

It is also referred to as paralytic shellfish toxin (PST) and is neurotoxic in nature that commonly associated with red tides due to marine dinoflagellates like (Gymnodinium sp., Pyrodinium sp., Alexandrium sp). Several genera of freshwater cyanobacteria like Anabaena, Lyngbya, and Aphanizomenon form this toxin [17]. Saxitoxins are a group of unsulfonated, monosulfonated, or disulfonated carbamate alkaloid neurotoxins and are commonly referred to as saxitoxins, goniototoxins, and C-toxins, respectively. It consists of a 3, 4-perhydropurine tricyclic system with two guanidine groups as shown in Fig 2B [18]. Being a neurotoxin, it acts as an open, reversible, voltage-gated sodium channel blocker and prevents the flow of sodium ions through the membrane thus causing the nervous shutdown. Because of their presence in bivalve shellfishes like oysters and scallops, commercial and recreational shellfish harvesting has been banned in countries (Western Europe, East Asia, Australia, the US, and South Africa) [19].

2.5 Anatoxin-a

It is a potent commonly occurring cyanotoxin that is neurotoxic in nature. With a 165 Da molecular mass, it is considered to be the smallest cyanotoxin with the structure of bicyclic secondary amine as shown in Fig 2C [20]. It is also referred to as Very Fast Death Factor (VFDF) secondary metabolites. Several genera of cyanobacteria produce this toxin including Anabaena, Planktothrix, and Aphanizomenon globally. Toxicity of anatoxin-a includes coordination loss, fasciculation of muscles, convulsions, and can be fatal due to respiratory paralysis. Its mode of action is via the nicotinic

acetylcholine receptor (nAchR), which mimics the binding of its natural ligand, acetylcholine. It poses a great risk to animals and humans due to its high toxicity and potential presence in drinking water [21]. It was tested in male mice using intraperitoneal injection, which leads to excessive salivation, cyanosis, dyspnea, fatigue, etc before death, which is highly neurotoxic in nature [22].

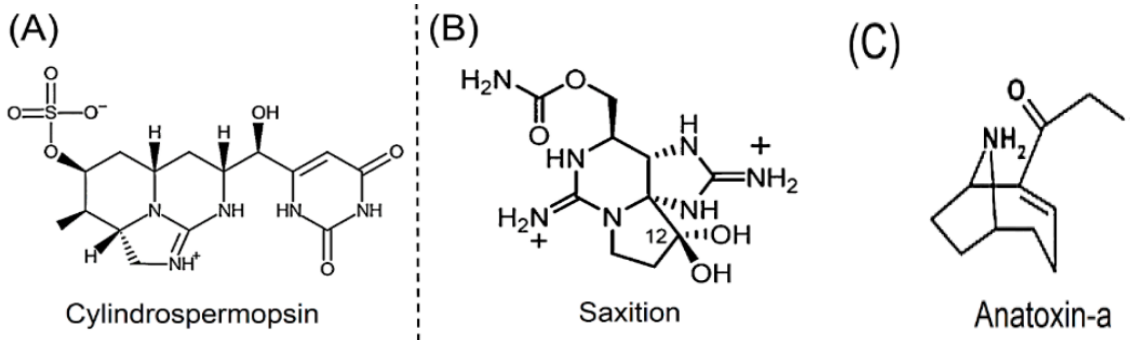

Figure 2. (A) Cylindrospermopsin, (B) Saxitoxin, (C) Anatoxin-a

3.Detection of cyanotoxins

A wide range of advanced methods is available for the detection and quantification of cyanotoxins. The methods include molecular assay, bioassay, biochemical analysis, and chemical analysis which target cyanotoxins in the water matrices and are carried out with multiple Environmental Protection Agency (EPA) standards.

