Recent trends in the detection of freshwater cyanotoxins with a critical note on its occurrence in Asia

Prabir Kumar Kulabhusan[1*], Katrina Campbell[1*]

[1]Institute for Global Food Security, School of Biological Sciences, Queen's University, 19 Chlorine Gardens, Belfast BT9 5DL, UK
*Corresponding author: katrina.campbell@qub.ac.uk; p.kulabhusan@qub.ac.uk

Abstract

Cyanobacteria are highly prevalent in slow-moving and nutrient-rich water bodies due to changing climatic conditions, eutrophication, and anthropogenic activities. Toxins of cyanobacterial blooms, i.e., cyanotoxins are also increasing at alarming rates in freshwater. Several efforts are being taken to detect cyanotoxins using molecular and analytical techniques to understand their occurrence and distribution, however, these studies are discrete and localized. The detection of cyanotoxins within water bodies is a long-established challenge. In this article, conventional methods of detection and the recently used nanostructure based immuno-sensors are described. Several studies are considered where aptamers are utilised as the biorecognition probe and we have discussed their use in colorimetric, electrochemical and optical sensors for the detection of cyanotoxins. Furthermore, this article also reviews the current field deployable diagnostics for the real-time monitoring of cyanotoxins. Future work on fundamental studies, development of highly specific aptamers for recognising different congeners of cyanotoxins, integration of sensors into portable or lab-on-a-chip devices could be interesting and useful research in this direction. Moreover, several studies related to the occurrence and distribution of cyanotoxins in the freshwater bodies of Asia are reviewed, with potential threats identified. This article also adds a note on the geographical distribution and detection of cyanotoxins in Asia. Thus, we have provided an overview of various evolving detection systems that could be employed in identified problematic underdeveloped regions to improve management strategies to monitor and control cyanotoxins.

Keywords: Cyanotoxins; Freshwater; Detection methods; Biosensors; Aptamers; Asia

Introduction

Freshwater ecosystems such as rivers, lakes, and reservoirs have high ecological merit and play a significant role in controlling the global climate. More importantly, they provide crucial resources and have socio-economic value for the human population. However, due to industrialization, soil erosion, deposition of effluents, the water quality has adversely affected biodiversity. Furthermore, activities like inadequate treatment of sewage, agricultural run-off, excessive use of fertilizers, and eutrophication have led to the proliferation of algae and cyanobacteria, thus impacting the freshwater ecosystems. These resources are not only exposed to anthropogenic pressure but are also equally vulnerable to natural toxins [1]. Globally, the massive proliferation of harmful cyanobacterial blooms (cyanoHABs) is a major factor that may result in the accumulation of toxins. Cyanobacteria are the oldest, photosynthetic, Gram-negative microorganisms existing in fresh, brackish and marine water as well as terrestrial environments. However, cyanobacteria produce several secondary toxic metabolites known as cyanotoxins that are released directly into the water when the cells die [2]. These cyanotoxins adversely affect the water quality in aquaculture and farming, posing serious sanitary hazards to public and animal health. The United States, Australia, and Europe and other parts of the world have frequently reported elevated levels of cyanotoxins in drinking water. This trend has continually increased during the past two decades [3]. Several studies have indicated the accumulation of cyanotoxins in water bodies from small ponds to large water reservoirs. Moreover, the frequency of cyanoHABs accumulation has also been gradually increasing [4].

Cyanotoxins are categorized into five different types (a) hepatotoxins (cylindrospermopsin (CYN), microcystins (MCs), and nodularins (NOD)); (b) neurotoxins (anatoxin-a (ATX), saxitoxins (STX) and β-N-methylamino-L-alanine (BMAA)); (c) dermatotoxins; (d) cytotoxins; and irritant toxins like lipopolysaccharides [5]. Different genera of cyanobacteria also produce the same type of toxins [6]. For example, genera such as *Nostoc*, *Microcystis*, *Plankothrix,* and *Anabaena* produce microcystins. Furthermore, toxic and non-toxic strains of cyanobacteria coexist even within the same species. However, the factors that trigger the toxin production or the dominance of toxic strains versus non-toxic strains are less understood [7]. Global warming and rapid eutrophication have favoured the growth of toxic cyanobacterial species in water bodies [8].

Asia is a major producer of fish (90 %) through aquaculture, with a projected 89 % of world aquaculture by 2029 [9]. Therefore, the water quality of aquaculture production in Asia is of high importance to ensure both food safety and food security. However, due to the extensive

use of the freshwater system, there is an increase in nutrient load, giving rise to frequent cyanobacterial blooms. These cyanobacterial toxins have been observed frequently in many Chinese lakes, such as Lakes Taihu, Chaohu, and Dianchi [10], Sri Lanka, India, Singapore, and other Asian countries.

Considering the impact of cyanotoxins in freshwater bodies and their deleterious effects on health, it is necessary to understand the occurrence, distribution, and biological significance of these toxins particularly in regions such as Asia where aquaculture is projected to increase. Therefore, detection methods for cyanotoxins are vital as a fundamental tool for gathering further knowledge as required for a better understanding. This review article examines the current detection methods and those evolving methodologies as frontline screening tools deployable in the field for on-site analysis. Currently, the application of aptamers and aptasensors in environmental monitoring and food/water safety are an immense area to explore. Hence, it is important to examine the current aptamer based analytical sensors and on-field diagnostic platforms that are used for monitoring cyanotoxins (**Figure 1**).

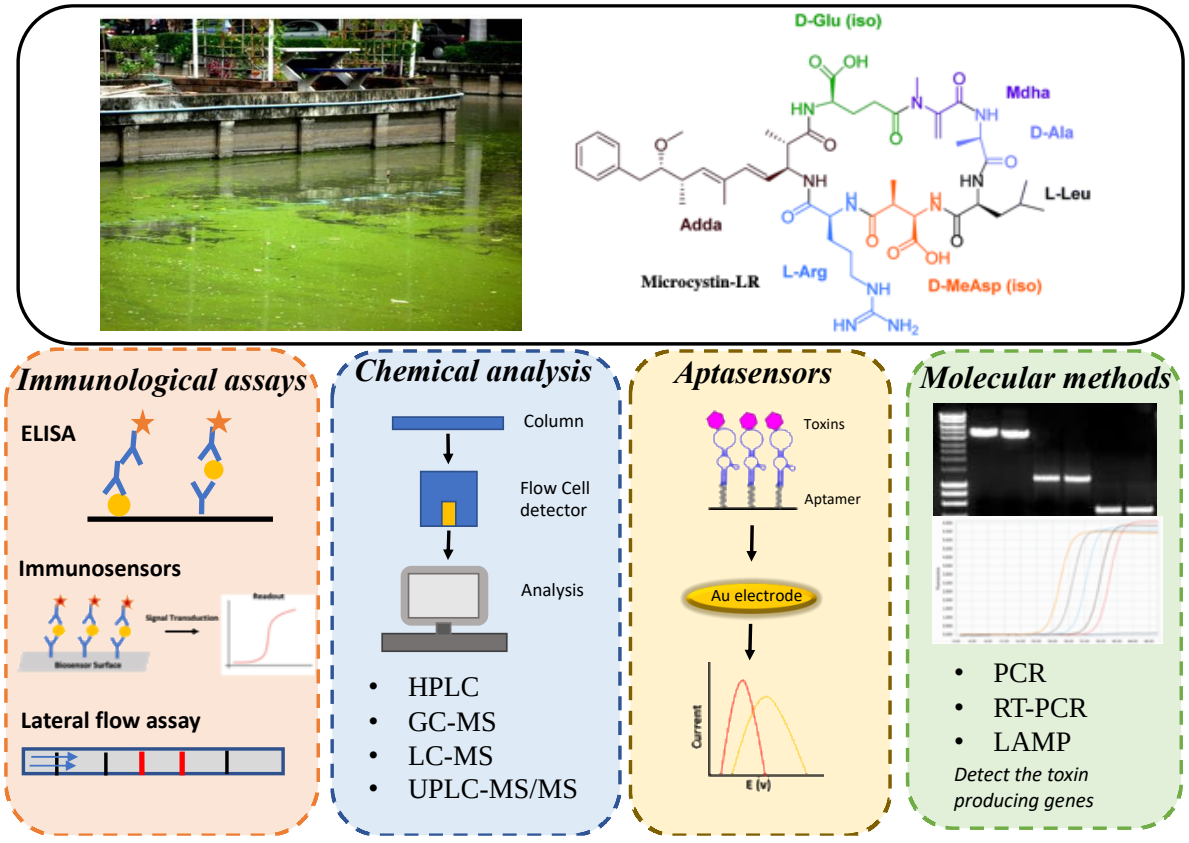


Figure 1: Schematic representation of various methods for the detection of cyanotoxins from fresh water. The freshwater pond image used here adopted from [11].

2. Occurrence of freshwater toxins in Asia

Cyanobacterial toxins are widely distributed in fresh and marine waters and have been reported several times in various countries, including the Arctic and arid desert areas. They are more frequently reported in Europe, North America, and Australia, while Asia, South America, and Africa have reported fewer occurrences. However, the data for cyanotoxin distribution and diversity may be unbalanced in different regions between the developed and undeveloped countries due to the variations in infrastructure and capability for frequent analysis [5]. Cyanotoxins including MCs, CYNs, STXs, NODs and ATXs are commonly found in every continent. However, to date Antarctica has not reported any occurrence of BMAA or ATXs [5].

Herein, we mainly focus on the distribution of freshwater cyanotoxins in Asia. Cyanotoxins such as MCs, CYNs, NODs, STXs, and ATXs have been distributed in tropical and temperate coastal areas, inland lakes, as well as in rivers of China, Japan, Korea, Bangladesh, Singapore, Saudi Arabia, Sri Lanka, India, Philippines, Thailand, Vietnam, Israel, and Turkey. Wan et al., studied MCs concentrations in 30 subtropical lakes in eastern China during the summer of 2018 [12]. The results indicated that 28 out of 30 lakes showed the presence of MCs and the highest concentration (26.7 μg/L) being found in Lake Chaohu, followed by Lake Taihu (3.11 μg/L). Similarly, He et al., 2018 studied spatiotemporal variations of MC-RR and MC-LR in the Yulin River for every month of 2016 and found that MC-RR and MC-LR's maximum concentration was 3.55 μg/L and 1.0 μg/L respectively [13]. The findings also revealed that MCs concentration was highest during the flood season and subsequently decreased from the estuary to upstream. The ecological risk assessment also demonstrated that MC-LR had adverse effects on the benthic invertebrates. Furthermore, MCs concentration was higher than the WHO guideline value, set at 1.0 μg/L, in Lake Taihu, China, from 2005 to 2014 [14]. *M. aeruginosa* also occurred prevalently in India. In a study, cyanobacterial blooms were monitored in Kerala, wherein *M. aeruginosa* was found in the freshwater ponds of Kochi, mainly during early and late summers [15]. Genus *Microcystis* were also detected from various water bodies of Maldah District, West Bengal, India [16]. The occurrence of various cyanotoxins in Asia are highlighted in the map (**Figure. 2)**.

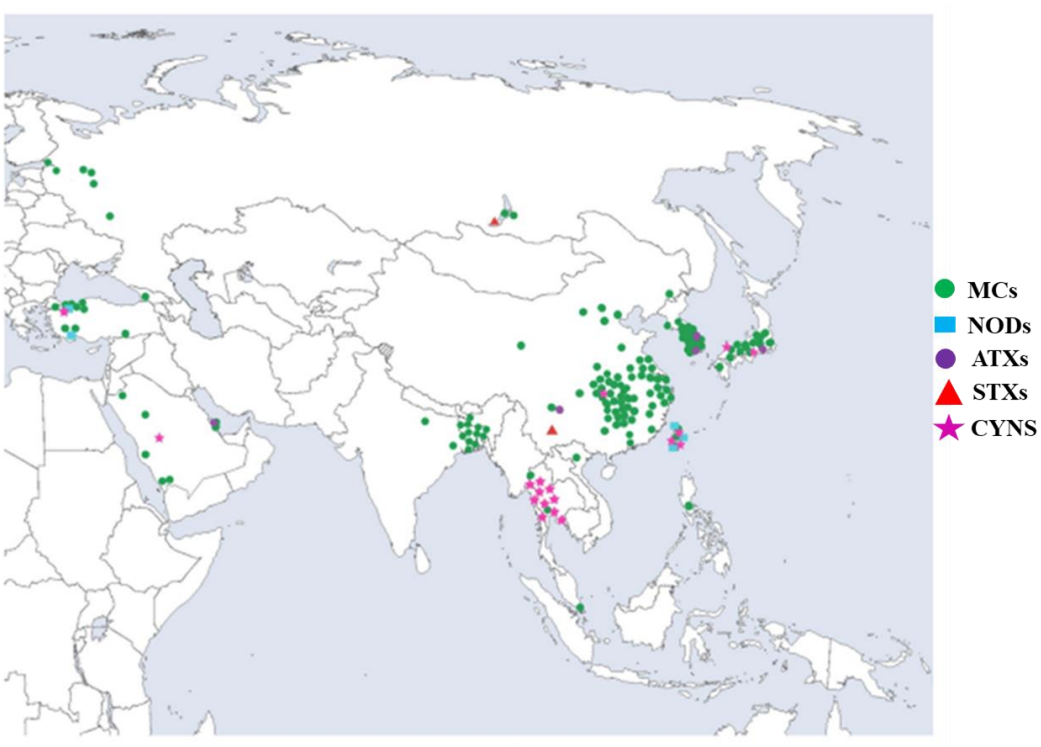


Figure 2: Geographical distributions of the different cyanotoxins in Asia. The image is adopted from [17]**.**

Bajpai et al., 2009 isolated a freshwater cyanobacterium, i.e., *Nostoc* sp. BHU001 from the agricultural ponds of Banaras Hindu University, India. This was the first report of MCs producing *Nostoc* strain from India which produces more MC-LR, -WR, -AR, -LA and methylated MC-LR. The MCs contents were found to be 25.2 µg/g (ELISA) dry weight of the total cyanobacterium and LC-MS was used to detect the MCs congeners amongst which 54% was MC-LR [18]. Dixit et al., 2017 isolated fourteen cultivable cyanobacteria from the Ganga River. The new isolates, *Oscillatoria spp.* RBD01 and *Leptolyngbya spp.* RBD05 were found to be toxic and showed the presence of MCs [19]. Besides MCs, CYNs and their homologues were mainly found in China, Japan, Saudi Arabia, Vietnam, Thailand, Israel, and Turkey, while NODs were reported only in China and Turkey. STXs were reported in China, Korea, India, Singapore, Bangladesh, and Turkey. ATXs have been found in Korea, Qatar, India, China, Japan, Turkey, and BMAA reported in China, Japan, and Qatar [5]. The details of the cyanobacterial species and the toxins recorded from different Asian countries are presented in **Table 1**.

Recent reports highlight that there is a frequent occurrence of cyanobacterial toxic blooms and suspected fish kills in the freshwater of Sri Lanka [20,21]. Furthermore, it has also been observed that there are patchy distributions of thick cyanobacterial scum in irrigation water

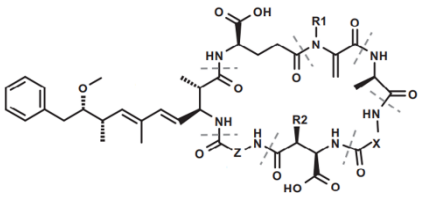
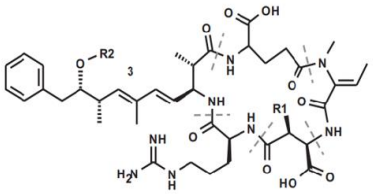
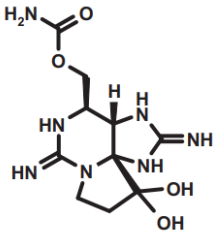
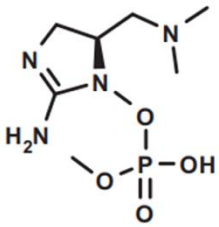
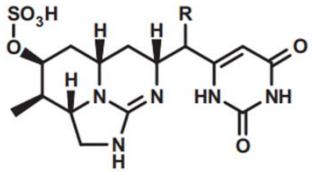
bodies in Sri Lanka particularly in the dry season [22]. Jayatissa et al., have performed an extensive study in 17 reservoirs of Sri Lanka over a two year period and found the abundance of cyanobacteria, the dominant species being *Microcystis spp* [22]. They also suggested that the fraction of cyanobacteria in irrigation waters was higher than that in hydropower reservoirs and half of the bloom material contains the MCs up to 81 μg/L. The study by Senanayake et al. also suggested that *M. aeruginosa* was recorded in the wet zone reservoir as well as over a wide distribution in all the other climatic regions of Sri Lanka [23] which indicates the potential cyanotoxin threat to the freshwater bodies of Sri Lanka. Recent evidence also suggested that cyanotoxins play a significant role in Chronic Kidney Disease (CKDu), particularly in Sri Lanka, India, and Nicaragua. The aetiology of CKDu occurs where well water is the primary source of drinking water. The study revealed that *M. aeruginosa*, *Cylindrospermopsis spp*., and *Anabaena spp*. were found in most of the drinking water reservoirs and that there was a strong correlation between the water of CKDu endemic areas and the cell density of cyanobacterial species [20]. However, more detailed investigations are imperative to derive a relationship between CKDu and the presence of cyanotoxins in drinking water.

