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Review

Cyanobacterial Blooms: Current Knowledge and New Perspectives

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Abstract: Cyanobacteria are ancient prokaryotes responsible for bloom formation in many freshwater resources worldwide. These dense agglomerations are a result of the rise of nutrient input (N and P) or temperature. The toxin content and illness associated with contact impair human health with repercussions in water quality. Produced by a wide variety of cyanobacteria species, CyanoBlooms are in need of a literature review to achieve a global scenario of its current impacts on freshwater resources aiming at changing behaviors towards CyanoBlooms globally and by making communities more resilient to this recurrent problem. With a global distribution, recent data highlight the impacts of climate change on CyanoBlooms occurrence, namely through the rise of temperature and nutrient input from storms and heavy rainfall. With current worldwide regulations based on the enumeration of the nutrient input of freshwater ecosystems, the increase in field monitoring regarding CyanoBlooms occurrence is demanded since evaluation of this parameter may conceal these massive agglomerations resulting in human health episodes and cyanotoxin outbreaks.

Keywords: cyanobacteria; cyanotoxins; analytical methods; molecular methods; artificial intelligence

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1. Introduction

Cyanobacteria are ancient prokaryotes responsible for oxygen production in the Earth's atmosphere. Their emergence dates back from 2.8 billion years ago [1]. Inhabitants of a wide range of ecosystems, from the Polar regions to other habitats such as cold and hot deserts, hot springs, and flood plain soils [2–5], cyanobacteria are microorganisms that possess a global occurrence. As primary producers of aquatic and terrestrial food chains, cyanobacteria currently have increasing biotechnological applications and as a source of new drugs for medical treatment [6]. In nature, the toxic and non-toxic forms of cyanobacteria can co-exist, but it is under a CyanoBloom that the amount of toxic forms increases, producing and releasing to the water column potent toxins designated as cyanotoxins. A CyanoBloom can be defined and characterized as an increase in cyanobacterial biomass in a relatively short period of time (few days to 2 weeks) and with the dominance (above 80%) of one or a few species of the phytoplankton community [7]. Factors such as eutrophication, anthropogenic pressure, or increase of temperature are associated with the proliferation of cyanobacteria and consequently of bloom formation. For these reasons, Cyanobacterial Blooms are in need of further insights due to their possible linkage to climate change. In addition, as toxin producers, CyanoBlooms, particularly in freshwater resources, are in need of constant vigilance and study since the changes in composition of blooms which may reflect toxin profile alterations have been reported, causing severe cyanotoxin outbreaks and associated human illness and animal deaths [8].

Freshwater systems have drinking, irrigation or recreational impacts and possess a diverse range of exposure routes to intoxications for humans and animals. Though climate change effects are more studied in the marine areas, the impacts of this phenomenon on freshwater ecosystems where CyanoBlooms may appear demand the need to intensify surveillance programs through analysis of bloom composition, occurrence, and toxicity. With statistical models, it is possible to predict future CyanoBlooms episodes by correlating the data (cyanobacterial biomass) with other environmental data [9]. In a CyanoBloom, toxins can be released to the water column where microcystins, cylindrospermopsins, anatoxins, and saxitoxins are of main concern [10]. Therefore, due to the underlined toxicity, nuisance, and linkage to climate change, a review is demanded of the current literature and knowledge to improve behaviors towards CyanoBlooms and make communities more resilient to this phenomenon fostering water quality and public health to tackle CyanoBlooms globally.