3.1 Molecular Assays

Over two decades, the advancement in molecular assays based on polymerase chain reaction (PCR) has evolved hastily. Genes that are responsible for the production of cyanotoxin can be detectable by cyanobacterial genome sequencing, which makes it feasible for PCR amplification of the target gene [23]. The first approach is based on targeting a particular gene called the conventional PCR method which helps for detecting potential cyanobacterial toxins from the bloom. The second approach is qPCR which is based on quantitative analysis that targets a gene responsible for cyanotoxin biosynthesis. The third approach is the duo combination method of PCR & DNA microarray which can detect potentially toxic cyanobacterial species from the blooms and can detect the particular gene responsible for the biosynthesis of cyanotoxins. However, because of various technical and biological issues, detection of actual toxin concentration is inconsistent [24]. Using LC-MS/MS, the sample water was analyzed for detection and quantitative cyanotoxins, i.e., anatoxin, cylindrospermopsin, nodularin, and 12 microcystin variants. Cyanotoxins were detected from more than 50% of the water sample [25].

3.2 Bioassays using microbes, plants & animals

Detection of cyanobacterial toxins using biotic components such as microbes, plants, or animals plays an important role within a limited time in environmental samples. Assessment based on some bacterial species (*Aeromonas hydrophila, Bacillus subtilis*) found to be sensitive. The n-hexane extracts of *Cylindrospermum majus*, and *Limnothrix redekei* and methanol extracts of *Anabaena variabilis* and *Pseudanabaena catenata* inhibit the growth of *Bacillus subtilis* [25]. However further investigation is necessary for implementing bacterial strain as host. Recent trends for evaluating cyanotoxins are based on LD50 assays obtained from surviving infected animals such as Mice. However, due to ethical issues, high cost, and low sensitivity, animal trials are replacing by other alternative methods. Immortalized and primary cell lines extracted from animal tissues were used for cytotoxicity tests [26]. It has been

found that MCs can inhibit growth and chlorophyll content in *Solanum tuberosum* cultures. Appropriate information on toxic effects can be obtained by biological analysis, which is not possible with the aid of physicochemical analysis. Although the duo-combination method can clearly indicate the presence of a toxin, and its effects [27]. Using flesh and liver sample of fish (*Cyprinus caprio*),12 MCs variants were analyzed by LC-MS in an ex-situ environment. MCs were not detected; thus, it shows the absence of MCs [25]. *Dinophysis acuminate* is a harmful algal bloom species that is responsible for paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP) in human beings. However, an investigation of *D.acuminate* toxicity on larval oysters (*Crassostrea virginica*) was found severely toxic [28].

3.3 Biochemical assay

It is based on the method that relies on the interaction between biological macromolecules and cyanotoxins. Examples may include enzyme inhibition assays, immunoassays, and receptor bioassays.

3.3.1 Enzyme inhibition assay

Protein phosphatases Inhibitory Assay (PPIA) is one of the general enzyme inhibition assays used for the detection of cyanotoxins. This is highly conserved in eukaryotes and actively involved in many cellular activities [29]. By estimating the degree of inhibition of protein phosphatases it can detect cyanotoxins as well as their toxicity level. Because of its strong sensitivity level, it does not get affected by sample matrices. [30]. PPIA is widely used to analyze microcystins (MCs) concentration because it is comparatively less expensive and even faster than other assays [31].

3.3.2 Immuno assay

Is a biochemical test that measures the concentration of an analyte. The best standard method for cyanotoxin analysis is ELISA, fluorescence immunoassay (FIA), and immuno-chromatography assay (ICA). ELISA has been used to detect MCs, NODs, CYNs, ATX, and STX in samples [32]. Performing the analysis is very simple, does not require professionally qualified personnel, and is cost-effective. However, ELISA is difficult to identify specific toxin variants and may not always be as accurate as physicochemical test methods [33]. Fluorescent Immunoassays (FIA) is a biochemical technique used for detecting the binding of analytes (drugs, hormones, proteins, and other compounds). It has higher sensitivity detection and requires simplified reagents and a less difficult assay design. It is used for the detection & quantification of cyanotoxins. Fluorescence Polarization Immunoassay (FPIA) is a technique that is primarily based on FIA that specifically quantifies a target analyte. The fundamental precept of FPIA is based on the distinction in fluorescence polarization of the labeled analyte-antibody complex [34]. Immuno-chromatography Assay (ICA) additionally referred to as lateral waft test is a separation method of pattern analyte based on migration on solid matrix through the capillary float. Lateral go with the flow Enzyme Immunochromatography assay (LFICA) conjugated with molecular imprinting method is used for speedy detection of MC-LR in water [35].