Table 1: Summary of Cyanobacterial toxins recorded in Asia						
Country	**Location**	**Dominant cyanobacteria**	**Toxin found**	**Amount of toxins (ng/mL)**	**Toxin test**	**Reference**
China	Lake Chaohu	*M. aeruginosa*	MCs	1.07	HPLC	[24]
	Hunan Province/ Changsha City	*Microcystis* spp. YFM1	MC-LR,MC-RR,MC-YR	NA	HPLC-ESI-MS	[25]
	Hunan Province/ Dong Ting lake	*Microcystis* spp. YFM2	MCs	92.88 µg/10^7 cells	ELISA	[26]
	Beijing City	*Cylindrospermopsis raciborskii*	CYNs	NA	PCR and 16s rRNA	[27]
	Lake Taihu	*Microcystis sp.*	MC-LR	20-44	ELISA	[28]
Bangladesh	Dhaka City	*M. aeruginosa*	MC-RR,-LF	0.25 - 0.22	HPLC/MS	[29]
	Ishakha Lake	*M.aeruginosa* *M. wesenbergii* and *Anabaena circinalis*	MCs	37.46	ELISA	[30]
	Aquaculture pond in Gazipur, Dhaka.	*M. aeruginosa*	MC-RR, MC-YR and MC-LR	33.2(MC-LR), 9.03 (MC-RR), 5.23 (MC-YR)	HPLC-MS	[31]
India	Muttukadu backwaters, Chennai, Tamil Nadu	*M. aeruginosa*	MCs	NA	Microtox® bioluminescence assay	[32]
	Manjalar Dam, Theni District, Tamil Nadu	*M. aeruginosa*	MC-LR and [D-Asp^3] MC-LR	NA	HPLC-GC/MS	[33]
Thailand	Khon Kaen, Chanthaburi, Chiang Mai,	*M. aeruginosa*	MCs	3.62±0.43 (Cold seasons) and 3.10±0.97 (hot	Microcystin-Adda ELISA kit	[34]

	Bangkok, and Pathum Thani			seasons)		
Singapore	Water reservoir	*Cylindrospermopsis, Planktolyngbya, Pseudanabaena, and Microcystis spp*	CYNs and ATXs	0.4 (CYNs) and 0.1 (ATXs)	LC-MS/MS	[35]
Turkey	Lake Kovada	*M. aeruginosa,* Synechococcus *sp., Phormidium limosum*, *Phormidium formosa* and *Planktothrix limnetica*	MC–LR, MC–RR, MC–LA, MC–LW, MC–LF	98.9 (MC–LW) 0.5 (MC-LR)	ELISA and HPLC	[36]
	Lake Uluabat	*Microcystis spp.*	MC-LR, MC-RR, MC-LA, MC-LY, MC-LW, MC-LF, MC-YR, MC-WR, MC-HtyR,	0.2–330	LC-MS/MS, LC-UV-MS, LC-HRMS and ELISA	[37]
	Kucukcekmece Lagoon	*Microcystis spp.*	MC-YR and MC-LR	0.06 -24.2	HPLC-PDA	[38]
Iran	Anzali wetland	*Anabaena*	MC-LR	0.18–3.02	HPLC-UV	[39]
Japan	Artificial ponds in Okinawa	*M. aeruginosa*	MC-LR, MC-RR, MC-LA, MC-FR and MC-WR	NA	^{1}H NMR spectrometry, LC-MS	[40]
Vietnam	Mekong Delta	*M. aeruginosa*	MC-LR and MC-RR, MC-dmLR, MC-LW, and MC-LF	11,039	LC-MS	[41]
NA: Not available						

3. Nature and biological significance

Toxic cyanobacteria are found worldwide, especially in inland and coastal water environments. The most common toxic cyanobacteria in freshwater are *Microcystis* spp., *Planktothrix rubescens*, *Cylindrospermopsis raciborskii*, *Nostoc* spp., *Oscillatoria* spp., *Schizothrix* spp., and *Synechocystis* spp. amongst other genera and species [42]. Therefore, it is usually presumed that toxins are present in most of the cyanobacterial populations. **Table 2** presents the overview of the structures of freshwater cyanotoxins along with their structure, short-term and long-term health effects with provisional guideline values.

Table 2: Overview of freshwater toxins structures, producing organisms, and health effects					
	MCs	**NODs**	**STXs**	**ATXs**	**CYNs**
Structure					
Producing organisms	*Anabaena, Aphanocapsa, Microcystis, Nostoc, Oscillatoria, Plalnktothrix*	*Nodularia spumigena*	*Anabaena, Aphanizomenon, Cylindriospermopsis , Lyngbya*	*Anabaena, Aphanizomenon, Oscillatoria, Plalnktothrix*	*Aphanizomenon, Cylindrospermopsis, Umezakia*
Source	Drinking water, Irrigation water	Drinking water, Irrigation water	Drinking water, Irrigation water	Drinking water, Irrigation water	Drinking water, Irrigation water
Short-term health effects	Gastrointestinal liver inflammation, leading to death, Pneumonia dermatitis	Similar to MCs	Tingling, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death	Tingling, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death	Gastrointestinal liver inflammation, Pneumonia dermatitis
Long-term health effects	Tumour promoter, liver failure leading to death	Similar to MCs	Unknown	Cardiac arrhythmia leading to death	Malaise, liver failure leading to death
Provisional guideline values (µg/mL)	1 (Drinking water, life time) 12 (Drinking water. Short term exposure) 24 (Recreational)	1 (Drinking water)	3 (Drinking water) 30 (Recreational)	30 (Drinking water) 60 (Recreational)	0.7 (Drinking water, life time exposure) 6 (Recreational)
The structures refer to microcystin-LR, nodularin, saxitoxin, anatoxin-a and cylindropermopsin respectively. MCs: microcystins; NOD: nodularins; CYNs: cylindrospermopsin; ANA: anatoxins; STX: saxitoxins Short term exposure: refers to periods of about two weeks until enhanced drinking-water treatment or other measures can be implemented to achieve concentrations below the lifetime guideline value.					

4. Detection methods of cyanotoxins

Several biochemical and molecular approaches are established for the detection of cyanotoxins and their congeners from water bodies. Conventionally, microscopic analysis was employed for monitoring harmful algal communities; however, this approach does not distinguish between toxic and non-toxic strains. Thus, the risk assessment and monitoring of water are not possible by these methods. Furthermore, to determine the actual toxic effects of cyanotoxins, several bio-toxicity assays were performed. Yoshida et al.,1997, performed an acute toxicity assessment by intra-peritoneally injecting toxins into mice, known as the mouse bioassay (MBA) [72]. The lethal dosages (LD_{50}) by this intraperitoneal route range from 50 and 600 μg/kg for MC-LR and MC-RR, respectively [73]. The MBA effectively uses a whole animal to provide an accurate and realistic approach for determining MC toxicity; however, the application is limited and discontinued in many countries [73]. The MBA method has also been employed to investigate MC toxicity in Malpas dam (New England region of Australia) [74] and Paraná River (Argentina) [75]. However, considering the ethical concerns of using animals, the MBA assay is not feasible for determining toxicity during the routine assessment. Several bioassays have also been performed by using cyanotoxins in culture media to crustaceans (*Artemia*, *Daphnia*), insects (*Drosophila melanogaster*), protozoa (*Brachionus*), and bacteria (*Vibrio and E.coli*) [76]. Lately, several molecular methods (PCR and RT-PCR), immunoassays (ELISA), and biochemical methods such as protein phosphate inhibition assays (PPIA), protein synthesis inhibition (PSI) assays were employed for the screening and monitoring of cyanotoxins from water resources [77,78]. Furthermore, laboratory based systems including chromatographic methods such as liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) are employed for toxin detection due to their high selectivity and resolution to identify toxins. Recently, with the advances in nanotechnology, various nanomaterial-based detection systems, plasmonic platforms, and portable biosensors have been employed. These methods are employed to detect toxins from complex environmental samples due to their high sensitivity and specificity. However, the commercial availability of nanomaterial-based biosensors for cyanotoxin detection are limited. Recently, several aptamer based sensors and electrochemical sensors and on-site detection techniques have been developed.

4.1 Molecular methods

Since 1990, molecular methods have been widely adopted to monitor the presence of cyanobacteria in the aquatic systems. These methods incorporate the polymerase chain

reaction (PCR) and their variants such as one-step, multiplex, and real-time PCR (RT-PCR). These techniques detect the genes responsible for toxins synthesis present in cyanobacteria. Several non-PCR based approaches such as fluorescence *in situ* hybridization (FISH) [79] and DNA microarrays [80] have also been used to detect toxin producing cyanobacteria.

However, the identification of a cyanobacterial genus or species by molecular analysis does not confirm its potential for toxin production. Literature studies suggest that different strains of one species can be morphologically identical. However, they may differ in their toxicity [81] as found in *M. aeruginosa*, which has toxic and non-toxic strains [82]. Attempts are made to identify strains using PCR to amplify phycocyanin intergenic spacer (PC-IGS) between β and α subunits of phycocyanin operon [83], 16S-23S rRNA internally transcribed spacer region [84]. PCR was also used to detect other gene segments of the ribulose bisphosphate carboxylase [85] and RNA polymerase β subunit gene (rpoB) using taxon-specific primers [86]. Weller et al., 2011 studied population dynamics and the distribution of cyanobacterial species in three New Zealand lakes. The study revealed the presence of microcystin synthetase genes (*mcyE*), which produced MCs in toxicogenic cyanobacterial strains. The PCR amplification of the 820-bp-long region of the *mcyE* indicated *Microcystis* spp, *Coelosphaerium* spp, *Aphanocapsa* spp, and *Anabaena spp* [87].

Similarly, Feng et al., 2019 isolated and characterized a cyanobacterial strain present in surface water samples of Dong Ting lake, Hunan province, China. The gene amplification of mcyA-C genes and phylogenetic tree detected the strain as *Microcystis spp*. YFM2 and was 98% similar to *Microcystis aeruginosa* FACHB 940 [26]. Cyanobacteria is also detected by polyphasic identification using microscopy and DNA-based analysis. Lee et al., 2014 studied cyanobacterial isolates (n=39) from freshwater sites of Australia [88]. They employed 16S rDNA, *rpo*C1, and *cpc*BA-IGS approaches for molecular characterization; however, only 26 % (10/39) and 31 % (12/39) isolates agreed with the morphological and molecular data up to species and genus level, respectively. Despite advancements in bioinformatics tools and taxonomic revisions, confident identification beyond the order lever is impossible.

RT-PCR or qPCR-based molecular techniques have been successfully developed to detect cyanotoxins and other toxigenic genes. Multiple sample analysis in a single run with less turn-around time is capable with this technique. Michinaka et al., 2012 developed a microwave-based method for qPCR pre-treatment and TaqMan probes to quantify toxigenic and total MCs. The portable qPCR methods were validated using laboratory toxigenic strain *M. aeruginosa* PCC7820. Six drinking water reservoir sites in Taiwan (n=22) were tested with a detection limit of 1000 cells/mL [89]. Similarly, qPCR was developed to detect CYNs

for targeting *rpo*C1 and *cyr*J genes of *C. raciborskii* species which showed strong linearity between 10^2 and 10^6 copies per reaction. The method was applied to detect CYNs producing *C. raciborskii* from 34 subtropical reservoir sites of southern China [90]. However, most of the qPCR methods developed so far are mostly uniplex and can detect a single genetic target. Considering the complex nature of toxic cyanobacterial blooms, it is necessary to develop multiple sample analyses simultaneously [91,92]. The development of multiplex-qPCR capable of amplifying multiple targets simultaneously in a single reaction is an excellent approach. However, the successful development of a multiplex PCR assay depends upon primers, probes, and the reaction conditions to obtain the desired sensitivity. Ting Chiu et al., 2017 developed two sets of duplex qPCR, which quantified *Microcystis*, and *Cylindrospermopsis* [93]. The duplex qPCR system was developed and validated using samples collected from 29 reservoir sites of Taiwan (n=338). The result substantiated an excellent correlation (R^2 = 0.392−0.740) between the concentration of MCs and CYNs as determined by ELISA, cell counts from microscopy, and corresponding gene copies from qPCR. A similar type of multiplex-PCR was also developed to detect three *aoa* and *mcy* genes of MCs and CYNs synthesis, respectively, from the mixed cyanobacterial culture [94]. Multiplex PCR-based detection of cyanotoxins has several advantages, such as high sensitivity and throughput capacity, giving quick results. However, primer optimization for multiplex PCR is laborious and is sometimes prone to cross-oligo contamination. Several other qPCR and their variant methods are developed for the detection of STXs [95,96], CYNs [97], and ATXs [98]. Recently, the loop-mediated isothermal amplification (LAMP) method was developed, which amplifies the target gene with an optimum temperature ranging from 60 to 65°C. This technology has completely revolutionized the amplification of DNA due to the high primer specificity towards its target. Ramya et al., 2018, developed LAMP to identify microcystin synthetase B gene (*mcyB*) responsible for the production of MCs. The developed method was rapid (~2 h), and able to detect the toxin producing cyanobacteria from the seven water samples collected from the freshwater lakes and bird sanctuary from Chennai, Tamil Nadu, India [99].

The qPCR technique is promising for detecting less abundant (< 102 gene copies/mL) cyanobacterial toxic strains, helping in better understanding the bloom dynamics. Moreover, it is also able to detect toxin genes from the crude cell extracts using optimized protocols. However, a well-setup laboratory and trained personnel are needed for PCR operation. The qPCR may also sometimes give false-positive results due to mutated and partially deleted versions of the toxic biosynthesis genes [100]. However, PCR and qPCR should be used as

complementary tools with other analytical methods such as LC-MS/MS to monitor cyanoHABs.

4.2. Immunological methods

The property of antibodies to bind antigens has been extensively used to develop several commercial toxin detection methods. Enzyme-linked immune sorbent assay (ELISA) has been widely explored for detection purposes. It involves the interaction between antigens, i.e., the toxins and poly-/monoclonal antibodies, which act as recognition elements. Several industries (Abraxis, Enzo life science, etc.) are currently manufacturing cyanotoxin detection kits based on ELISA. Furthermore, different types of ELISAs, such as direct, indirect, and sandwich, have also been reported to detect MCs, NODs, STXs, and CYNs. After Brooks and Codd in 1987 developed the first polyclonal antibodies for MCs [101], other research groups reported raising both poly-/monoclonal antibodies for different toxin congeners. Some antibodies show broad specificity, which is advantageous for rapid screening but exhibit low specificity between the toxin congeners [102]. Yang et al., 2016 developed a multi-analyte immune-assay using antibodies with broad specificity and high affinity for different algal toxin congeners [103]. A conjugated carrier protein was prepared using the one-step active ester (AE) method, multistep thiolene click chemistry, and glutaraldehyde method. The monoclonal antibody (mAb2G5) not only detected NODs, but also all eight congeners of the MCs (MC-LR, MC-RR, MC-YR, MC-WR, MC-LA, MC-LF, MC-LY, and MC-LW) with high sensitivity. Indirect ELISA was also developed using the mAb2G5 that detected MC-LR and NODs, with a limit of detection (LOD) of 0.16 and 0.10 ng/mL, respectively, from the water samples. Similarly, Devlin et al., 2014 produced broad specificity monoclonal antibodies which displayed significant cross-reactivity with different congeners of MCs such as MC-LR (100%), MC-RR (108%), MC-YR (68%), MC-LA (69%), MC-LW (71%), MC-LF (68%), and Nodularin (94%) [104]. Another highly sensitive and competitive indirect ELISA (ciELISA) was developed for the simultaneous detection of nine different types of MCs and NODs [105]. This method employed heterogeneous coating strategies, which significantly enhanced the sensitivity of detection. The LOD of ciELISA for MC-LR was 0.0016 ng/mL and demonstrated high stability, reproducibility, and precision value for both MCs and NODs. Chen et al., 2020 developed an Open Sandwich ELISA (OS-ELISA) to quantify MC-LR using antigen-driven interaction enhancement between variable regions of an antibody [106]. The OS-ELISA employed an antibody VH fragment isolated from the phage display peptide library and showed a LOD of 0.13 ng/mL with a dynamic detection

range between 0.13 and 9952 ng/mL. Furthermore, the antibody-based microarray is another advanced technique for multi-analyte detection considering the reduced analysis time and cost. McNamee et al., 2014 developed an MBio biosensor based on planar waveguide microarray and fluorescence detection for five groups of harmful algal and cyanobacterial toxins in marine, brackish, and freshwater environments [107]. The toxins were domoic acid (DA), okadaic acid (OA and analogues), STXs, CYNs, and MCs and their analogues. The microarray was designed on a plastic slide; the light was directed from the bottom, while the reader permitted the fluorophore's excitation, enabling sensitive detection. The LOD of the microarray was 0.37, 0.44, 0.05, 0.08, and 0.40 ng/mL for DA, OA, STX, CYN, and MC, respectively, with a turn-around time of 15 min. Considering the alarming incidences of other freshwater toxins, i.e., CYNs, for the first time, Elliott et al., 2013 developed both mono- and polyclonal antibodies for CYNs [108]. The study employed a modified Mannich reaction to couple the toxin with two carrier proteins. The antibodies were characterized by competitive indirect ELISA and optical SPR biosensor. The sensitivity was found to be 0.027 to 0.131 ng/mL (ELISA) and 4.4 to 11.1 ng/mL (SPR) with low cross-reactivity towards the less toxic metabolite deoxyCYNs. The major limitations associated with ELISA are that it involves a relatively long procedure and requires skilled personnel to perform. Furthermore, antibodies employed in the ELISA are not capable of recognizing different variants or congeners of cyanotoxins and the results can vary quantitatively due to different cross reactivities. Therefore, the exact content of MCs and toxicity compared to LC-MS methods may not always be detected due to the variations in the specificity of the antibody. ELISA can detect MCs in solid samples (including fish tissues), however the extraction methods it uses (for the solid samples) are often not suited to non-solid samples [109,110]. Furthermore, false positives in ELISA are more likely to occur than false negatives as the assay may be compromised by matrix effects [111].