2. CyanoBlooms: Current Regulations

As ubiquitous microorganisms of freshwater resources, cyanobacteria tend to form blooms in the ecosystems, particularly after high levels of nutrients are found in the water or through the rise of temperature. Some strains find their ideal conditions for growth, compete, and proliferate intensively on the water surface causing visible masses of cyanobacteria that can be toxic and cause severe diseases (Figure 1). In both hemispheres, there have been reports on the occurrence of CyanoBlooms [11]. Climate alterations and increasing urbanization close to freshwater resources can exacerbate the occurrence of CyanoBlooms, leading to intoxication episodes due to unintentional contact of humans and domestic animals. In Portugal, recently, microcystins were found in the kidneys of death farmed cows after blooms were observed in a near water resource used for animal drinking [12]. Lack of knowledge and dissemination of the toxic properties of CyanoBlooms has led to intoxication episodes globally, constituting a problem in need of constant vigilance. Due to intensifications of CyanoBlooms, regulations on other cyanotoxins, apart from the common microcystins, were implemented in several countries. In fact, microcystins have currently an established guideline value of 1 μg/L in drinking water adopted by the World Health Organization (WHO) that implemented this provisional guideline [13]. In cylindrospermopsins, a guideline value for drinking water similar to microcystins has been proposed by Humpage and Falconer [14], despite the lack of recommendation by the WHO. However, on a global scale, three countries adopted the enumeration of cylindrospermopsins into their national legislation which include Australia (1 μg/L), New Zealand (1 μg/L), and Brazil (15 μg/L) [15]. Neurotoxins guideline values have also been adopted to the national legislation where New Zealand regulates both anatoxins (6 μg/L) and saxitoxins (3 μg/L), and Australia and Brazil regulate only saxitoxins (3 μg/L) [15]. These altogether reinforce the surveillance of freshwater systems through campaigns where screening of cyanotoxins can occur prior to bloom onset.

Figure 1. Photograph of a CyanoBloom (*Microcystis* sp.) in a river in the North of Portugal.

3. CyanoBlooms: Toxicity

Cyanotoxins are a chemically and biologically diverse group of secondary metabolites that are not produced by the primary metabolism of cyanobacteria. They are classified according to their mode of action in hepatotoxins (microcystins and nodularins), cytotoxins (cylindrospermopsin), neurotoxins (anatoxins and saxitoxins), and dermal toxins (aplysiatoxin and lyngbyatoxin) [16]. Some of these have proven effects on DNA damage (genotoxic) and promotion of cancer (carcinogenic) such as is the case for microcystins and cylindrospermopsins [17,18]. Human fatalities and animal deaths have been attributed to cyanotoxins worldwide. In Brazil, water contaminated with microcystins and cylindrospermopsins was responsible for the death of 60 patients in a dyalisis center that died after acute liver failure associated with the presence in the treatment water of these two cyanotoxins [19,20]. Another incident associated with cyanotoxins intoxication was reported in Australia where 148 people, mostly children, were hospitalized with symptoms of gastroenteritis [20]. Later, this episode was attributed to water contaminated with the cytotoxin cylindrospermopsins. Incidents with neurotoxins have been reported in France where dogs died after drinking water contaminated with anatoxins [21]. Cyanotoxins are synthetized non-ribosomically and are organized in gene clusters [22,23]. Their main characteristic is that they are non-strain specific meaning that more than one genus of cyanobacteria can synthetize the same cyanotoxin and the same genus can produce more than one type of cyanotoxin. Among all cyanotoxins, microcystins and cylindrospermopsins are the two most studied worldwide while the neurotoxins are the most dangerous [11].

3.1. Microcystins

Microcystins (MCs) (Table 1) are hepatotoxins characterized by a group of cyclic heptapeptides whose chemical structure is characterized as a cyclo(D-Ala-L-X-D-erythro-β-methylAsp-L-Z-Adda-D-Glu-N-methyldehydro-Ala), where Adda is translated as the β-amino acid (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, which is found solely in the Cyanobacteria group [24]. X and Z positions in MC chemical structure correspond to varied amino acid residues where the most common are leucine and arginine whose outcome is MCLR (Figure 2). With these variations, more than 200 structural isoforms of MCs can be produced by cyanobacteria [24]. MCs act as specific inhibitors of protein phosphatases type 1 and 2A resulting in increased phosphorylation of proteins in liver cells which may affect metabolic pathways or cell division [25,26]. MCs producing genera include *Chrysosporum*, *Dolichospermum*, *Limnothrix*, *Microcystis*, *Nostoc*, *Phormidium*, and *Planktothrix* [24]. CyanoBlooms of MCs due to its varied species production are still the most commonly reported, globally demonstrating that, despite global changes, this is the cyanotoxin of most concern in water quality and human health [27,28].