3.4. Chemical Assays

This is one of the most advanced and specific techniques that can evaluate the quantitative and qualitative analysis of cyanobacterial toxins in environmental samples, based on different physicochemical properties. Techniques such as HPLC, GC, NMR, etc can be used [36]. MALDI-TOF is a highly sensitive, rapid, and selective technique that allows the detection of compounds based on a molecular formula that can also distinguish the toxicity level of cyanobacterial toxins [37].

4.Recent advancement for mitigation of cyanotoxins

There are several factors that influence the removal or inactivation of cyanotoxin. These toxins exist intracellularly in the cytoplasm of cyanobacteria. However, it can be released by cell lysis or excretion and becomes extracellular cyanotoxins. As already mentioned above the impact of cyanotoxins on human health and other creatures. The strategy to treat or remove cyanotoxin is a matter of concern, although all species of cyanobacteria do not produce cyanotoxins. Treatment of endotoxin means that intact cyanobacterial cells must be removed, whereas treatment of extracellular toxin requires special tactics to meet the requirements. [38].

4.1 Membrane filtration

The membrane filtration method is used for microbiological analysis of water using a special filter 'millipore filter" of 0.45 µm to trap the microorganisms for their isolation and enumeration in a test water sample. There are several membrane filtration methods (reverse osmosis, ultrafiltration, and nanofiltration) that separate the contaminants based on the size and physicochemical characteristics of the membrane. About 80% of microcystins (MCs) removal can be possible through nanofiltration and reverse osmosis [38]. Microcystins (LR, RR, YR, and LA) & anatoxin removal by a tricep membrane with a cutoff of molecular mass of 200 Da i.e at least 96% removals of the cyanotoxins. Nanofiltration can be a promising method for anatoxin-a removal by reducing the molecular weight by 150-700 Da [39].

4.2 Biochar

Being an economic and carbon-rich adsorbent, biochar has been widely used for removing different pollutants from wastewater and water. Biochar can be generated by pyrolysis of municipal waste, agricultural waste, and animal manure under (300-1000°C) [40]. Its surface area, porosity, and surface functional groups highly influenced the potential of biochar adsorption. Limited studies focused on cyanotoxin adsorption. However, MC-LR has widely been studied for biochar adsorption [41]. Biochar shows greater carbonation, and surface area due to π–π electron donor-acceptor interaction as temperature level increases. The size of MC-LR also plays an important role in adsorption via the pore filling effect and is highly pH-dependent. It exists as a singly dissociated anion (MC-LR-) [42]. Its application is not very common, however, its excellent capacity for adsorbing water pollutants such as heavy metals, nutrients, and pharmaceutical effluents makes it a good adsorbent.

4.3 Coconut Shell

It is a very effective method using coconut shell-based activated carbon for the removal of organic matter, dyes, and metals [43]. It has a size of (0.8-2 nm). Because of the pore size, it affects the SA (surface area) for the process of adsorption [44]. 280 g of MC-LR per mg carbon in Milli-Q water samples were seen to be adsorbed by wood-based powdered activated carbon (PAC). The next was coal-based PACs with adsorptions of 116, 75, and 70 g of MC-LR per mg of carbon followed by coconut shell-based PACs at 40 and 20 g of MC-LR per mg of carbon. Similar results were observed in a study where micropores were dominant for coconut shell-based ACs, thereby reducing the adsorption capacity of MC-LR [45]. Therefore, the carbon pore size, mostly mesopores, dominants MC-LR adsorption rather than micropores, and the raw material of the adsorbent determine the percentage of the availability of the mesopores.

4.4 Lignin

It is a complex organic polymer made up of phenylpropanoid units. It bounds covalently with polysaccharides within the cell wall of plants that act as a key structural material in the supportive tissues of plants. ACs based on lignin can be prepared from lignocellulosic materials such as wood, agricultural wastes, grasses, etc [46]. For the study of MC-LR adsorption different sources of lignocellulosic materials have been used (coconut shell endocarp, sugarcane bagasse, pinewood residues, macadamia nutshells, unripe coconut mesocarp) as AC fibers by carbonation, followed by steam activation at 900°C. The study report shows that pinewood and sugarcane bagasse-based ACFs produced larger mesopore volumes, 1.06 and 0.39 cm3/g, respectively. The following order unripe coconut mesocarp > pinewood > macadamia nutshell > coconut shell endocarp > sugarcane bagasse shows secondary micropore volumes. At 99.27, 98.73, and 62.31%, respectively, within 40 min equilibrium time it removed MC-LR from pinewood, sugarcane, and coconut shell-based ACFs [47].