Advancements in the field of nanomaterials have allowed for rapid screening of cyanotoxins with improved sensitivity and robustness. Due to their unique structural and electronic properties, nanomaterials assist in amplifying signals during detection. The schematics of nanomaterials based immunosensors are depicted in **Figure. 4**. Amongst these, an electro-chemiluminescent (ECL)-based immunosensor consisting of gold nanoparticles (AuNPs) and CdS quantum dots (QDs) was developed to detect MC-LR [112]. The dynamic range of the detection was 0.01 to 50 ng/mL with a LOD of 0.0028 ng/mL. Gan et al., 2016 developed an immune sensor to detect MC-LR [113]. Carbon nanotube/cobalt silicate (CNT@Co silicate) and Fe_3O_4 nanoclusters/polydopamine/gold nanoparticles (Fe_3O_4@PDA–Au) were used as

labels for the secondary antibody and the HRP substrate, respectively. This method exhibited a linear response to MC-LR ranging from 0.005 to 50 ng/mL with a LOD of ng/mL. The AuNPs and gold nanorods (AuNRs) have been of particular interest in developing immunosensors. AuNPs have a high extension coefficient and inherent plasmonic properties using whereby different immune-sensing methods such as colorimetric and electrochemical have been developed [114,115]. Zhang et al., 2017 developed an electrochemical immunosensor using molybdenum disulphide (MoS_2) and AuNRs to detect MC-LR [116]. The MC-LR antibodies were immobilized on the MoS_2/AuNRs nanocomposite-modified gold electrode, which competed for the MC-LR antigen to form an antigen-antibody complex (**Figure 3A**). The assay displayed a linear detection range 0.01-20 ng/mL with a LOD of 0.005 ng/mL.

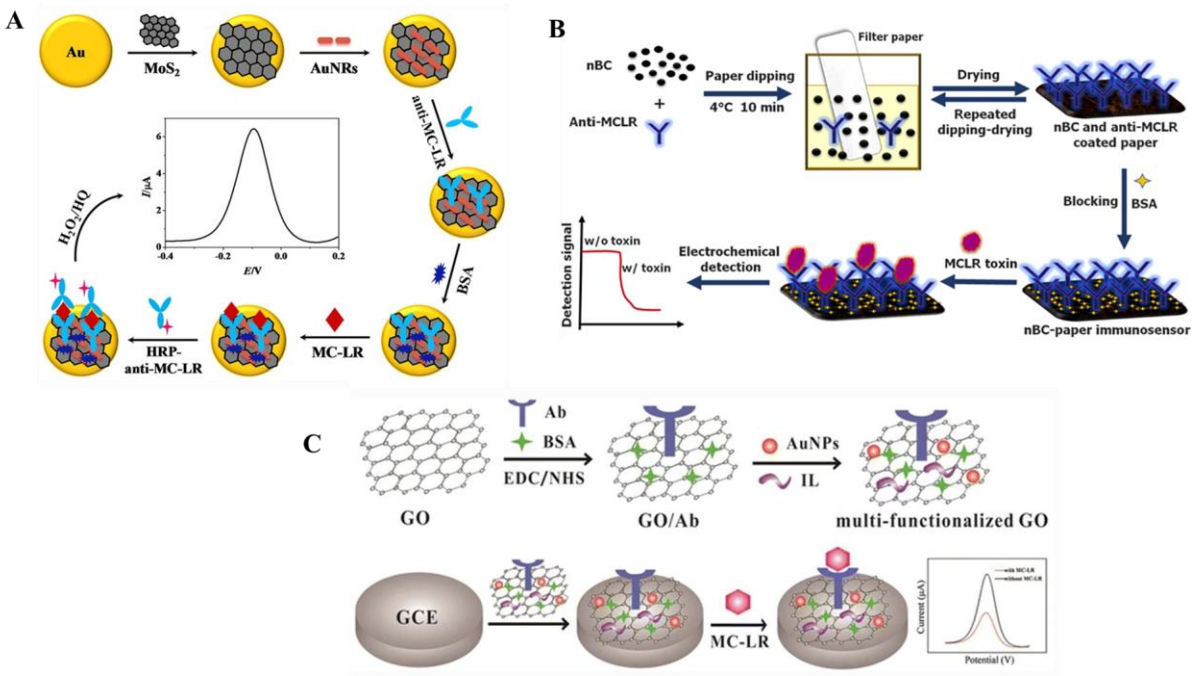


Figure. 3: Schematic representation of different types of immunosensors employing nanomaterials. (A) The MoS_2 and AuNRs for the detection of MC-LR [116]***. (B) Conductive nanobiochar for the detection of MC-LR*** [117]***. (C) The application of graphene oxide for label-free detection of MC-LR from water*** [118]***. The images are adopted with permission.***

Yao et al., 2020, developed conductive nanobiochar particles (nBC) for the sensitive detection of MC-LR toxins from the water sample [117]. MC-LR was quantified ampere-metrically using anti-MC-LR antibodies conjugated to the nBC (**Figure 3B**). The immunosensor exhibited high specificity, reproducibility, and storage stability with a LOD of 0.017 ng/mL. Furthermore, several label-free immunoassays were developed for the detection of cyanotoxins from the waterbodies. Hu et al., 2018 developed a label-free immunosensor for the detection of MC-LR by using multifunctional graphene oxide (**Figure 3C**) [118]. The

electrical conductivity of the sensor was enhanced by the addition of AuNPs and 1-butyl-3-methylimidazolium hexafluorophosphate. Differential pulse voltammetry detected MC-LR with an LOD of 0.1 ng/mL. Considering the vast application of nanomaterials in immunosensors, a summary of the different assay formats, nanomaterials and the recognition probe used, with their LOD for the detection of cyanotoxins from the water sample have been provided (**Table 3).**

Table 3: Different electrochemical immuno-sensors for the detection of cyanotoxins

Toxins	Matrix	Recognition element	Sensor platform	Linear range (ng/mL)	LOD (ng/mL)	References
MC-LR	Water	mAb	MoS_2/AuNCs and Au@PtNPs	0.001–1000	0.0003	[119]
MC-LR	Water	Abs	MGNnP-PEI and GCE	0.1-500	0.053	[120]
MC-LR	Water	Abs	AuNP/PPyMS and Electrochemical striping signal	0.0002 - 50	0.0001	[121]
MC-LR	Tap Water	Abs	SiO2@MSN and electro-chemical	0.0005-25	0.0003	[122]
MC-LR	Lake water	Abs	CdTe quantum dots and fluorescent immunosensor	0.0001- 3	0.00004	[123]
MC-LR	Water	Abs	3D graphene based electrode	0.05- 20	0.05	[124]
STXs	Water/ Shellfish	Abs	Cu-Graphene and Potentiometric	1-1000	1	[125]
MC-LR	Water	mAbs	Au-GCE	0.01-100	0.004	[126]
MC-LR	Water	mAbs	Graphene film	0.005-10	0.0023	[127]
STXs	Sea water	Abs	Antibody-Magnetic beads(recognition) and g-C3N4-PdNPs for oxidation	0.02-0.4	0.0012	[128]
MC-LR	Lake water	Abs	GH@PDA and and mesoporous carbon sphere enzyme label	0.01-10	0.0097	[129]
MC-LR	Reservoir water	Abs	SPCEs and SCEA	0.001–100	0.00011	[130]
MC-LR	Lake water	Abs	GNS@SiO_2 and SERS	0.01 -100	0.014	[131]
MC-LR	Water	Abs	AuNPs@MOF and GCE, competitive immunosensor	0.05 −75 000	0.02	[132]

mAbs: Monoclonal antibodies; MGNnP-PEI; Poly-ethyleneimine and magnetite nanoparticles, GCE; Glassy carbon electrode, PPyMS; polypyrrole microsphere, SiO_2@MSN; core-shell mesoporous silica, EIS; electrochemical impedance spectroscopy; g-C_3N_4-PdNPs; palladium-doped graphitic carbon nitride; GH@PDA: graphene hydrogel@polydopamine; SPCEs;Screen printed carbon electrode; SCEA; smartphone-controlled electrochemical analyser; GNS@SiO_2: Gold nanostar @silica shell; MOF: Metal organic framework.

To summarize, immunoassays and immunosensors are generally rapid and do not need much sample preparation for the detection. These methods detect the toxins in both active and inactive forms [133] but are predisposed to environmental matrix effects. The currently available commercial kits are used to detect individual cyanotoxins in water samples. However, there is not a single multiplex test kit available for the detection of cyanotoxins. The other limitation includes the difficulty in raising antibodies against toxins, causing concern during the development of high-throughput immunoassays. Strikingly, other recognition elements such as phage display peptides, antibodies, and DNA aptamers could overcome the limitations of antibodies and substantially contribute to the development of a robust detection system.

4.3 Chemical analysis

Chemical methods such as separation-based (LC) combined with ultraviolet (UV), or mass spectrometric detectors are used to quantify cyanotoxins in water samples. However, UV and fluorescence methods are not the preferred choice for detection due to the presence of other compounds with similar absorption spectra. Recently, Mass Spectroscopy (MS), Triple Quadrupole Mass Spectrometry (LC-MS/MS), Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight (MALDI-TOF)/MS, and Liquid Chromatography-Time-of-Flight (LC-TOF) MS are explicitly used for the identification and quantification of cyanotoxins. Liquid chromatography-mass spectrophotometry (LC-MS) based on electrospray ionisation (ESI) utilizes mass spectrometry (MS) along with physical separation properties of liquid chromatography (LC). These methods are highly specific and provide adequate sensitivity; however, these methods suffer due to the presence of interfering compounds such as salts, metals and inorganic or organic compound. Furthermore, these methods requires long processing time, skilled personnel expensive instrument and time taking protocols which constraints its application in resource limited settings [134]. In addition, these techniques require pre-concentration steps and clean up strategies before the analysis. Several sample extraction procedures such as liquid-liquid extraction[135], solid phase micro extraction

(SPME) [136], and solid phase extractions (SPE) [137] are currently being employed before performing any analytical techniques. Sometimes, the cyanobacterial cells are subjected to ultra-sonication, lyophilisation, and freeze-thawing to extract the total toxins. However, SPE is most commonly used due to the availability of various stationary phases and can be processed using lower solvent consumption. The SPE is used along with octadecyl silica (C18) and methanol which is the most frequent combination, showing recoveries higher than 85% MCs [138]. The selection of the extraction solvents is often carried out according to the physicochemical properties of the toxins and also the nature of the sample. The cyanobacterial blooms are usually rich in proteins and hence the extracts can result in complex mixtures. It has been shown that the application of water acidified with acetic acid, and methanol extraction suppressed the water-soluble proteins [139]. Another study reported the use of pure methanol as the versatile solvent for the extraction of different MCs (MC-LR, MC-LY, MC-LW, and MC-LF) [140]. The use of copolymer hydrophilic–lipophilic-balanced Oasis HLB cartridges (Waters, Milford, CA) has been successfully validated for the analysis of different toxins in bottled water [141].

In the liquid chromatography (LC), the cyanotoxins are separated by liquid under high pressure, packed with very small particles (3–50 μm). The application of ultra-high pressure with particles less than 2 microns facilitates faster analysis and higher separation efficiency.

MCs have UV absorption between 190 nm and 300 nm, with a maximum at 238 nm and detected with photodiode-array (PDA) detectors [53]. Before HPLC analysis, solid-phase extraction (SPE) was performed to clean up the samples, and the target analytes were concentrated within a pH range of 6-8 [142]. Li et al., 2017 developed a method for the rapid and accurate determination of MCs using magnetic solid-phase extraction and detection using HPLC with UV [143]. The limit of quantification of MC-RR was found to be 0.003 ng/mL, with a LOD of 0.001 ng/mL, which is much lower than that established by WHO for maximum concentration of MCs in drinking water. Further, the magnetic composite materials with cetylpyridinium chloride used for SPE are low in price and easy to use in solid-liquid separation. HPLC is integrated with high-resolution reverse-phase (RP) C18 columns (15 or 25 cm in length and 3 to 5 mm internal diameter) for better quantification. Furthermore, several other parameters such as flow rate, temperature, and column features (length, silanol activity) can be optimized for excellent separation and detection in HPLC.

However, the HPLC cannot differentiate between toxin congeners due to the same retention time of structurally similar toxins, which may co-elute. Furthermore, the additional peaks of co-elution of other organic compounds making the detection of low concentrations of toxin

difficult [144]. Besides, the lack of standards for MC congeners makes identification challenging. Therefore, quantification of the results are generally expressed as MC-LR equivalents. It is efficiently used for the detection and quantification of cyanotoxins from environmental samples. Teta et al., 2015 developed an LC-MS/MS-based method to quantify cyanotoxins from Green Lake, Seattle [145]. The method identified new cyclic peptides, namely, microcystin-MhtyR, which contributed to about half of the total MCs content in the bloom. A similar type of study was also carried out by Zervou et al., 2017, for the simultaneous detection of multiple cyanobacterial and algal toxins in water samples from Greece [146]. The SPE cartridge was used to extract and concentrate target compounds CYNs, ATX-a, NODs, 12 congeners of MCs, Okadaic acid, and Domoic acid. MCs were detected at a concentration ranging from 0.034 to 63 µg/L. Lachapelle et al., 2019, screened a wide range of cyanotoxins based on solid-phase extraction ultra-high performance liquid chromatography high-resolution mass spectrometry (SPE-UHPLC-HRMS) [147]. HRMS is increasingly being employed as a powerful technique for performing targeted analysis, with the added benefit of offering suspect-target and non-target screening, when certified standards are not available. The method employs high-throughput screening of CYNs, ATX-a, homoanatoxin-a, anabaenopeptins A and B, and 12 congeners of MCs from the lake samples in Canada. The method exhibited precision (<20%), accuracy (81–113%), and low relative matrix effects (< 29%) for environmental samples. The analysis of cyanotoxins like MCs, NODs, and CYNs using LC-MS techniques is relatively easy, whereas, due to the high polarity nature of the STXs, their detection by LC-MS is difficult. Real-time analysis of cyanotoxins by LC-MS suffers limitations due to the requirement of trained analysts and the need for careful instrumental observation due to the technique's high-level of sensitivity [148,149].

Gas chromatography coupled to the mass analyser (GC-MS) is another analytical method for detecting toxins. Moreover, due to the low volatile nature and high polarity of these toxins, few studies have reported detection using GC-MS. Prieto et al., 2020, developed analytical pyrolysis (Py) method before performing GC/MS for the detection of CYNs from raw and cooked fish [150]. The results indicated that Py-GC/MS could be a rapid and economical alternative for detecting and monitoring CYN and its degradation products (DP. *m/z* 290.1, 169.1 and 336.2). The degradation products ions at m/z 290.1 is found in samples cooked by microwaving and broiling whereas, the ions at m/z 169.1 and m/z 336.2 are present in all cooking process studied (microwaving, broiling, boiling and steaming).