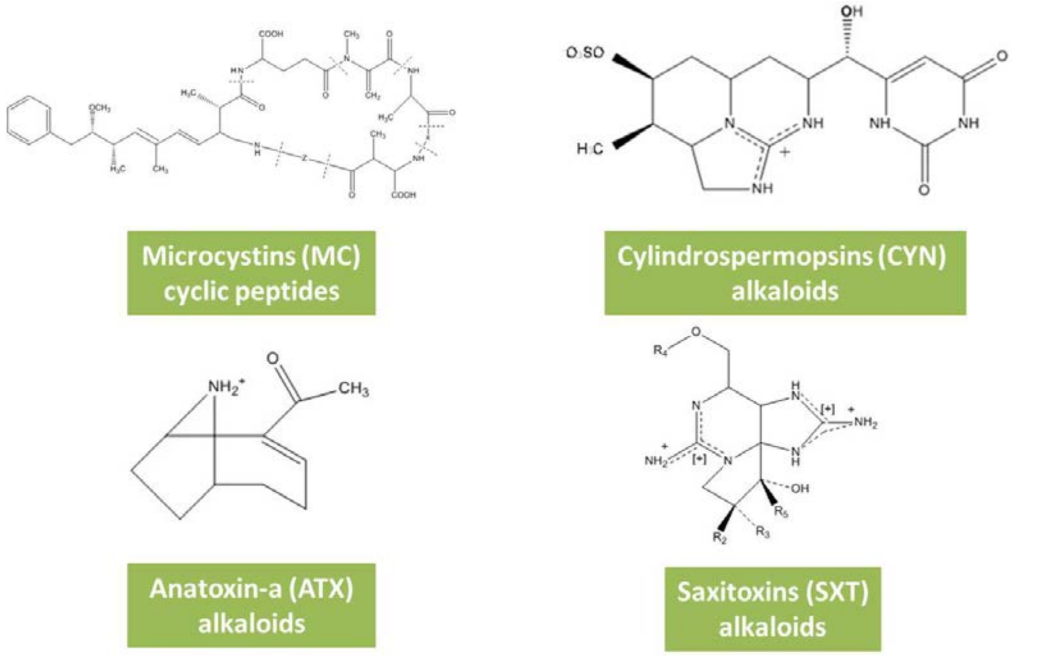

Figure 2. Chemical structure of cyanotoxins associated with CyanoBlooms.

3.2. *Cylindrospermopsins*

Cylindrospermopsins (CYN) (Table 1) are cytotoxins chemically characterized as alkaloids that can also affect the liver and nervous systems [29,30]. CYN chemical structure encompasses a guanidine group and a hydroxide group associated with a tricyclic-carbon skeleton (Figure 2) [31]. Despite CYN high stability, the molecule can suffer minor changes in its chemical structure, being currently found to possess four variants: 7-epi-CYN, 7-deoxy-CYN, 7-deoxy-desulfo-CYN and 7-deoxy-desulfo-12-acetyl-CYN [32]. CYN acts as a potent inhibitor of protein synthesis [33]. Initially described as a liver toxin, CYN is currently a cytotoxin also with genotoxic effects and a carcinogenic potential [17]. Produced by a variety of species including *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Aphanizomenon flos-aquae*, *Aphanizomenon gracile*, *Aphanizomenon klebahnii*, *Umezakia natans*, *Raphidiopsis curvata*, *Anabaena bergii*, *Anabaena planctonica*, *Anabaena lapponica* and *Lyngbya wollei* [34]. Blooms of CYN are at the moment poorly reported, despite CYN being considered the second most studied cyanotoxin worldwide. The Palm Island incident, after a bloom of *C. raciborskii* in the Soloman dam, is until now the only CYN CyanoBloom described, despite its production by diverse strains.

3.3. *Anatoxins*

Anatoxins (ATX) (Table 1) are a group of cyanotoxins with neurotoxic effects chemically characterized as an alkaloid composed of a bicyclic secondary amine (Figure 2) [35]. Anatoxin-a is highly unstable in nature, being easily converted into non-toxic metabolites, namely dihydroanatoxin-a and epoxyanatoxin-a [35]. The most studied metabolite is anatoxin-a with proven toxicity in the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermum*, *Oscillatoria*, *Microcystis*, *Raphidiopsis*, *Planktothrix*, *Artrospira*, *Nostoc* and *Phormidium* [35,36]. ATX outbreaks relate to an episode associated with blooms of *Phormidium* spp. that had neurotoxin production and resulted in the death of dogs after making contact with these cyanobacterium mats in both France and New Zealand [20,37]. In fact, with regard to ATX, no human fatalities have been reported until now, despite the several deaths reported in wild and domestic animals [38].