Conclusions

The emerging threat of toxic cyanobacteria to the aquatic environment and public water supplies is increasing globally. Protection of source water is alone insufficient for protecting the aquatic environment. With changing climate and an excessive load of nutrients, triggers the formation of algal blooms. Cyanobacterial toxins are multifaceted and it is an urgent need to control their toxicity. Based on their chemical structure, cyanotoxins are classified into cyclic peptides, alkaloids, lipopeptides, lipoglycan, and nonprotein amino acids. Commonly found are microcystins, anatoxins-a, and saxitoxins in the world. Several advanced detection methods are available such as molecular assay, biochemical assay, bioassay, and chemical assay. However, there is no single and specific method for the detection of all types of cyanobacterial toxins. Depending on the path of their exposure, these pollutants may lead to liver failure, seizures, respiratory arrest, an increase of tumors resulting in cancer, etc which can be fatal to human beings. Some cases of death are also reported from dogs, birds, and livestock. This is very important for the treatment and removal of cyanotoxins from water. Adsorption was found to be the widely used purification strategy, that depends on the hydrophobic and electrostatic interactions between adsorbent surface and cyanotoxins. The factors (surface area, porosity, surface chemistry, pH, water quality, and type of toxin) also determine the fate of adsorption. The micropores and mesopores of activated carbon can promote greater adsorption of cyanotoxins than macropores. This review article may help the society to understand the public health entanglements due to harmful cyanobacteria and its toxins, its identification, and multiple approaches for removing cyanotoxins from water in a sustainable manner.

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Disclosure statement

Conflict of Interest: The author declares that there are no conflicts of interest.

References

[1] H.W. Paerl, J. Huisman, Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports* (2009) 1, 27-37.

[2] WW. Carmichael, Cyanobacteria secondary metabolites the cyanotoxins. *J. Appl. Bacteriol*. (1992) 72, 445-459.
[3] J. Demay, C. Bernard, A. Reinhardt and B. Marie, Natural products from cyanobacteria: Focus on feneficial activities. *Mar. Drugs* (2019),17, 320.
[4] J. Huisman, G.A. Codd, H.W Paerl, B.W Ibelings, J.M.H Verspagen, P.M Visser, Cyanobacterial blooms. *Nat. Rev. Microbiol*. (2018),16,471-483.
[5] M.K Agrawal, S.K Ghosh, D. Bagchi, J. Weckesser ,M. Erhard, S. Bagchi, Occurrence of microcystin containing toxic water blooms in Central India. *J. Microbiol. Biotechn* (2012), 16:212-218.
[6] S.N Bagchi, Cyanobacterial toxins. *J. Sci. and In. Res*. (1999), 55:715-727.
[7] D.J. Griffiths, M.L Saker, The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin. *Environ. Toxicology* (2003), 18:78-93.
[8]F.M Buratti, M. Manganelli, S. Vichi, M. Stefanelli, S. Scardala, E. Testai, E. Funari, Cyanotoxins: Producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Arch. Toxicol*. 91 (2017) 1049–1130.
[9] H.K Hudnell (2008) *Adv Exp Med Biol* 619:980.
[10] A. Catherine, C. Bernard, L. Spoof, M. Bruno, Microcystins and nodularins. *Handb. Cyanobacterial Monit. Cyanotoxin Anal*. (2017), 11, 107–126, doi:10.1002/9781119068761.ch11.
[11] International Agency for Research on Cancer (IARC). Ingested Nitrate and Nitrite and Cyanobacterial Peptide Toxins: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; *WHO Press: Geneva, Switzerland*, (2010), Volume 94.
[12] S. Vichi, M. Stefanelli, Cyanotoxins: Producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Arch. Toxicol*. (2017), 91, 1049–1130.
[13] I. Humans, IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. *IARC monographs on the evaluation of carcinogenic risks to human*s (2010), 94, v-vii, 1, doi: 10.1136/jcp.48.7.691-a.
[14] G.B McGregor, B.C Sendall, Iningainema pulvinus gen nov., sp nov. (*Cyanobacteria, Scytonemataceae*) a new nodularin producer from Edgbaston Reserve, north-eastern Australia. *Harmful Algae* (2017), 62, 10–19, doi:10.1016/j.hal.2016.11.021.
[15] Y. Yang, G. Yu, Y. Chen, N. Jia, R. Li, Four decades of progress in cylindrospermopsin research: The ins and outs of a potent cyanotoxin. *J. Hazard. Mater.* (2021), 406, 124653.
[16] L.A Pearson, B.A Neilan, The molecular genetics of cyanobacterial toxicity as a basis for monitoring water quality and public health risk. *Curr. Opin. Biotechnol*. (2008), 19, 281–288, doi:10.1016/j.copbio.2008.03.002.
[17] M.N Mons, H.P Van Egmond, G.J.A Speijers, Paralytic shellfish poisoning; a review. *RIVM Report* (1998), 388802,005.
[18] J.L Wong, R. Oesterlin, H. Rapoport, The structure of saxitoxin. *J. Am. Chem. Soc.* (1971), 93, 7344–7345.
[19] I. Chorus, Current Approaches to Cyanotoxin Risk Assessment, *Risk Management and Regulations in Different Countries*. (2012).
[20] J.P Devlin, O.E Edwards, Anatoxin-a, a toxic alkaloid from Anabaena flos-aquae NRC-44h. *Can. J. Chem*. (1977), 55, 1367–1371, doi:10.1139/v77-189.
[21] J.K Fawell, R.E Mitchell, D.J Everett, R.E Hill *Hum Exp Toxicol.* (1999), 18:162.