MALDI-TOF is a sophisticated, rapid, selective, and sensitive technique for the quantification of cyanotoxins. The mass-to-charge (m/z) ratio of an ion is measured by determining the time required to travel the length of the flight tube. The majority of the toxins are peptides which are easily detected by MALDI-TOF. HPLC combined with MALDI-TOF can provide considerable support for detecting trace amounts of cyanotoxins from environmental samples. Some of the examples of chemical methods and applications for the analysis of cyanotoxins from various sources are listed in **Table 4**.

Over the last few decades, tremendous progress has been made on the detection of cyanotoxins using various analytical techniques. However, there is no single method available to identify and quantify toxins and their congeners simultaneously from the complex sample. Therefore, more efficient, cost-effective, and real-time monitoring strategies are required to detect cyanobacteria and their toxins.

Table 4: Different chemical methods for the detection of cyanotoxins							
Toxins	**Matrix**	**Sample preparation method**	**Recovery (%)**	**Instrumental approach**	**LOQ**	**LOD**	**References**
MC-LR, MC-RR, MC-YR, MC-LW, MC-LF, NODs, and CYNs	Water	SPE	64-115	UHPLC MS/MS; RP18 column	0.046 µg/L - 0.053 µg/L	0.05 µg /L	[151]
STXs	Aquatic species	IAC and SPE	79.3- 102.9	LC-MS/MS/TSK-Gel amide column	0.4 µg/kg	0.1 µg/kg	[152]
STXs	Rat plasma sample	magnetic solid-phase extraction (Fe_3O_4/ l-Cys MSPE)	NA	LC–MS/MS	5 ng/mL	0.5 ng/mL	[153]
STXs and Neo-STXs	Urine	SPE -HILIC	81.5–117 (STXs) 89.0–118 (Neo-STXs)	LC–MS/MS	0.5 ng/mL (STX) and 2 ng/mL for (Neo-STXs)	0.2 ng/mL(STX) and 1 ng/mL (Neo-STXs)	[154]
STXs	Human urine	Immuno-magnetic	80	LC-MS	1.00–100 ng/ml	NA	[155]
MCs, CYNs, and ATXs	Soil	Online SPE	60 - >90	UHPLC-tandem mass spectrometry	NA	0.001–0.3 ng/g	[156]
CYNs, ATX-a, NODs and MC-LR, MC-RR, MC-YR, MC-LA, MC-LY, MC-LW, MC-LF	Water	SPE using a polymeric cartridge and a graphitized non-porous carbon cartridge	66.6–87.3	UHPLC-HRMS	1–50 µg/L	4 and 150 pg/L	[157]
ATX-a, CYN, and HATX-a	Freshwater	SPE	73–97	UPLC- MS/MS	NA	0.6 to 15 ng/L	[158]
CYN,NOD,MC-RR, MC-	Fish	SPE	62.3-101.2	DSPE-LC-MS/MS	NA	5-10 µg/kg	[159]

YR, and MC-LR	tissues						
MC-LR, MC-YR, MC-RR, MC-LA, MC-LW, and MC-LF	Water	SPE	97.1- 100.9	HPLC-Orbitrap MS	NA	1 ng/ L	[160]
MC-LR, MC-RR, MC-YR, MC-LW, MC-LF	Vegetables	MSPD	1.9- 96.5	HPLC-MS	NA	13 μg/kg	[161]
MC-RR, MC-LR	Fish, Plant and soil sample	SPE	94-98	UPLC-MS/MS	NA	0.026 μg/g	[162]
MC-LR	Human urine	Immunocapture	100	LC–MS/MS	NA	0.00455 ng/mL	[163]
MC-LR, MC-RR, MC-LA, MC-LF, MC-LW, and MC-YR	Mouse urine, Mouse plasma, and Human serum	SPE	90.4 to 104.3	HPLC-orbitrap-MS And UHPLC-QqQ-MS/MS	0.13 μg/L (MC-LR, RR,YR) ∼0.50 μg/L (MC-LA, LF, and LW)	NA	[164]
MC-LR and NODs	Fish (Liver and Plasma)	SPE	NA	LC/MS/MS	NA	25 ng/g	[165]
MCs-LR, MC-RR, MC-YRand CYNs	Mussels	SPE	70.37–114.03	UPLC-MS/MS	0.23-0.40 ng/g	0.01-0.39 ng/g	[166]
MC-RR, MC-YR, MC-LR, MC-LY, MC-LW and MC-LF, ANA-a and CYN	Water	SPE	91–101	LC-HESI-MS/MS	NA	0.01–0.02 μg/L	[167]
MC-LR, MC-RR,MC-YR and CYN	Vegetables	SPE	41–93	UPLC-MS/MS	5–50 ng/g	0.06–0.42 ng/g	[168]
NA: Not available; IAC; Immuno-ffinity Column, HILIC; Hydrophilic Interaction Chromatography. DSPE-LC-MS/MS; Dispersive Solid Phase Extraction-Liquid Chromatography-tandem mass spectrometry, MSPD; Matrix Solid-Phase Dispersion							

5. Need for development of ASSURED detection methods

The real-time monitoring and detection of toxins is a challenging task due to the heterogeneous nature of cyanobacterial blooms, the presence of different variants of cyanotoxins, and the low molecular weight cyanotoxin congeners. Cyanotoxins are identified and detected using LC-MS, GC-MS Reverse phase-HPLC, Nuclear magnetic resonance (NMR), ELISA. However, they are generally performed in the centralized laboratory and are not well-suited for on-site detection. Thus, there is a requirement for a sensitive, rapid, and on-site detection kit for the successful control of cyanotoxins. Lateral flow immunoassays (LFIA) test strips have gained considerable attention due to their on-field application and cost-effectiveness. The LFIA employs poly- or monoclonal antibodies as the recognition probe, and the assay is performed on a nitrocellulose (NC) membrane. The principle lies in the target analyte movement and binding to the recognition probe on the NC membrane. The fabrication of the LFIA is carried out by assembling four different parts, namely, sample pad, conjugate pad, NC membrane, and absorbent pad, all of which are laminated on a plastic card [169]. The assay is performed using two different formats, i.e., competitive and sandwich, which have distinct features and advantages depending upon the target analytes. The conventional LFIA uses either latex beads or AuNPs, whereas, fluorescent nanomaterials (QDs and UCNPs) are employed as detection labels to enhance the sensitivity. The results of the LFIA can be interpreted by the naked eye without any special expertise and instruments. However, LFIA can be quantified using an LFIA reader or other software (e.g., Image J). These tests can also be used to detect cyanotoxins and have the potential of multiplexing, i.e., detection of several cyanotoxins in one test strip. LFIA efficiently meets the affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free, and deliverable (ASSURED) criteria of end-users. Several LFIA methods described in the literature for detecting cyanotoxins vary based on the recognition probe, detection label, and readout of the signal.

Melnik et al., 2017, raised an antibody MC10E7 from a murine hybridoma. The single-chain format of this antibody was expressed in yeast and a chimeric full-size version in the leaves of *Nicotiana tabacum* and *Nicotiana benthamiana* [170]. The plant-derived antibody was used to develop an LFA (dipstick assay) for the sensitive detection of [Arg4]-MCs. The LOD of freshwater samples collected from various sites was found to be 100-300 ng/L. Plant-based antibodies can also reduce the cost of antibodies which is the most expensive component of the assay. Similarly, Lawton et al., 2010 employed the recombinant antibodies for the development of LFA/ ImmunoStrip® which can detect the MCs and NODs [171]. The ImmunoStrip® are used for the detection of 10 µg/L MCs and all 7 congeners of MCs and

NODs were detected below 1 ng/mL. These tests are successfully validated from a range of laboratory algal cultures and the water sample from irrigation ponds.

Liu et al., 2019, reported a lateral flow fluorescent microsphere immunochromatographic assay (FM-ICA) combined with UV light to detect MC-LR from freshwater food samples [172]. An europium (Eu) nanosphere was used as a label in this developed assay. The LOD and IC_{50} were 0.0542 ng/mL and 0.5613 ng/mL, respectively (**Figure 4A and B**). The FM-ICA assay was simple and rapid, which can be used to screen freshwater samples contaminated with cyanotoxins.

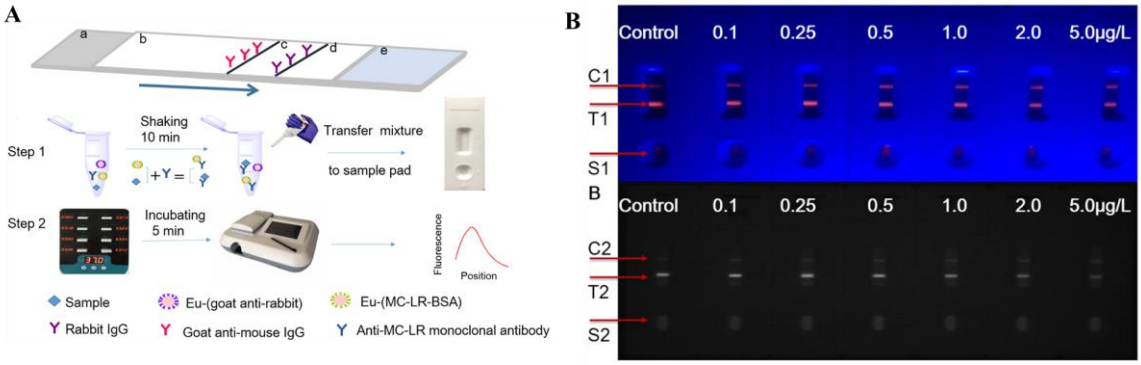


Figure. 4 (A) Schematics illustrates the FM-ICA (B) Visualization of FM-ICA strips under UV light (365 nm) under gel imager system. C1 and C2 are control and T1 and T2 test lines. The images are adopted with permission from [172].

Akter et al., 2019 developed a sandwich-based LFIA method for the screening of MCs and NODs-R [173]. The assay consisted of an antibody fragment as a recognition element, which enabled visual detection of major hepatotoxins, i.e., MC-LR, MC-dmLR, MC-RR, MC-dmRR, MC-YR, MC-LY, MC-LF MC-LW, and NOD-R at and below the concentration of 4 ng/mL. Along with the detection of toxins, the identification of cyanobacteria is equally essential. Li et al., 2019 developed a recombinase polymerase assay (RPA) method coupled with LFA strips [174]. The LF-RPA detected eight cyanobacterial species with high specificity and distinguished them from five non-cyanobacterial species. The method could also detect cyanobacterial DNA in water samples containing as low as 0.01 cell/mL, which was more sensitive than the commonly used PCR. Although, LFA is suitable for the on-site detection of cyanotoxins, their lower sensitivity as compared to other methods hinders its further application. To increase the sensitivity of LFA, Wu et al., 2019 employed a molecularly imprinted polymer (MIP) to initially separate MC-LR from complex matrices and then LFIA was performed [175]. MIPs are a facile analytical technique used to separate the target analytes from surrounding interference. The signal generated in the LFA test zone was enhanced by the oxidation reaction between TMB and HRP, which subsequently led to

blue colour formation. The optimized LFA detected MC-LR in a wide linear range (0.1 - 100 ng/mL) with a LOD of 0.04 ng/mL. The practicality of the assay was also evaluated by spiking the MC-LR with tap water. However, MIPs have limited applications for small molecules and require large quantities of cyanotoxins to generate the MIP. The major advantages of LFA include visual interpretation of the results without having any sophisticated instruments and can be operated by untrained users. Nonetheless, the colorimetric LFIA is limited to sensitivities of the human eye. With the recent advancement in optical sensors and electronics, these LFIAs could be coupled with smartphones for the quantification of target analytes. Furthermore, the algorithm for colour processing increases the assay sensitivity and provide a fast communication strategies with central laboratories for immediate action [176]. Thus, these tests generally offer first-line screening for the assessment of water quality and can be used by municipal authorities and the general public. Besides the above advantages, LFA still warrants modification in terms of sensitivity, specificity, and simultaneous detection of multiple toxins.

6. Aptamer-based detection of cyanotoxins

Aptamers, also known as chemical antibodies, are short, engineered oligonucleotide strands of DNA or RNA which mimic antibodies. Aptamers are generated *in vitro* by Systematic Evolution of Ligands through Exponential Enrichment (SELEX) against a wide range of biomolecules, microbes, proteins, and chemicals. As compared to their counterpart, i.e., antibodies, aptamers are highly stable, specific, and are easy to modify [177]. Their low production cost and minimal batch-to-batch variation make them a suitable probe for the development of various detection platforms. Moreover, aptamers can be immobilized at a higher density which is a critical requirement for developing immunoassays and microarrays [178]. Due to the inherent low stability of RNA aptamers, these are less suitable for the detection of environmental analytes. However, the stability can be enhanced by covalent modification and insertion of unnatural nucleic acid bases. For the very first time, in 2001, aptamers were developed for the detection of MCs. These aptamers demonstrated specific binding to MCs and detected them in the range of 50- 1000 µg/mL by surface plasmon resonance (SPR) [179]. However, the aptamers have lack of sensitivity and later the sensitivity was improved using high affinity aptamers. Andy Ng et al., 2012, selected DNA aptamers (Kd=28 to 60 nM) from a random oligonucleotide library, which displayed high affinity and specificity to MC-LR, MC-YR and MC-LA [180]. These aptamers were used in a sensitive electrochemical biosensor, which exhibited congener-specific MCs detection with

LOD as low as 0.01 ng/mL. The aptamer-based detection is usually performed by directly binding the toxins to the aptamers without any other complementary strands. The binding signal is derived from the conformational changes that occur between the target analyte and the aptamers. However, the main challenge is to optimize the signal-to-noise ratio of the detection platform to quantify the target accurately. Colorimetric methods are suitable and advantageous for the detection of targets as they are rapid, low in cost, and easy to operate. These methods generally employ antibodies for the detection of various target analytes. Nonetheless, the use of aptamers for the detection of cyanotoxins using colorimetric assays has been promising.

Li et al., 2016 developed an aptamer-AuNPs based sensor wherein the SPR properties of the AuNPs were exploited [181]. In the presence of MC-LR, the aptamers changed their conformation and dissociated from the surface of AuNPs. This dissociation led to the aggregation of AuNPs, which resulted in the colour change from red to blue. The assay displayed a rapid and sensitive detection platform for MC-LR with a LOD of 0.36 ng/mL. This aptamer-based colorimetric assay enabled monitoring of MC-LR in water samples without the need for any sophisticated instrument.

Furthermore, fluorescence-based detection (fluorescent label or FRET) methods are also employed for the detection of various cyanotoxins. Taghdisi et al., 2017 developed a fluorescent-based aptasensor to detect MC-LR from water and serum samples [182]. The assay comprised of single-walled carbon nanotubes (SWCNTs), DAP-10 as a specific aptamer for dapoxyl (fluorescent dye), and MC-LR aptamer (Apt) as a sensing ligand (**Figure 5A**). The method differentiated fluorescent intensity in the presence and absence of MC-LR and was highly specific towards them. The LOD of the fluorescent aptasensor was found to be 0.13 and 0.167 ng/mL (135-168 pM) for the tap water and serum samples, respectively (**Figure 5B and C**).