Table 1. Summary of the cyanobacterial toxicity associated with CyanoBlooms.

Cyanotoxins	Chemical Structure	Effect	Strains	Maximum Permissible Concentration	References
Microcystins	Cyclic peptides	Hepatotoxins	*Chrysosporum*, *Dolichospermum*, *Limnothrix*, *Microcystis*, *Nostoc*, *Phormidium* and *Planktothrix*	1 µg/L	[12,24,25]
Cylindrospermopsins	Alkaloids	Cytotoxins	*Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Aphanizomenon flos-aquae*, *Aphanizomenon gracile*, *Aphanizomenon klebahnii*, *Umezakia natans*, *Raphidiopsis curvata*, *Anabaena bergii*, *Anabaena planctonica*, *Anabaena lapponica* and *Lyngbya wollei*	1 µg/L	[14,29,34]
Anatoxins	Alkaloids	Neurotoxins	*Anabaena*, *Aphanizomenon*, *Cylindrospermum*, *Oscillatoria*, *Microcystis*, *Raphidiopsis*, *Planktothrix*, *Artrospira*, *Nostoc* and *Phormidium*	6 µg/L	[14,35,36]
Saxitoxins	Alkaloids	Neurotoxins	*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya* and *Planktothrix*	3 µg/L	[14,29,39]

3.4. Saxitoxins

Saxitoxins (SXT) (Table 1) are a neurotoxic alkaloid where the chemical structure is represented by tetrahydropurines with varied amino acids in the R positions (Figure 2) [39,40]. Its tricyclic structure brings to saxitoxins a high resistance to extreme environmental conditions [40]. Currently presenting 58 variants, saxitoxins can be produced by varied genera of marine dinoflagellates including *Alexandrium*, *Gymnodinium*, and *Pyrodinium*. In cyanobacteria, *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, and *Planktothrix* genera are the most frequently found [29,39,40]. Despite its reported production in several genera, SXT CyanoBlooms are currently only reported in dinoflagellates and intoxications normally associated with seafood consumption [29].

4. CyanoBlooms: Evaluation Methods

4.1. Microscopy

Assessing cyanobacteria species or genera can be performed by applying microscopy methods (Figure 3). The most traditional is light microscopy and in this the inspection for cyanobacteria composing CyanoBlooms can be achieved. Although microscopy is the most common method of all the main limitations, it requires extensive expertise and is unable to identify toxic taxa. Microscopy methods though greatly applied are becoming surpassed by other methods that permit the enumeration of cyanotoxins, most of them required by legislation. Despite their disadvantages, they have been used in the inspection of CyanoBloom samples [8].

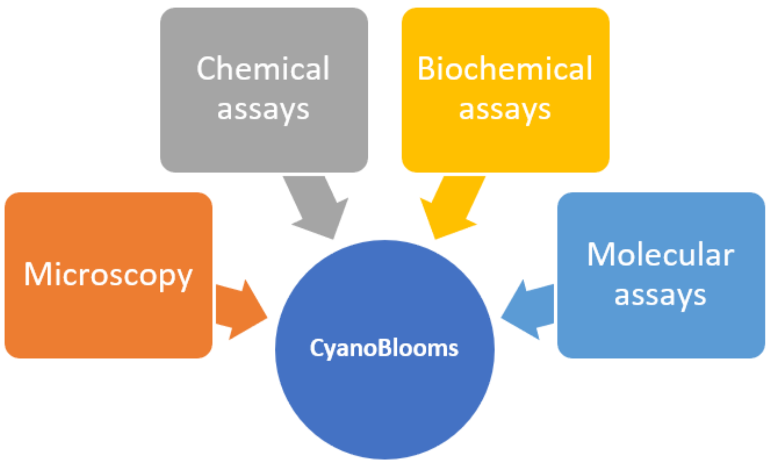

Figure 3. Current methods available in CyanoBloom investigations.