[22] M.F Fiore, S.T de Lima, W.W Carmichael, S.M.K McKinnie, J.R Chekan, B.S Moore, Guanitoxin, re-naming a cyanobacterial organophosphate toxin. *Harmful Algae* (2020), 92, 101737.

[23] J.F. Humbert, Molecular tools for the detection of toxigenic cyanobacteria in natural ecosystems. *Handb. Cyanobacterial Monit. Cyanotoxin Anal*. (2017), 28, 280–283, doi:10.1002/9781119068761.

[24] S. Singh, A. Srivastava, H.M. Oh, C.Y Ahn, G.G Choi, R.K. Asthana, Recent trends in development of biosensors for detection of microcystin. *Toxicon* (2012), 60, 878–894, doi:10.1016/j.toxicon.2012.06.005.

[25] N.A. Hammoud, S.K. Zervou, T. Kaloudis, C. Christophoridis, A. Paraskevopoulou, T.M. Triantis, K. Slim, J. Szpunar, A. Fadel, R. Lobinski, Investigation of the Occurrence of Cyanotoxins in Lake Karaoun (Lebanon) by Mass Spectrometry, Bioassays and Molecular Methods. *Toxins* (2021), 13, 716. https://doi.org/10.3390/ toxins13100716.

[26] A. Straser, M. Filipic, B. Zegura, Cylindrospermopsin induced transcriptional responses in human hepatoma HepG2 cells. *Toxicol. In Vitro* (2013), 27, 1809–1819, doi:10.1016/j.tiv.2013.05.012.

[27] J. McElhiney, L.A Lawton, C. Leifert, Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. *Toxicon* (2001), 39, 1411–1420, doi:10.1016/s0041- 0101(01)00100-3.

[28] SKD Pease M.L Brosnahan, M.P Sanderson J.L Smith, Effects of Two Toxin-Producing Harmful Algae, *Alexandrium catenella* and *Dinophysis acuminata* (Dinophyceae), on Activity and Mortality of Larval Shellfish. *Toxins (Basel).* (2022) May 10; 14(5):335. doi: 10.3390/toxins14050335.

[29] S.R Pereira, V.M. Vasconcelos, A. Antunes, The phosphoprotein phosphatase family of Ser/Thr phosphatases as principal targets of naturally occurring toxins. *Crit. Rev. Toxicol*. (2011), 41, 83–110, doi:10.3109/10408444.2010.515564.