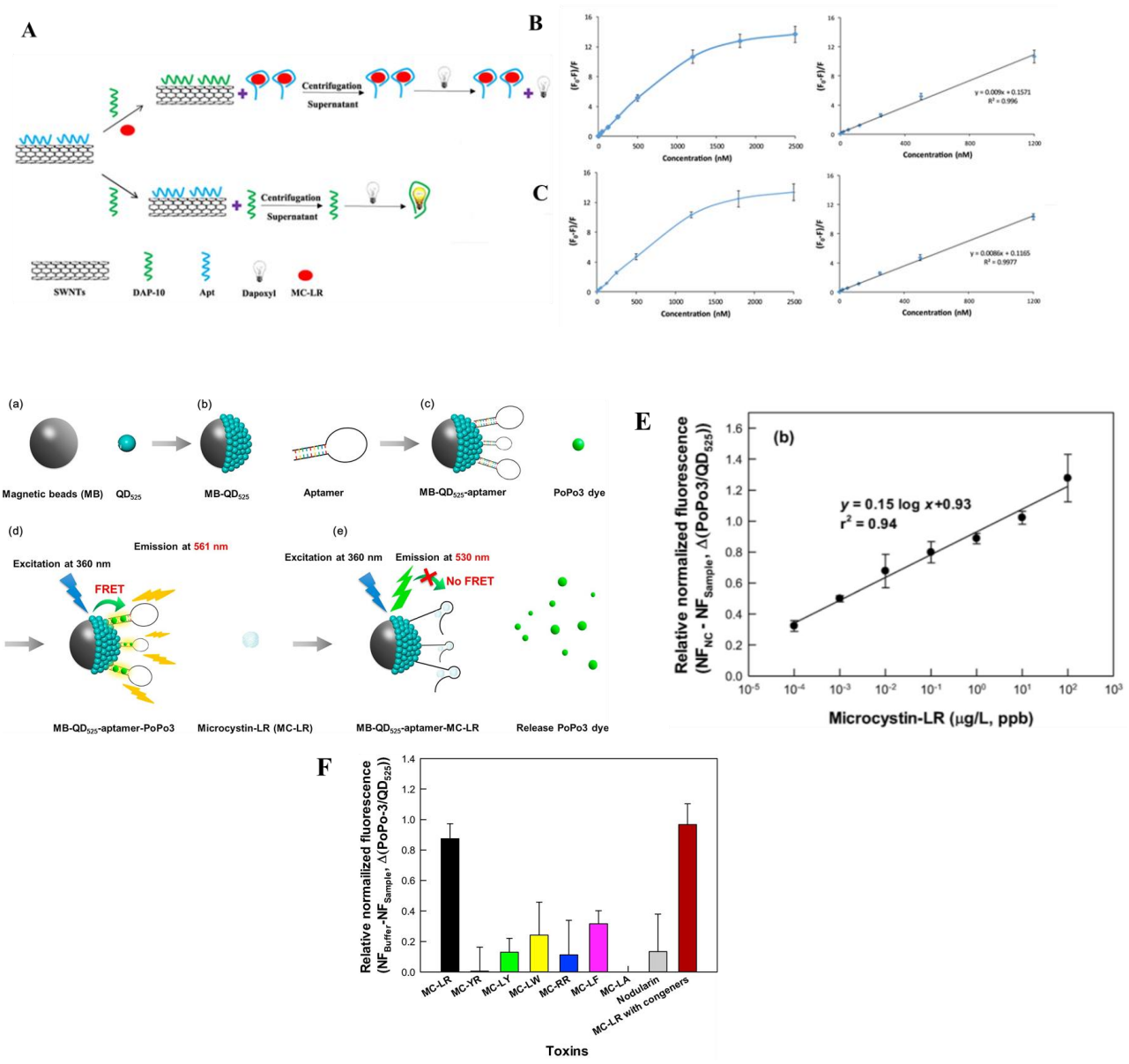


Figure.5 (A) Schematic represented the fluorescent-based sensor for MC-LR. (B) The fluorescent intensity measured in the presence of various concentrations of MC-LR (C) The performance of aptasensor after addition of MC-LR in serum. F_0 and F are the fluorescence intensities at 460 nm before and after the addition of various amounts of MC-LR, respectively. (D) Illustration of FRET-based aptasensor for MC-LR. (E) Sensitivity of FRET sensor (F) Specificity of the FRET based sensor at 1 µg/L of MCs and NODs (n=3). The images (A), (B), and (C) are adopted from[182] *and the images (D), (E), and (F) are adopted from* [183] *with permission.*

Similarly, FRET is yet another method used for cyanotoxin detection. It is based upon non-radiative energy transfer from an excited donor fluorophore to an acceptor fluorophore. Hee Lee et al., 2019 recently developed a QD-aptasensor for quantitative detection of MC-LR from eutrophic water samples (**Figure 5D**) [183]. The assay used QD525 as a fluorescent probe along with PoPo3 dye for the donor-acceptor pair. The binding of the MC-LR target to aptamer led to conformational changes in aptamer, increasing the donor-acceptor distance, which resulted in altered FRET-based fluorescence. This FRET-based aptasensor exhibited a

LOD of 0.1ng/L displays specificity towards different congeners such as MC-YR, MC-LY, MC-LW, MC-RR, MC-LF, MC-LA, and NODs (**Figure 5F**). The LOD was found to be 10^{-4} µg/L much below the WHO guidelines for drinking water (**Figure 5E**). The developed method also showed a positive correlation ($r = 0.981$ and $r = 0.862$) with conventional ELISA for both laboratory culture and environmental samples. Several reports have already suggested the development of a fluorescent-based sensor for the detection of freshwater and marine toxins [184,185]. However, the methods suffer from some limitations due to the interference of temperature, pH, and concentration of ions [186,187].

Furthermore, most of the commonly used fluorophores have a short fluorescent life-time (sec) and therefore demand specific storage conditions. As a result, fluorescent-based assays are expensive and are generally unsuitable for on-site analysis of the target. With the advent of nanotechnology, several fluorescent nanoparticles are employed to overcome the limitations of organic fluorophores.

Electrochemical methods also use aptamers which are immobilized on the electrode surface for the detection of cyanotoxins. The toxins are quantified by measuring the signals such as current, voltage potential, or impedance. A DNA aptamer-based impedance biosensor for the detection of ATX was developed [188]. The binding of ATX to aptamers led to significant decrease in electron transfer resistance due to conformational change of aptamers. The LOD of the sensor was found to be 0.1 ng/mL with a wide linear range of ATX concentrations (0.2- 25 ng/mL) and displayed high stability and specificity. The same research group also developed a DNA aptamer-based label-free impedimetric sensor for the detection of CYNs (**Figure 6A**). Aptamers were selected from a random DNA library which exhibited a high affinity for CYNs in the nanomolar range (20.25 ± 2.7 nM). The aptamers were assembled on the gold electrode surface to fabricate the sensor and the LOD was found to be 41 ng/mL with a linear range of detection from 0.04 to 33 ng/mL [189]. Liu et al. 2019, used chemiluminescence method and aptamers to detect MC-RR by using Cu/Co nanorods as CL catalyser (**Figure 6B**). The thiolated aptamers were immobilized on the Cu/Co and that led to a conformational change after MC-RR binding. The CL intensity depended on the concentration of MC-RR, and the LOD of the assay was found to be 3.3×10^{-11} M [190].

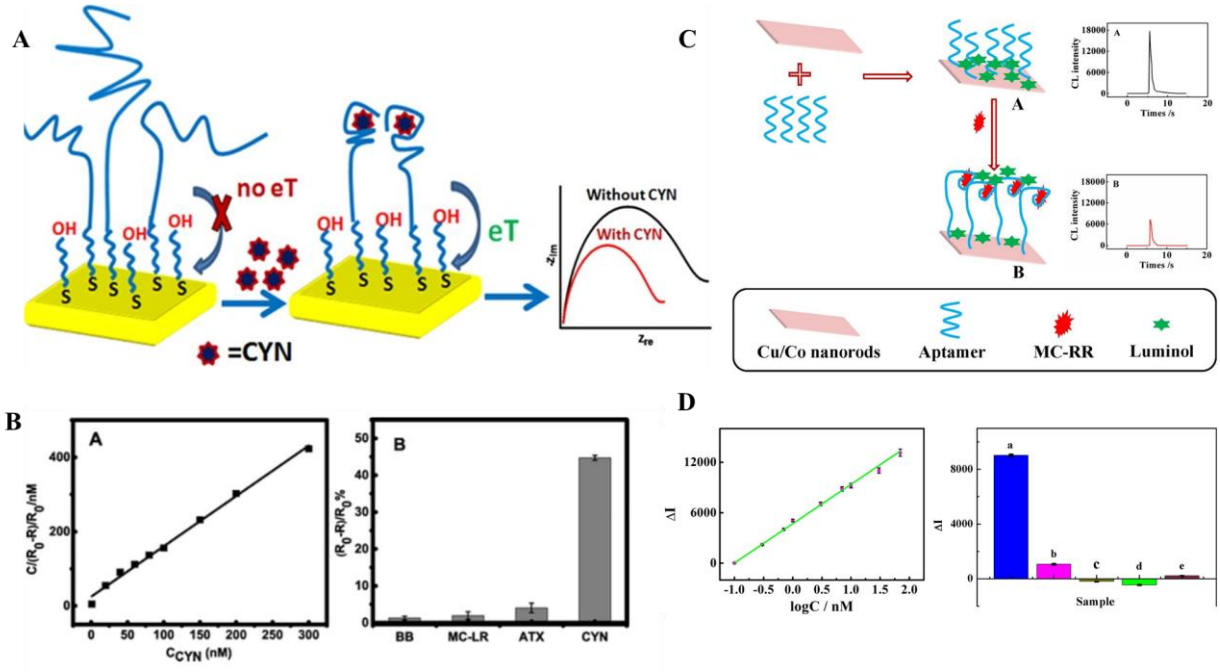


Figure.6 (A) Schematic representation of the aptamer based impedimetric sensor for the detection of CYNs. (B) The specificity of the sensor towards the CYNs (C) Schematic of the chemiluminescence detection platform using the aptamers for MC-RR. (D) The sensitivity and the validation of specificity for the MC-LR. The images** A and B **are** adopted from* [189] *and C and D from* [190] ***with permission.

Assays based on surface enhanced Raman spectroscopy (SERS) have been studied which employ SPR and LSPR properties of the nanomaterials demonstrating high sensitivity as compared to other detection methods. The SERS tags are made up of noble or transition metals that enhance the spectral signal stability and reproducibility. He et al., 2019 developed an aptasensor based on SERS to detect MC-LR using AuNPs as a signal probe and magnetic nanoparticles as a capture probe [191]. The SERS-based assay demonstrated a LOD of 0.002 ng/mL with a dynamic linear range from 0.01 to 200 ng/mL. The reliability of the method was also confirmed by detecting MC-LR from spiked tap water samples. Li et al., recently detected MC-LR using dual-modal aptasensor based on fluorescence and SERS [192]. The sensor design was based on the high affinity of the cyanine (Cy3) dye-modified complementary DNA (Cy3-cDNA) strand towards the plasmonic gold nanostars (GNSs). The binding of MC-LR to the aptamers triggered the dissociation of Cy3-cDNA/aptamer duplexes, which switched "off" the Cy3 fluorescence and turned "on" SERS due to the proximity of Cy3 dye to the GNS surface. Both fluorescence and SERS intensities varied linearly with the MC-LR concentration. The last decade has witnessed the application of aptamers for the detection of cyanotoxins on various detection platforms. However, most of these platforms were designed mainly for MCs and their congeners. Studies on the development of aptasensors for the detection of CYNs and ATXs are scarce in the literature.

Table 5 represents examples of aptamer-based detection of cyanotoxins, their method, working range, and the LOD.

Table 5: Aptamer-based methods for the detection of cyanotoxins						
Source	**Cyanotoxins**	**Sequence of the aptamer (5'-3')**	**Sensing system**	**Working range (ng/mL)**	**LOD (ng/mL)**	**References**
Tap water	MC-LR	GGC GCC AAA CAG GAC CACCAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC	Microcantilever array	1–500	50	[193]
Water	MC-LR	GGCGCCAAACAGGACCACCATG ACAATTACCCATACCACCTCATTA TGC CCCATCTCCGC	Glassy carbon electrode and CV	0.1-1.1	0.04	[194]
Spiked water	MC-LR	GGCGCCAAACAGGACCACCATGACAATT ACCCATACCACCTCATTATGCCCCATCTC CGC	Competitive displacement assay and molecular beacon	0.4-199	0.004	[195]
Water	MC-LR	GGC GCC AAA CAG GAC CAC CAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC	Cu/Au/Pt trimetallic nanoparticles/colorimetric platform	0.004-10	0.003	[196]
Spiked water sample	MC-LR	GGC GCC AAA CAG GAC CAC CAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC	Enzyme-free fluorescent detection	0.005 -1200	0.00003	[197]
Water	MC-LR	GGCGCCAAACAGGACCACCATGACAATT ACCCATACCACCTCATTATGCCCCATCTC CGC	Fluorescent nanoparticles	0.01–50	0.002	[198]
Water	CYNs	ATCAGGCAACAACCGATGGTC CGGCCACCCTAACAACCAGCCCACCCAC CACCCCGCCG	thionine–graphene nanocomposite modified GCE/$[Fe(CN)_6]^{4-/3}$	0.39-78	0.117	[199]
Tap water	CYNs	GGCATCAGGCAACAACCGATGGTCCGGC CACCCTAACAACCAGCCCACCCACCACC CCGCCG	GO and Fluorescent sensing	NA	0.007	[200]

Water	NOD-R	AAGGAGCAGCGTGGAGGATACCGTGTGG TATGATTCTAGGCTCGAAGTCGTGCATCT GCATTAGGGTGTGTCGTCGTGGT	Label-free BLI	0.033-0.1	0.13	[201]
Water	STXs	GGTATTGAGGGTCGCATCCCGTGGAAAC ATGTTCATTGGGCGCACTCCGCTTTC TGTAGATGGCTCTAACTCTCCTCT	AuNPs based colorimetric sensing	0.000002-29.9	0.000002	[202]
Spiked shellfish, ribbon fish and water	STXs	AACCTTTGGTCGGGCAAGG TAGGT	Optical BLI	10 - 2000	0.5	[203]
Blue mussels and Asia green mussels and water	STXs	GGTATTGAGGGTCGCATCCCGTGGAAAC ATGTTCATTGGGCGCACTCCGCTTTC TGT AGATGGCTCTAACTCTCCTCT	Gold electrode modified with MWCNT/Amperometric	0.26-8.9	0.11	[204,205]
CV; Cyclic voltammetry, SERS; Surface-enhanced Raman spectroscopy, EIS; Electrochemical impedance spectroscopy, GO; Graphene Oxide, BLI; Bio-layer Interferometry, MWCNT; Multi-walled Carbon nanotube, SWCNT; Single-walled carbon nanotube, FRET; Fluorescence resonance energy transfer						

Finally, aptamer based detection has several promising advantages, but still there are key challenges to be addressed. Nearly most of these aptasensors were designed to detect MC-LR, however, it is necessary to develop aptamers for other freshwater toxins. The cyanotoxins are small molecules, henceforth screening of aptamers is generally a time consuming and complex process. Further, the binding of aptamers are also influenced by several factors such as salt concentrations, pH, thus this may hinder the selection efficiency [206]. MCs have many structural variants and it is difficult to screen and access the aptamers binding for each analogue which is a tedious process. Today, only a few DNA aptamers are generated for cyanotoxins, however, the practical applicability and market penetration is very scarce. For wider application of aptamers, it should be validated by national and international regulatory agencies, related to public health, and food and water safety [207]. To summarize, we believe that aptamer based cyanotoxin detection may turn out to be a strategic approach compared to traditional methods for the implementation of safety and corrective measures.

Summary and conclusion

It is widely accepted that due to increasing eutrophication, anthropogenic activities, and significant changes in climate, cyanobacterial blooms and their toxins have posed a severe threat to water bodies. According to WHO provisional guideline value for drinking water (1 µg/L for MC-LR), several countries have developed their regulation strategies to tackle this problem. In addition, many countries have implemented the management of cyanobacterial blooms or alert level frameworks for recreational waterbodies. However, more scientific challenges and experimentally validated toxicological data are required to determine the threshold level for other alarming cyanotoxins.

Evidence from the literature suggests that the cyanotoxins are responsible for intoxication and death of livestock and pets worldwide. Furthermore, negative impacts of the cyanotoxins have been observed such as closure of water supplies and recreational sites. An incident took place in Caruaru, Brazil [208] wherein patients on dialysis were acutely exposed to cyanotoxins in the water supply which highlighted to the researchers the potential exposure problems. However, the lethal effects to humans by consuming drinking water containing cyanotoxins is less unlikely, as toxins are removed during the process of water purification. Though globally these water purification systems are aging and often under repaired. In addition, these cyanotoxins sometimes get dissolved in the surrounding water medium. Evidence suggests that MCs and NODs are associated with causing chronic health effects including tumour formation and reproductive toxicity. Some recent studies also suggested

that cyanotoxins can be taken up by higher plants, hampering their growth and metabolism. Thus, the presence of cyanotoxins in the agricultural irrigation system may affect the crops.

Consumption of fish from HABs affected water is an exposure route to the cyanotoxins. It has been reported that 40 % of the global finfish production comes from the freshwater bodies. Cyanotoxins detected in these freshwater fish reduces human consumption, thus impacting the aquaculture industry. In addition, the biomagnification of cyanotoxins, particularly MCs, has been reported in rice plants (*Oryza. sativa*, BG358) and other leafy vegetables (*I. aquatica*) in Sri Lanka [209]. The study demonstrated that the highest MC-LR accumulation was found in *O. sativa*, BG358 in the laboratory, however, in the field, highest MC-LR contamination was found in *I. aquatica*. Although, the concentrations were below the recommended tolerable daily intake (TDI) level, the study provided a warning for further study and necessary actions. Thus, extensive field studies on toxin biomagnification are critical in translating the laboratory estimated concentrations to the more uncontrolled environment [210,211].