4.2. Chemical Assays

Detection and enumeration of cyanotoxins from a complex matrix such as CyanoBlooms can be achieved by applying chemical methods (Figure 3). These englobe mainly High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LCMS) coupled with MS that permit in a few days from sampling the retrieval of the cyanotoxins molecule present in a CyanoBloom or in a water sample. As high specific methods, limitations encompass the lack of detection and enumeration of all main cyanotoxins in a given sample. The most recent chemical method developed englobes the screening through the LCMS technique of nine cyanotoxins in pure and natural waters including CYN, ATX, and six variants of MC including the common MCLR [41]. Chemical methods have also been applied in cyanotoxin screening in samples such as food supplements [42] and also in CyanoBloom samples [43]. These methods were the first to be applied in cyanotoxins investigations, being associated with the report of the first cyanotoxin ever enumerated, the anatoxin-a, from a cattle poisonous bloom of *Anabaena flos-aquae* [44].

4.3. Biochemical Assays

Biochemical assays englobe the most commonly known as Enzyme-Linked Immunosorbent Assay (ELISA) that possess a higher sensitivity and lower specificity to cyanotoxins than the chemical assays (Figure 3). Despite these specificities, these methods are commonly used in water samples to screen for cyanotoxin presence in the study of ecosystems for cyanotoxin occurrence [8]. These methods also allow, similarly to the chemical methods, the detection and enumeration of cyanotoxins if the proper commercial cyanotoxin kit is used, meaning that, if multiple toxins are present, a kit for each cyanotoxin needs to be purchased. Abraxis currently commercializes ELISA kits for all main cyanotoxins with a time response of a few hours.

4.4. Molecular Assays

Discovered since the mid 2000s, molecular methods constitute an alternative method for cyanotoxin detection (Figure 3). The potential of cyanotoxicity of these methods are a limitation since the presence of toxicity genes does not represent actual toxicity due to the reported presence of a gene inactivation [45]. Despite toxicity genes being described in the environment, the producing capability of the strains needs to be clarified by other methods, namely the chemical ones. As the gene clusters of each cyanotoxin are annotated and sequenced in varied strains, the development of new tools (primers) that in a general or specific manner contribute to the environmental detection of toxigenic strains is increasing. Polymerase chain reaction (PCR) methods are the most commonly applied and developed, being normally associated with primer development [8,46]. These methods further allow other inferences through phylogenetic analysis and possible biogeographic investigations [47,48].

4.5. Artificial Intelligence Methods

In CyanoBlooms and in cyanotoxins research, there is no known application of artificial intelligence in their study. However, the outcome of interactive rooms portraying ecosystems with CyanoBlooms may permit surpassing limitations such as poor education and foster a change in behaviors to tackle CyanoBlooms health impairment. Promising machines with artificial intelligence on the identification and toxicity of CyanoBlooms are lacking at the moment.

5. Future Perspectives

Evaluating nutrient input is mandatory but not satisfactory since evaluation leads to classification of freshwater resources as sensible to eutrophication, but several factors can contribute to CyanoBlooms. These include rise of temperature, nutrient input by agricultural runoff and sewage discharges and finally climate change storms where heat events and rainfall can also lead to the rise of nutrients in freshwater resources. For this, the monitoring of freshwater resources for CyanoBlooms occurrence is relevant to infer on the effects of climate change and on the effects on human health contributing to altering behaviors towards Cyanobacteria Blooms globally and simultaneously making communities more resilient to this frequent problem, namely by the implementation of artificial intelligence methods. Therefore, continued research is required to assess possible relations between nutrient input and the occurrence of CyanoBlooms with particular emphasis on current climate conditions.

6. Conclusions

As toxin producers and a health hazard, CyanoBlooms are required to be continuously studied and reported globally. Under the scenario of global changes, the risk of CyanoBloom occurrence may increase, requiring a literature review to surpass limitations in its study and foster new challenges and behaviors. As an environmental assessment, its monitoring frequency should be increased since the presence of nutrients may hamper CyanoBloom reports that can occur after climate change phenomena such as heat waves or storms. In view of this relationship, much needs to be investigated with artificial intelligence methods assisting in the alert and education of communities. Therefore, with this review, the essential information on CyanoBlooms is summarized, globally reinforcing their environmental procurement and the monitorization for their occurrence, toxicity, and composition.

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