[30] T. Heresztyn, B.C. Nicholson, Determination of cyanobacterial hepatotoxins directly in water using a protein phosphatase inhibition assay. *Water Res*. (2001), 35, 3049–3056.

[31] K.H Oh, K.M Beak, Y. Shin, Y.C. Cho, Assessing the microcystins concentration through optimized protein phosphatase inhibition assay in environmental samples. *J Microbiol*. (2022) Jun;60(6):602-609. doi: 10.1007/s12275-022-2020-4.

[32] I. Cunha, R. Biltes, M. Sales, V. Vasconcelos, Aptamer-based biosensors to detect aquatic phycotoxins and cyanotoxins. *Sensors* (2018), 18, 2367, doi:10.3390/s18072367.

[33] L.N Sangolkar, S.S Maske, T. Chakrabarti, Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria. *Water Res*. (2006), 40, 3485–3496, doi:10.1016/j.watres.2006.08.010.

[34] D.S Smith, S.A Eremin, Fluorescence polarization immunoassays and related methods for simple, high throughput screening of small molecules. *Anal. Bioanal. Chem*. (2008), 391, 1499–1507, doi:10.1007/s00216-008-1897-z.

[35] Z. Wu, D. He, B. Cui, Z. Jin, Ultrasensitive detection of microcystin-LR with gold immune chromatographic assay assisted by a molecular imprinting technique. *Food Chem*. (2019), 283, 517– 521, doi:10.1016/j.foodchem.2019.01.064.

[36] C. Moreira, V. Ramos, J. Azevedo, V. Vasconcelos, Methods to detect cyanobacteria and their toxins inthe environment. *Appl. Microbiol. Biotechnol.* (2014), 98, 8073–8082, doi:10.1007/s00253-014-5951-9.

[37] R. Nicolau, M. Leloup, D. Lachassagne, E. Pinault, G. Feuillade Cathalifaud, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) coupled to XAD fractionation: Method to algal organic matter characterization. *Talanta* (2015), 136, 102–107, doi:10.1016/j.talanta.2015.01.011.
[38] J.K. Fawell J. Hart H.A. James, W. Parr, *Water Supply* 11 (1993) 109–122
[39] A.J. Gijsbertsen Abrahamse, W. Schmidt, I. Chorus, S.G.J. Heijman, *J. Membr. Sci.* 276 (2006) 252–259
[40] M. Ahmad, A.U. Rajapaksha ,J.E. Lim, M. Zhang, N. Bolan, D. Mohan, M.Vithanage, Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* (2014), 99, 19-33.
[41] C.Peiris, S.R. Gunatilake, T.E. Mlsna, Biochar based removal of antibiotic sulfonamides and tetracyclines in aquatic environments: A critical review. *Bioresour. Technol*. 246 (2017) 150–159.
[42] G. Liu, Y. Qian, S. Dai, N. Feng, Adsorption of microcystin LR and LWon suspended particulate matter (SPM) at different pH. *Water Air Soil Pollut*. 192 (2008) 67–76.
[43] A. Demirbas, Agricultural based activated carbons for the removal of dyes from aqueous solutions: A review. *J. Hazard. Mater*. 167 (2009) 1–9.
[44] Y. Liu, Treatment of the Cyanotoxins Cylindrospermopsin, Microcystin-LR, and Anatoxin-a by Activated Carbon in Drinking Water; *University of Waterloo: Waterloo, ON, Canada*, (2017).
[45] J.A Park, S.M. Jung, J.W. Choi, J.H. Kim, S. Hong, S.H. Lee, Mesoporous carbon for efficient removal of microcystin-LR in drinking water sources, Nak-Dong River, South Korea: Application to a field-scale drinking water treatment plant. *Chemosphere* 193 (2018) 883-891.
[46] D.S.E, Chemical Modification of Lignocellulosic Materials, *1st ed.; Hon, (Ed.) Routledge*: *New York, NY, USA*, (1996); p. 384.
[47] E. Cavalcanti, D.A. Júnior, M. Orlando, A. Méndez, Removal of Cyanobacteria Toxins from DrinkingWater by Adsorption on Activated Carbon Fibers 2. *Experimental Procedures. Mater. Res.* 11 (2008) 371–3.

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