The constant monitoring of cyanotoxins from drinking and recreational water bodies has become essential to minimize the health risks. For this, sensitive, rapid and high-throughput screening methods are required and need to be optimized and validated for their on-field applications. Several methods that have been devised, including advanced nanomaterial-based biosensors with different specific recognition probes. The most applied analytical methods for the detection of cyanotoxins are LC/MS, GC-MS, molecular approaches for the detection of the gene responsible for cyanotoxins and other antibody-based techniques such as ELISA. Molecular methods such as PCR and RT-PCR have been employed not to detect toxins but to provide important information and understanding of the geographical and temporal distribution of HABs. These molecular methods are highly sensitive and can detect toxin-producing organisms from environmental samples. These techniques can differentiate between the toxic and non-toxic cyanobacterial species based upon 16s rRNA genes or phycocyanin intergenic spacer (PC-IGS). Similarly, the qPCR also helps to detect and quantify cyanobacterial toxicogenic genes and provides in-depth information about toxin production at mRNA and protein levels. The change in toxin production/inhibition due to the various environmental conditions can also be assessed using qPCR methods. Due to the high sensitivity and specificity, these methods can replace the laborious microscopy and mouse bioassay minimising the use of animals for the toxicity studies. However, certain limitations are associated with these methods such as cost and the requirement of sophisticated instruments and skilled human resources.

The analytical methods such as HPLC-UV, Fluorescence, Photodiode array (PDA) and LC-MS are used to identify and quantify different toxin variants, mainly in the centralized laboratory. These methods are robust and sensitive enough to detect toxins in ultra-low concentrations from the complex environmental samples. Moreover, these methods are not portable for on-site application and should be made affordable for the detection of toxins and their variants.

Recently, much attention has been given to emerging biosensors due to their striking features such as high specificity and sensitivity, rapid response and portability. Several biosensor strategies also satisfy the ASSURED criteria devised by the WHO for the development of diagnostic assays. Biosensors employ several detection approaches such as electrochemical (labelled and label-free detection), optical, fluorescent, and colorimetric methods. Depending upon the recognition probe and the transduction method, the biosensors have different LODs and LOQs. In this review article, we have emphasized immunosensors and aptamer-based biosensors for the detection of cyanotoxins. Aptamers can be generated for small toxin molecules and are also capable of detecting and differentiating different toxin congeners. However, the major challenges of aptasensors are production of low signals and high signal/noise ratio. This can be addressed by the application of different assay formats such as sandwich or competitive assays using a novel class of nanomaterials or modifications on the transducer surface. The biosensors developed for cyanotoxins are usually tested in buffer or water, spiked with toxins. However, detection of toxins from the environmental samples is altogether a different challenge. The presence of other contaminants or matrix effects always influences the analytical performance of a sensor. Further, the stability of the sensor after repeated use needs to be improved for commercialization. Certain biosensors need specialized sample pre-treatment procedures, which deters their on-site application. Overall, optimization of biosensors with pico/femto-molar detection from the real sample requires more efforts. Currently, to the best of our knowledge, there are no biosensors that integrate sample collection to quantification within a single system. In our opinion, the application of a lab-on-a-chip for sample processing along with the biosensing element makes the system more robust and offers the possibility of real-time monitoring. There is a pressing need for fully integrated sensitive biosensors with high specificity, which would significantly contribute to the public health system.

To summarize, there has been lot of studies conducted for the detection of MCs and the associated cyanobacterial blooms. However, the fate of other toxins such as CYNs, ATXs and STXs should be investigated and validated to devise methods suitable for proper

regulatory approval. Extensive research on potential toxicity assessment and a model for the prediction of blooms relative to aquaculture safety also needs to be addressed.

Declaration of Competing Interest:

The authors report no declarations of interest.

Acknowledgements:

The authors would like to thank UK GCRF BBSRC project “A Scalable Bio-based Solution to Eliminate Cyanotoxins in Drinking Water; grant reference BB/S011579/1) for providing financial support.

References:

[1] T.D. Bucheli, Phytotoxins: Environmental micropollutants of concern?, Environ. Sci. Technol. 48 (2014) 13027–13033. https://doi.org/10.1021/es504342w.

[2] S. Bogialli, C. Bortolini, I.M. Di Gangi, F.N. Di Gregorio, L. Lucentini, G. Favaro, P. Pastore, Liquid chromatography-high resolution mass spectrometric methods for the surveillance monitoring of cyanotoxins in freshwaters, Talanta. 170 (2017) 322–330. https://doi.org/10.1016/j.talanta.2017.04.033.

[3] M.Y. Cheung, S. Liang, J. Lee, Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health, J. Microbiol. 51 (2013) 1–10. https://doi.org/10.1007/s12275-013-2549-3.

[4] L.C. Backer, J.H. Landsberg, M. Miller, K. Keel, T.K. Taylor, Canine cyanotoxin poisonings in the United States (1920s-2012): Review of suspected and confirmed cases from three data sources, Toxins (Basel). 5 (2013) 1597–1628. https://doi.org/10.3390/toxins5091597.

[5] X. Du, H. Liu, L. Yuan, Y. Wang, Y. Ma, R. Wang, X. Chen, M. Losiewicz, H. Guo, H. Zhang, The Diversity of Cyanobacterial Toxins on Structural Characterization, Distribution and Identification: A Systematic Review, Toxins (Basel). 11 (2019) 530. https://doi.org/10.3390/toxins11090530.

[6] G. Zanchett, E.C. Oliveira-Filho, Cyanobacteria and cyanotoxins: From impacts on aquatic ecosystems and human health to anticarcinogenic effects, Toxins (Basel). 5 (2013) 1896–1917. https://doi.org/10.3390/toxins5101896.

[7] C. Hu, C. Rea, Z. Yu, J. Lee, Relative importance of Microcystis abundance and diversity in determining microcystin dynamics in Lake Erie coastal wetland and downstream beach water, J. Appl. Microbiol. 120 (2016) 138–151. https://doi.org/10.1111/jam.12983.

[8] I.Y. Massey, M. Al osman, F. Yang, An overview on cyanobacterial blooms and toxins production: their occurrence and influencing factors, Toxin Rev. (2020). https://doi.org/10.1080/15569543.2020.1843060.

[9] Fish | OECD-FAO Agricultural Outlook 2020-2029 | OECD iLibrary, (n.d.). https://www.oecd-ilibrary.org/sites/4dd9b3d0-en/index.html?itemId=/content/component/4dd9b3d0-en (accessed March 26, 2021).

[10] L. Peng, Y. Liu, W. Chen, L. Liu, M. Kent, L. Song, Health risks associated with

consumption of microcystin-contaminated fish and shellfish in three Chinese lakes: Significance for freshwater aquacultures, Ecotoxicol. Environ. Saf. 73 (2010) 1804–1811. https://doi.org/10.1016/j.ecoenv.2010.07.043.

[11] R.P. Rastogi, D. Madamwar, A. Incharoensakdi, Bloom dynamics of cyanobacteria and their toxins: Environmental health impacts and mitigation strategies, Front. Microbiol. 6 (2015) 1254. https://doi.org/10.3389/fmicb.2015.01254.

[12] X. Wan, A.D. Steinman, Y. Gu, G. Zhu, X. Shu, Q. Xue, W. Zou, L. Xie, Occurrence and risk assessment of microcystin and its relationship with environmental factors in lakes of the eastern plain ecoregion, China, Environ. Sci. Pollut. Res. 27 (2020) 45095–45107. https://doi.org/10.1007/s11356-020-10384-0.

[13] Q. He, L. Kang, X. Sun, R. Jia, Y. Zhang, J. Ma, H. Li, H. Ai, Spatiotemporal distribution and potential risk assessment of microcystins in the Yulin River, a tributary of the Three Gorges Reservoir, China, J. Hazard. Mater. 347 (2018) 184–195. https://doi.org/10.1016/j.jhazmat.2018.01.001.

[14] X. Su, Q. Xue, A.D. Steinman, Y. Zhao, L. Xie, Spatiotemporal dynamics of microcystin variants and relationships with environmental parameters in lake Taihu, China, Toxins (Basel). 7 (2015) 3224–3244. https://doi.org/10.3390/toxins7083224.

[15] R. Mohan, T. Sathish, K.B. Padmakumar, Occurrence of potentially toxic cyanobacteria Microcystis aeruginosa in aquatic ecosystems of central Kerala (south India), Ann. Limnol. 56 (2020). https://doi.org/10.1051/limn/2020015.

[16] P. Gupta, Occurrence of Genus Microcystis Lemmerm. from water bodies of Maldah district, West Bengal, India, Trop. Plant Res. 6 (2019) 233–240. https://doi.org/10.22271/tpr.2019.v6.i2.33.

[17] Z. Svirčev, D. Lalić, G. Bojadžija Savić, N. Tokodi, D. Drobac Backović, L. Chen, J. Meriluoto, G.A. Codd, Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings, Arch. Toxicol. 93 (2019) 2429–2481. https://doi.org/10.1007/s00204-019-02524-4.

[18] R. Bajpai, N.K. Sharma, L.A. Lawton, C. Edwards, A.K. Rai, Microcystin producing cyanobacterium Nostoc sp. BHU001 from a pond in India, Toxicon. 53 (2009) 587–590. https://doi.org/10.1016/j.toxicon.2009.01.023.

[19] R.B. Dixit, A.K. Patel, K. Toppo, S. Nayaka, Emergence of toxic cyanobacterial species in the Ganga River, India, due to excessive nutrient loading, Ecol. Indic. 72 (2017) 420–427. https://doi.org/10.1016/j.ecolind.2016.08.038.

[20] S.A. Kulasooriya, Toxin producing freshwater cyanobacteria of Sri Lanka, Ceylon J.

Sci. 46 (2017) 3. https://doi.org/10.4038/cjs.v46i1.7413.

[21] F. Hossain, R.R. Ratnayake, S.A. Kulasooriya, K.L.W. Kumara, Culturable cyanobacteria from some selected water bodies located in the major climatic zones of Sri Lanka, Ceylon J. Sci. 46 (2017) 47. https://doi.org/10.4038/cjs.v46i1.7417.

[22] L.P. Jayatissa, E.I.L. Silva, J. McElhiney, L.A. Lawton, Occurrence of toxigenic cyanobacterial blooms in freshwaters of Sri Lanka, Syst. Appl. Microbiol. 29 (2006) 156–164. https://doi.org/10.1016/j.syapm.2005.07.007.

[23] P.A.A.P.K. Senanayake, S. Kumburegama, C.S. Wijesundara, S.K. Yatigammana, What drives the dominance and distribution of Cyanobacteria and Dinoflagellata in reservoirs of Sri Lanka?, Sri Lanka J. Aquat. Sci. 26 (2021) 5. https://doi.org/10.4038/sljas.v26i1.7585.

[24] Z. Yang, F. Kong, M. Zhang, Groundwater contamination by microcystin from toxic cyanobacteria blooms in Lake Chaohu, China, Environ. Monit. Assess. 188 (2016). https://doi.org/10.1007/s10661-016-5289-0.

[25] P. Liu, J. Wei, K. Yang, I.Y. Massey, J. Guo, C. Zhang, F. Yang, Isolation, molecular identification, and characterization of a unique toxic cyanobacterium Microcystis sp. found in Hunan Province, China, J. Toxicol. Environ. Heal. - Part A Curr. Issues. 81 (2018) 1142–1149. https://doi.org/10.1080/15287394.2018.1532716.

[26] H. Feng, T. Clara, F. Huang, J. Wei, F. Yang, Identification and characterization of the dominant *Microcystis* sp. cyanobacteria detected in Lake Dong Ting, China, J. Toxicol. Environ. Heal. Part A. 82 (2019) 1143–1150. https://doi.org/10.1080/15287394.2019.1700604.

[27] J. Xie, G. Yu, X. Xu, S. Li, R. Li, The morphological and molecular detection for the presence of toxic Cylindrospermopsis (Nostocales, Cyanobacteria) in Beijing city, China, J. Oceanol. Limnol. 36 (2018) 263–272. https://doi.org/10.1007/s00343-018-6283-x.

[28] H. Sakai, A. Hao, Y. Iseri, S. Wang, T. Kuba, Z. Zhang, H. Katayama, Occurrence and Distribution of Microcystins in Lake Taihu, China, Sci. World J. 2013 (2013) 1–7. https://doi.org/10.1155/2013/838176.

[29] M.S.*. A.S.. G.B.. S.V. and L.B. Ahmed, Detection of microcystins in nile tilapia (Oreochromis niloticus) from a eutrophic pond containing Microcystis aeruginosa bloom in Dhaka, Bangladesh, Bioresearch Commun. 6 (2020) 887–893. http://www.bioresearchcommunications.com/index.php/brc/article/view/147 (accessed March 4, 2021).

[30] M.A. Affan, H.E.S. Touliabah, S.M. Al-Harbi, N.I. Abdulwassi, A.J. Turki, M.M. Haque, S. Khan, R.A. Elbassat, Influence of environmental parameters on toxic cyanobacterial bloom occurrence in a Lake of Bangladesh, Rend. Lincei. 27 (2016) 473–481. https://doi.org/10.1007/s12210-016-0502-1.

[31] M.S.A.S. Hiller, B. Luckas, (PDF) Microcystis aeruginosa Bloom and the Occurrence of Microcystins (Heptapeptides Hepatotoxins) From an Aquaculture Pond in Gazipur, Bangladesh, Turkish J. Fish. Aquat. Sci. 8 (n.d.) 37–41. https://www.researchgate.net/publication/242603323_Microcystis_aeruginosa_Bloom_and_the_Occurrence_of_Microcystins_Heptapeptides_Hepatotoxins_From_an_Aquaculture_Pond_in_Gazipur_Bangladesh (accessed April 15, 2021).

[32] B. Balaji Prasath, P. Santhanam, R. Nandakumar, T. Jayalakshmi, Detection of cyanotoxins of cyanobacterial (Microcystis aeruginosa) strain using Microtox® Bioluminescence Bioassay, in: Basic Appl. Phytoplankt. Biol., Springer Singapore, 2018: pp. 211–219. https://doi.org/10.1007/978-981-10-7938-2_10.

[33] A.M.P. Anahas, M. Gayathri, G. Muralitharan, Isolation and characterization of microcystin-producing microcystis aeruginosa MBDU 626 from a freshwater bloom sample in Tamil Nadu, South India, in: Microbiol. Res. Agroecosystem Manag., Springer India, 2013: pp. 235–248. https://doi.org/10.1007/978-81-322-1087-0_16.

[34] R. Prasertphon, P. Jitchum, R. Chaichana, Water chemistry, phytoplankton diversity and severe eutrophication with detection of microcystin contents in thai tropical urban ponds, Appl. Ecol. Environ. Res. 18 (2020) 5939–5951. https://doi.org/10.15666/aeer/1804_59395951.

[35] F. Abbas, C. Porojan, M.A.D. Mowe, M. Lehane, S.M. Mitrovic, R.P. Lim, D.C.J. Yeo, A. Furey, Sample extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method development and validation for the quantitative detection of cyanobacterial hepatotoxins and neurotoxins in Singapore's reservoirs, Mar. Freshw. Res. 71 (2020) 673–688. https://doi.org/10.1071/MF19157.

[36] F. Gurbuz, J.S. Metcalf, A.G. Karahan, G.A. Codd, Analysis of dissolved microcystins in surface water samples from Kovada Lake, Turkey, Sci. Total Environ. 407 (2009) 4038–4046. https://doi.org/10.1016/j.scitotenv.2009.02.039.

[37] M. Yilmaz, A.J. Foss, C.O. Miles, M. Özen, N. Demir, M. Balcı, D.G. Beach, Comprehensive multi-technique approach reveals the high diversity of microcystins in field collections and an associated isolate of Microcystis aeruginosa from a Turkish lake, Toxicon. 167 (2019) 87–100. https://doi.org/10.1016/j.toxicon.2019.06.006.

[38] M. Albay, A. Matthiensen, G.A. Codd, Occurrence of toxic blue-green algae in the Kucukcekmece lagoon (Istanbul, Turkey), Environ. Toxicol. 20 (2005) 277–284. https://doi.org/10.1002/tox.20118.

[39] S. Rezaitabar, A. Esmaili Sari, N. Bahramifar, Z. Ramezanpour, Transfer, tissue distribution and bioaccumulation of microcystin LR in the phytoplanktivorous and carnivorous fish in Anzali wetland, with potential health risks to humans, Sci. Total Environ. 575 (2017) 1130–1138. https://doi.org/10.1016/j.scitotenv.2016.09.199.

[40] T. Ikehara, K. Kuniyoshi, H. Yamaguchi, Y. Tanabe, T. Sano, M. Yoshimoto, N. Oshiro, S. Nakashima, M. Yasumoto-Hirose, First Report of Microcystis Strains Producing MC-FR and -WR Toxins in Japan, Toxins (Basel). 11 (2019) 521. https://doi.org/10.3390/toxins11090521.

[41] B. Trung, T.S. Dao, E. Faassen, M. Lürling, Cyanobacterial blooms and microcystins in Southern Vietnam, Toxins (Basel). 10 (2018). https://doi.org/10.3390/toxins10110471.

[42] N. Salmaso, C. Bernard, J.-F. Humbert, R. Akçaalan, M. Albay, A. Ballot, A. Catherine, J. Fastner, K. Häggqvist, M. Horecká, K. Izydorczyk, L. Köker, J. Komárek, S. Maloufi, J. Mankiewicz-Boczek, J.S. Metcalf, A. Quesada, C. Quiblier, C. Yéprémian, Basic Guide to Detection and Monitoring of Potentially Toxic Cyanobacteria, in: Handb. Cyanobacterial Monit. Cyanotoxin Anal., John Wiley & Sons, Ltd, Chichester, UK, 2017: pp. 46–69. https://doi.org/10.1002/9781119068761.ch6.

[43] V. Vogiazi, A. De La Cruz, S. Mishra, V. Shanov, W.R. Heineman, D.D. Dionysiou, A Comprehensive Review: Development of Electrochemical Biosensors for Detection of Cyanotoxins in Freshwater, ACS Sensors. 4 (2019) 1151–1173. https://doi.org/10.1021/acssensors.9b00376.

[44] N. Bouaïcha, C.O. Miles, D.G. Beach, Z. Labidi, A. Djabri, N.Y. Benayache, T. Nguyen-Quang, Structural Diversity, Characterization and Toxicology of Microcystins, Toxins 2019, Vol. 11, Page 714. 11 (2019) 714. https://doi.org/10.3390/TOXINS11120714.

[45] A. Zastepa, Z.E. Taranu, L.E. Kimpe, J.M. Blais, I. Gregory-Eaves, R.W. Zurawell, F.R. Pick, Reconstructing a long-term record of microcystins from the analysis of lake sediments, Sci. Total Environ. 579 (2017) 893–901. https://doi.org/10.1016/j.scitotenv.2016.10.211.

[46] J.L. Graham, K.A. Loftin, M.T. Meyer, A.C. Ziegler, Cyanotoxin mixtures and taste-

and-odor compounds in cyanobacterial blooms from the midwestern united states, Environ. Sci. Technol. 44 (2010) 7361–7368. https://doi.org/10.1021/es1008938.

[47] IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins - PubMed, (n.d.). https://pubmed.ncbi.nlm.nih.gov/21141240/ (accessed March 4, 2021).

[48] W.J. Fischer, S. Altheimer, V. Cattori, P.J. Meier, D.R. Dietrich, B. Hagenbuch, Organic anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin, Toxicol. Appl. Pharmacol. 203 (2005) 257–263. https://doi.org/10.1016/j.taap.2004.08.012.

[49] J. Zhao, H. qiang Chen, H. fang Yang, Y. Li, D. jiao Chen, Y. jing Huang, L. xiong He, C. fen Zheng, L. qiao Wang, J. Wang, N. Zhang, J. Cao, J. yi Liu, W. qun Shu, W. bin Liu, Epigenetic silencing of ALX4 regulates microcystin-LR induced hepatocellular carcinoma through the P53 pathway, Sci. Total Environ. 683 (2019) 317–330. https://doi.org/10.1016/j.scitotenv.2019.05.144.

[50] L. Chen, J. Chen, X. Zhang, P. Xie, A review of reproductive toxicity of microcystins, J. Hazard. Mater. 301 (2016) 381–399. https://doi.org/10.1016/j.jhazmat.2015.08.041.

[51] World Health Organization (WHO), Cyanobacterial toxins: microcystins, Guidel. Drink. Qual. Guidel. Safe Recreat. Water Environ. (2020) 63. https://apps.who.int/iris/bitstream/handle/10665/338066/WHO-HEP-ECH-WSH-2020.6-eng.pdf?sequence=1&isAllowed=y.

[52] 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. https://doi.org/10.1007/s00204-016-1913-6.

[53] L. Spoof, J. Meriluoto, Rapid separation of microcystins and nodularin using a monolithic silica C18 column, J. Chromatogr. A. 947 (2002) 237–245. https://doi.org/10.1016/S0021-9673(01)01605-3.

[54] Y. Chen, D. Shen, D. Fang, Nodularins in poisoning, Clin. Chim. Acta. 425 (2013) 18–29. https://doi.org/10.1016/j.cca.2013.07.005.

[55] H. Ufelmann, T. Krüger, B. Luckas, D. Schrenk, Human and rat hepatocyte toxicity and protein phosphatase 1 and 2A inhibitory activity of naturally occurring desmethyl-microcystins and nodularins, Toxicology. 293 (2012) 59–67. https://doi.org/10.1016/j.tox.2011.12.011.

[56] G. Chen, L. Wang, W. Li, Q. Zhang, T. Hu, Nodularin induced oxidative stress

contributes to developmental toxicity in zebrafish embryos, Ecotoxicol. Environ. Saf. 194 (2020). https://doi.org/10.1016/j.ecoenv.2020.110444.

[57] M. Karjalainen, J.P. Pääkkönen, H. Peltonen, V. Sipiä, T. Valtonen, M. Viitasalo, Nodularin concentrations in Baltic Sea zooplankton and fish during a cyanobacterial bloom, Mar. Biol. 155 (2008) 483–491. https://doi.org/10.1007/s00227-008-1046-4.

[58] J. Lee, S. Lee, X. Jiang, Cyanobacterial Toxins in Freshwater and Food: Important Sources of Exposure to Humans, Annu. Rev. Food Sci. Technol. 8 (2017) 281–304. https://doi.org/10.1146/annurev-food-030216-030116.

[59] I. Ohtani, R.E. Moore, M.T.C. Runnegar, Cylindrospermopsin: A Potent Hepatotoxin from the Blue-Green Alga Cylindrospermopsis raciborskii, J. Am. Chem. Soc. 114 (1992) 7941–7942. https://doi.org/10.1021/ja00046a067.

[60] I.R. Falconer, A.R. Humpage, Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins, in: Environ. Toxicol., Environ Toxicol, 2006: pp. 299–304. https://doi.org/10.1002/tox.20194.

[61] D. Gutiérrez-Praena, S. Pichardo, Á. Jos, F.J. Moreno, A.M. Cameán, Biochemical and pathological toxic effects induced by the cyanotoxin Cylindrospermopsin on the human cell line Caco-2, Water Res. 46 (2012) 1566–1575. https://doi.org/10.1016/j.watres.2011.12.044.

[62] D. Gutiérrez-Praena, Á. Jos, S. Pichardo, I.M. Moreno, A.M. Cameán, Presence and bioaccumulation of microcystins and cylindrospermopsin in food and the effectiveness of some cooking techniques at decreasing their concentrations: A review, Food Chem. Toxicol. 53 (2013) 139–152. https://doi.org/10.1016/j.fct.2012.10.062.

[63] S. Pichardo, A.M. Cameán, A. Jos, In vitro toxicological assessment of cylindrospermopsin: A review, Toxins (Basel). 9 (2017). https://doi.org/10.3390/toxins9120402.

[64] K. Hercog, M. Štampar, A. Štern, M. Filipič, B. Žegura, Application of advanced HepG2 3D cell model for studying genotoxic activity of cyanobacterial toxin cylindrospermopsin, Environ. Pollut. 265 (2020) 114965. https://doi.org/10.1016/j.envpol.2020.114965.

[65] W. World Health Organization, Cyanobacterial toxins: Saxitoxins Background document for development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments, (2019) 2–16.

[66] L.E. Llewellyn, Saxitoxin, a toxic marine natural product that targets a multitude of receptors, Nat. Prod. Rep. 23 (2006) 200–222. https://doi.org/10.1039/B501296C.

[67] K. O'Neill, I.F. Musgrave, A. Humpage, Low dose extended exposure to saxitoxin and its potential neurodevelopmental effects: A review, Environ. Toxicol. Pharmacol. 48 (2016) 7–16. https://doi.org/10.1016/j.etap.2016.09.020.

[68] D. Andrinolo, L.F. Michea, N. Lagos, Toxic effects, pharmacokinetics and clearance of saxitoxin, a component of paralytic shellfish poison (PSP), in cats, Toxicon. 37 (1999) 447–464. https://doi.org/10.1016/S0041-0101(98)00173-1.

[69] E. Valério, S. Chaves, R. Tenreiro, Diversity and impact of prokaryotic toxins on aquatic environments: A review, Toxins (Basel). 2 (2010) 2359–2410. https://doi.org/10.3390/toxins2102359.

[70] L. V. D'Anglada, Editorial on the special issue "Harmful Algal Blooms (HABs) and public health: Progress and current challenges," Toxins (Basel). 7 (2015) 4437–4441. https://doi.org/10.3390/toxins7114437.

[71] M. Rutkowska, J. Płotka-Wasylka, T. Majchrzak, W. Wojnowski, H. Mazur-Marzec, J. Namieśnik, Recent trends in determination of neurotoxins in aquatic environmental samples, TrAC - Trends Anal. Chem. 112 (2019) 112–122. https://doi.org/10.1016/j.trac.2019.01.001.

[72] T. Yoshida, Y. Makita, S. Nagata, T. Tsutsumi, F. Yoshida, M. Sekijima, S. Tamura, Y. Ueno, Acute oral toxicity of microcystin-LR, a cyanobacterial hepatotoxin, in mice, Nat. Toxins. 5 (1998) 91–95. https://doi.org/10.1002/1522-7189(1997)5:3<91::aid-nt1>3.0.co;2-h.

[73] I.Y. Massey, P. Wu, J. Wei, J. Luo, P. Ding, H. Wei, F. Yang, A Mini-Review on Detection Methods of Microcystins, Toxins (Basel). 12 (2020) 641. https://doi.org/10.3390/toxins12100641.

[74] M. Masango, J. Myburgh, C. Botha, L. Labuschagne, D. Naicker, A comparison of in vivo and in vitro assays to assess the toxicity of algal blooms, Water Res. 42 (2008) 3241–3248. https://doi.org/10.1016/j.watres.2007.10.033.

[75] M. Forastier, Y. Zalocar, … D.A.-R. de biologia, undefined 2016, Occurrence and toxicity of Microcystis aeruginosa (Cyanobacteria) in the Paraná River, downstream of the Yacyretá dam (Argentina), Scielo.Sa.Cr. (n.d.). https://www.scielo.sa.cr/scielo.php?pid=S0034-77442016000100203&script=sci_arttext (accessed March 6, 2021).

[76] B. Maršálek, L. Bláha, Comparison of 17 biotests for detection of cyanobacterial toxicity, Environ. Toxicol. 19 (2004) 310–317. https://doi.org/10.1002/tox.20020.

[77] C. Moreira, V. Ramos, J. Azevedo, V. Vasconcelos, Methods to detect cyanobacteria

and their toxins in the environment, Appl. Microbiol. Biotechnol. 98 (2014) 8073–8082. https://doi.org/10.1007/s00253-014-5951-9.

[78] C. Moore, J. Juan, Y. Lin, C. Gaskill, B. Puschner, Comparison of Protein Phosphatase Inhibition Assay with LC-MS/MS for Diagnosis of Microcystin Toxicosis in Veterinary Cases, Mar. Drugs. 14 (2016) 54. https://doi.org/10.3390/md14030054.

[79] L. Brient, N. Ben Gamra, M. Periot, M. Roumagnac, P. Zeller, M. Bormans, A. Méjean, O. Ploux, I.C. Biegala, Rapid Characterization of Microcystin-Producing Cyanobacteria in Freshwater Lakes by TSA-FISH (Tyramid Signal Amplification-Fluorescent In Situ Hybridization), Front. Environ. Sci. 5 (2017) 43. https://doi.org/10.3389/fenvs.2017.00043.

[80] Y. Blanco, M. Moreno-Paz, V. Parro, Experimental protocol for detecting Cyanobacteria in liquid and solid samples with an antibody microarray chip, J. Vis. Exp. 2017 (2017) e54994. https://doi.org/10.3791/54994.

[81] J.A. Baker, B. Entsch, B.A. Neilan, D.B. McKay, Monitoring changing toxigenicity of a cyanobacterial bloom by molecular methods, Appl. Environ. Microbiol. 68 (2002) 6070–6076. https://doi.org/10.1128/AEM.68.12.6070-6076.2002.

[82] K. MeiÎ²ner, E. Dittmann, T. BÃ¶rner, Toxic and non-toxic strains of the cyanobacterium Microcystis aeruginosa contain sequences homologous to peptide synthetase genes , FEMS Microbiol. Lett. 135 (1996) 295–303. https://doi.org/10.1111/j.1574-6968.1996.tb08004.x.

[83] S.G. Kim, S.K. Rhee, C.Y. Ahn, S.R. Ko, G.G. Choi, J.W. Bae, Y.H. Park, H.M. Oh, Determination of cyanobacterial diversity during algal blooms in Daechung Reservoir, Korea, on the basis of cpcBA intergenic spacer region analysis, Appl. Environ. Microbiol. 72 (2006) 3252–3258. https://doi.org/10.1128/AEM.72.5.3252-3258.2006.

[84] J.R. Johansen, J. Mareš, N. Pietrasiak, M. Bohunická, J. Zima, L. Štenclová, T. Hauer, Highly divergent 16S rRNA sequences in ribosomal operons of Scytonema hyalinum (Cyanobacteria), PLoS One. 12 (2017) e0186393. https://doi.org/10.1371/journal.pone.0186393.

[85] K. Rudi, O.M. Skulberg, K.S. Jakobsen, Evolution of cyanobacteria by exchange of genetic material among phyletically related strains, J. Bacteriol. 180 (1998) 3453–3461. https://doi.org/10.1128/jb.180.13.3453-3461.1998.

[86] V. Gaget, S. Gribaldo, N.T. De Marsac, An rpoB signature sequence provides unique resolution for the molecular typing of cyanobacteria, Int. J. Syst. Evol. Microbiol. 61 (2011) 170–183. https://doi.org/10.1099/ijs.0.019018-0.

[87] D. Weller, Detection, identification and toxigenicity of cyanobacteria in New Zealand lakes using PCR-based methods, New Zeal. J. Mar. Freshw. Res. 45 (2011) 651–664. https://doi.org/10.1080/00288330.2011.570769.

[88] E. Lee, U.M. Ryan, P. Monis, G.B. McGregor, A. Bath, C. Gordon, A. Paparini, Polyphasic identification of cyanobacterial isolates from Australia, Water Res. 59 (2014) 248–261. https://doi.org/10.1016/j.watres.2014.04.023.

[89] A. Michinaka, H.K. Yen, Y.T. Chiu, H.W. Tsao, T.F. Lin, Rapid on-site multiplex assays for total and toxigenic Microcystis using real-time PCR with microwave cell disruption, Water Sci. Technol. 66 (2012) 1247–1252. https://doi.org/10.2166/wst.2012.308.

[90] L. Lei, M. Lei, Y. Lu, L. Peng, B.P. Han, Development of real-time PCR for quantification of Cylindrospermopsis raciborskii cells and potential cylindrospermopsin-producing genotypes in subtropicalreservoirs of southern China, J. Appl. Phycol. 31 (2019) 3749–3758. https://doi.org/10.1007/s10811-019-01898-3.

[91] W. Zhang, I. Lou, W.K. Ung, Y. Kong, K.M. Mok, Application of PCR and real-time PCR for monitoring cyanobacteria, Microcystis spp. and Cylindrospermopsis raciborskii in Macau freshwater reservoir, Front. Earth Sci. 8 (2014) 291–301. https://doi.org/10.1007/s11707-013-0409-4.

[92] S.H. Te, E.Y. Chen, K.Y.H. Gin, Comparison of quantitative PCR and droplet digital PCR multiplex assays for two genera of bloom-forming cyanobacteria, Cylindrospermopsis and Microcystis, Appl. Environ. Microbiol. 81 (2015) 5203–5211. https://doi.org/10.1128/AEM.00931-15.

[93] Y.T. Chiu, Y.H. Chen, T.S. Wang, H.K. Yen, T.F. Lin, A qPCR-based tool to diagnose the presence of harmful cyanobacteria and cyanotoxins in drinking water sources, Int. J. Environ. Res. Public Health. 14 (2017). https://doi.org/10.3390/ijerph14050547.

[94] Á. Barón-Sola, Y. Ouahid, F.F. del Campo, Detection of potentially producing cylindrospermopsin and microcystin strains in mixed populations of cyanobacteria by simultaneous amplification of cylindrospermopsin and microcystin gene regions, Ecotoxicol. Environ. Saf. 75 (2012) 102–108. https://doi.org/10.1016/j.ecoenv.2011.08.022.

[95] R. Ruvindy, C.J. Bolch, L. MacKenzie, K.F. Smith, S.A. Murray, qPCR Assays for the Detection and Quantification of Multiple Paralytic Shellfish Toxin-Producing Species of Alexandrium, Front. Microbiol. 9 (2018) 3153. https://doi.org/10.3389/fmicb.2018.03153.

[96] S.A. Murray, R. Ruvindy, G.S. Kohli, D.M. Anderson, M.L. Brosnahan, Evaluation of sxtA and rDNA qPCR assays through monitoring of an inshore bloom of Alexandrium catenella Group 1, Sci. Rep. 9 (2019) 1–12. https://doi.org/10.1038/s41598-019-51074-3.

[97] N. Rigamonti, L. Aubriot, F. Martigani, S. Bonilla, C. Piccini, Effect of nutrient availability on cylindrospermopsin gene expression and toxin production in Cylindrospermopsis raciborskii, Aquat. Microb. Ecol. 82 (2018) 105–110. https://doi.org/10.3354/ame01877.

[98] L.T. Kelly, S.A. Wood, T.G. McAllister, K.G. Ryan, Development and application of a quantitative PCR assay to assess genotype dynamics and anatoxin content in Microcoleus autumnalis-dominated mats, Toxins (Basel). 10 (2018) 431. https://doi.org/10.3390/toxins10110431.

[99] M. Ramya, M. Kayalvizhi, G. Haripriya, P. Rathinasabapathi, Detection of microcystin-producing cyanobacteria in water samples using loop-mediated isothermal amplification targeting mcyB gene, 3 Biotech. 8 (2018). https://doi.org/10.1007/s13205-018-1402-0.

[100] A.B.F. Pacheco, I.A. Guedes, S.M.F.O. Azevedo, Is qPCR a reliable indicator of cyanotoxin risk in freshwater?, Toxins (Basel). 8 (2016). https://doi.org/10.3390/toxins8060172.

[101] G.C. WP Brooks, IMMUNOLOGICAL AND TOXICOLOGICAL STUDIES ON MICROCYSTIS-AERUGINOSA PEPTIDE TOXIN, Br. Phycol. J. 22 (n.d.) 301.

[102] M. Picardo, D. Filatova, O. Nuñez, M. Farré, Recent advances in the detection of natural toxins in freshwater environments, TrAC - Trends Anal. Chem. 112 (2019) 75–86. https://doi.org/10.1016/j.trac.2018.12.017.

[103] H. Yang, R. Dai, H. Zhang, C. Li, X. Zhang, J. Shen, K. Wen, Z. Wang, Production of monoclonal antibodies with broad specificity and development of an immunoassay for microcystins and nodularin in water, Anal. Bioanal. Chem. 408 (2016) 6037–6044. https://doi.org/10.1007/s00216-016-9692-8.

[104] S. Devlin, J.P. Meneely, B. Greer, K. Campbell, V. Vasconcelos, C.T. Elliott, Production of a broad specificity antibody for the development and validation of an optical SPR screening method for free and intracellular microcystins and nodularin in cyanobacteria cultures, Talanta. 122 (2014) 8–15. https://doi.org/10.1016/j.talanta.2013.12.065.

[105] N. Lu, L. Ling, T. Guan, L. Wang, D. Wang, J. Zhou, T. Ruan, X. Shen, X. Li, Y. Sun,

H. Lei, Broad-specificity ELISA with a heterogeneous strategy for sensitive detection of microcystins and nodularin, Toxicon. 175 (2020) 44–48. https://doi.org/10.1016/j.toxicon.2019.12.003.

[106] L. Chen, R. Tan, Y. Zhou, L. Zhang, S. Zhang, X. Li, Y. Cong, H. Li, P. Sun, H. Ueda, J. Dong, Development of an Open sandwich ELISA for the detection of microcystin-LR, Microchem. J. 158 (2020) 105325. https://doi.org/10.1016/j.microc.2020.105325.

[107] S.E. McNamee, C.T. Elliott, B. Greer, M. Lochhead, K. Campbell, Development of a planar waveguide microarray for the monitoring and early detection of five harmful algal toxins in water and cultures, Environ. Sci. Technol. 48 (2014) 13340–13349. https://doi.org/10.1021/es504172j.

[108] C.T. Elliott, C.H. Redshaw, S.E. George, K. Campbell, First development and characterisation of polyclonal and monoclonal antibodies to the emerging fresh water toxin cylindrospermopsin, Harmful Algae. 24 (2013) 10–19. https://doi.org/10.1016/j.hal.2012.12.005.

[109] L. Geis-Asteggiante, S.J. Lehotay, L.L. Fortis, G. Paoli, C. Wijey, H. Heinzen, Development and validation of a rapid method for microcystins in fish and comparing LC-MS/MS results with ELISA, Anal. Bioanal. Chem. 2011 4018. 401 (2011) 2617–2630. https://doi.org/10.1007/S00216-011-5345-0.

[110] E.P. Preece, B.C. Moore, M.E. Swanson, F.J. Hardy, Identifying best methods for routine ELISA detection of microcystin in seafood, Environ. Monit. Assess. 2015 1872. 187 (2015) 1–10. https://doi.org/10.1007/S10661-014-4255-Y.

[111] S.B. Watson, A. Zastepa, G.L. Boyer, E. Matthews, Algal bloom response and risk management: On-site response tools, Toxicon. 129 (2017) 144–152. https://doi.org/10.1016/J.TOXICON.2017.02.005.

[112] J.J. Zhang, T.F. Kang, Y.C. Hao, L.P. Lu, S.Y. Cheng, Electrochemiluminescent immunosensor based on CdS quantum dots for ultrasensitive detection of microcystin-LR, Sensors Actuators, B Chem. 214 (2015) 117–123. https://doi.org/10.1016/j.snb.2015.03.019.

[113] C. Gan, L. Ling, Z. He, H. Lei, Y. Liu, In-situ assembly of biocompatible core-shell hierarchical nanostructures sensitized immunosensor for microcystin-LR detection, Biosens. Bioelectron. 78 (2016) 381–389. https://doi.org/10.1016/j.bios.2015.11.072.

[114] H. Aldewachi, T. Chalati, M.N. Woodroofe, N. Bricklebank, B. Sharrack, P. Gardiner, Gold nanoparticle-based colorimetric biosensors, Nanoscale. 10 (2018) 18–33. https://doi.org/10.1039/c7nr06367a.

[115] G. Liu, M. Lu, X. Huang, T. Li, D. Xu, Application of gold-nanoparticle colorimetric sensing to rapid food safety screening, Sensors (Switzerland). 18 (2018). https://doi.org/10.3390/s18124166.

[116] Y. Zhang, M. Chen, H. Li, F. Yan, P. Pang, H. Wang, Z. Wu, W. Yang, A molybdenum disulfide/gold nanorod composite-based electrochemical immunosensor for sensitive and quantitative detection of microcystin-LR in environmental samples, Sensors Actuators, B Chem. 244 (2017) 606–615. https://doi.org/10.1016/j.snb.2017.01.030.

[117] L. Yao, L. He, Y. Yang, Y. Zhang, Z. Liu, L. Liang, Y. Piao, Nanobiochar paper based electrochemical immunosensor for fast and ultrasensitive detection of microcystin-LR, Sci. Total Environ. 750 (2021) 141692. https://doi.org/10.1016/j.scitotenv.2020.141692.

[118] Y.X. Hu, F. Shang, Y. Liu, S. Wang, Y. Hu, Z. Guo, A label-free electrochemical immunosensor based on multi-functionalized graphene oxide for ultrasensitive detection of microcystin-LR, Chem. Pap. 72 (2018) 71–79. https://doi.org/10.1007/s11696-017-0258-9.

[119] P. Pang, X. Teng, M. Chen, Y. Zhang, H. Wang, C. Yang, W. Yang, C.J. Barrow, Ultrasensitive enzyme-free electrochemical immunosensor for microcystin-LR using molybdenum disulfide/gold nanoclusters nanocomposites as platform and Au@Pt core-shell nanoparticles as signal enhancer, Sensors Actuators, B Chem. 266 (2018) 400–407. https://doi.org/10.1016/j.snb.2018.03.154.

[120] R. Almeida de Oliveira, N. Zanato, I. Cruz Vieira, Label-free Immunosensor for the Determination of Microcystin-LR in Water, Electroanalysis. 32 (2020) 2166–2173. https://doi.org/10.1002/elan.202060041.

[121] J. Zhang, Z. Xiong, Z. Chen, Ultrasensitive electrochemical microcystin-LR immunosensor using gold nanoparticle functional polypyrrole microsphere catalyzed silver deposition for signal amplification, Sensors Actuators, B Chem. 246 (2017) 623–630. https://doi.org/10.1016/j.snb.2017.02.134.

[122] C. Gan, B. Wang, J. Huang, A. Qileng, Z. He, H. Lei, W. Liu, Y. Liu, Multiple amplified enzyme-free electrochemical immunosensor based on G-quadruplex/hemin functionalized mesoporous silica with redox-active intercalators for microcystin-LR detection, Biosens. Bioelectron. 98 (2017) 126–133. https://doi.org/10.1016/j.bios.2017.06.038.

[123] B. Liu, X.P. Wang, X.Y. Sun, C.X. Yang, P. Li, A sensitive immunosensor for

microcystin-(leucine-arginine) based on fluorescent self-assembled multilayers, Anal. Methods. 9 (2017) 282–286. https://doi.org/10.1039/c6ay02686a.

[124] W. Zhang, C. Han, B. Jia, C. Saint, M. Nadagouda, P. Falaras, L. Sygellou, V. Vogiazi, D.D. Dionysiou, A 3D graphene-based biosensor as an early microcystin-LR screening tool in sources of drinking water supply, Electrochim. Acta. 236 (2017) 319–327. https://doi.org/10.1016/j.electacta.2017.03.161.

[125] S. Bratakou, G.-P. Nikoleli, C.G. Siontorou, D.P. Nikolelis, S. Karapetis, N. Tzamtzis, Development of an Electrochemical Biosensor for the Rapid Detection of Saxitoxin Based on Air Stable Lipid Films with Incorporated Anti-STX Using Graphene Electrodes, Electroanalysis. 29 (2017) 990–997. https://doi.org/10.1002/elan.201600652.

[126] L. Hou, Y. Ding, L. Zhang, Y. Guo, M. Li, Z. Chen, X. Wu, An ultrasensitive competitive immunosensor for impedimetric detection of microcystin-LR via antibody-conjugated enzymatic biocatalytic precipitation, Sensors Actuators, B Chem. 233 (2016) 63–70. https://doi.org/10.1016/j.snb.2016.04.034.

[127] W. Zhang, B. Jia, H. Furumai, Fabrication of graphene film composite electrochemical biosensor as a pre-screening algal toxin detection tool in the event of water contamination, Sci. Rep. 8 (2018) 1–10. https://doi.org/10.1038/s41598-018-28959-w.

[128] X. Jin, J. Chen, X. Zeng, L.J. Xu, Y. Wu, F.F. Fu, A signal-on magnetic electrochemical immunosensor for ultra-sensitive detection of saxitoxin using palladium-doped graphitic carbon nitride-based non-competitive strategy, Biosens. Bioelectron. 128 (2019) 45–51. https://doi.org/10.1016/j.bios.2018.12.036.

[129] C. Gan, Z. Sun, L. Ling, Z. He, H. Lei, Y. Liu, Construction of portable electrochemical immunosensors based on graphene hydrogel@polydopamine for microcystin-LR detection using multi-mesoporous carbon sphere-enzyme labels, RSC Adv. 6 (2016) 51662–51669. https://doi.org/10.1039/c6ra07881h.

[130] T. Guan, W. Huang, N. Xu, Z. Xu, L. Jiang, M. Li, X. Wei, Y. Liu, X. Shen, X. Li, C. Yi, H. Lei, Point-of-need detection of microcystin-LR using a smartphone-controlled electrochemical analyzer, Sensors Actuators, B Chem. 294 (2019) 132–140. https://doi.org/10.1016/j.snb.2019.05.028.

[131] M. Li, S.K. Paidi, E. Sakowski, S. Preheim, I. Barman, Ultrasensitive Detection of Hepatotoxic Microcystin Production from Cyanobacteria Using Surface-Enhanced Raman Scattering Immunosensor, ACS Sensors. 4 (2019) 1203–1210. https://doi.org/10.1021/acssensors.8b01453.

[132] K. Zhang, K. Dai, R. Bai, Y. Ma, Y. Deng, D. Li, X. Zhang, R. Hu, Y. Yang, A competitive microcystin-LR immunosensor based on Au NPs@metal-organic framework (MIL-101), Chinese Chem. Lett. 30 (2019) 664–667. https://doi.org/10.1016/j.cclet.2018.10.021.

[133] R. Kurmayer, G. Christiansen, J. Fastner, T. Borner, Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium Planktothrix spp., Environ. Microbiol. 6 (2004) 831–841. https://doi.org/10.1111/j.1462-2920.2004.00626.x.

[134] V. Vogiazi, A. De La Cruz, S. Mishra, V. Shanov, W.R. Heineman, D.D. Dionysiou, A Comprehensive Review: Development of Electrochemical Biosensors for Detection of Cyanotoxins in Freshwater, ACS Sensors. 4 (2019) 1151–1173. https://doi.org/10.1021/acssensors.9b00376.

[135] C. Giménez-Campillo, M. Pastor-Belda, N. Campillo, N. Arroyo-Manzanares, M. Hernández-Córdoba, P. Viñas, Determination of Cyanotoxins and Phycotoxins in Seawater and Algae-Based Food Supplements Using Ionic Liquids and Liquid Chromatography with Time-Of-Flight Mass Spectrometry, Toxins 2019, Vol. 11, Page 610. 11 (2019) 610. https://doi.org/10.3390/TOXINS11100610.

[136] V. Rodríguez, M. Yonamine, E. Pinto, Determination of anatoxin-a in environmental water samples by solid-phase microextraction and gas chromatography-mass spectrometry, J. Sep. Sci. 29 (2006) 2085–2090. https://doi.org/10.1002/JSSC.200500488.

[137] G.P. Mashile, P.N. Nomngongo, Recent Application of Solid Phase Based Techniques for Extraction and Preconcentration of Cyanotoxins in Environmental Matrices, Http://Dx.Doi.Org/10.1080/10408347.2016.1225255. 47 (2016) 119–126. https://doi.org/10.1080/10408347.2016.1225255.

[138] S. Perez, D.A.-T.T. in A. Chemistry, undefined 2005, Recent advances in the sample preparation, liquid chromatography tandem mass spectrometric analysis and environmental fate of microcystins in water, Elsevier. (n.d.). https://www.sciencedirect.com/science/article/pii/S0165993605001147 (accessed September 3, 2021).

[139] J.M.-A.C. Acta, undefined 1997, Chromatography of microcystins, Elsevier. (n.d.). https://www.sciencedirect.com/science/article/pii/S0003267097001311 (accessed September 3, 2021).

[140] L. Lawton, C. Edwards, G.C.- Analyst, undefined 1994, Extraction and high-

performance liquid chromatographic method for the determination of microcystins in raw and treated waters, Pubs.Rsc.Org. 11 (1994) 9. https://pubs.rsc.org/en/content/articlehtml/1994/an/an9941901525 (accessed September 3, 2021).

[141] A. Mata, J. Ferreira, B. Oliveira, M.B.-F. Chemistry, undefined 2015, Bottled water: Analysis of mycotoxins by LC–MS/MS, Elsevier. (n.d.). https://www.sciencedirect.com/science/article/pii/S0308814614020019 (accessed September 3, 2021).

[142] H. Thuret-Benoist, V. Pallier, G. Feuillade-Cathalifaud, Quantification of microcystins in natural waters by HPLC-UV after a pre-concentration step: validation of the analytical performances and study of the interferences, Environ. Toxicol. Pharmacol. 72 (2019). https://doi.org/10.1016/j.etap.2019.103223.

[143] Q. Li, L. Lian, X. Wang, R. Wang, Y. Tian, X. Guo, D. Lou, Analysis of microcystins using high-performance liquid chromatography and magnetic solid-phase extraction with silica-coated magnetite with cetylpyridinium chloride, J. Sep. Sci. 40 (2017) 1644–1650. https://doi.org/10.1002/jssc.201601407.

[144] H.R. ez. Shamsollahi, M. Alimohammadi, R. Nabizadeh, S. Nazmara, A.H. ossei. Mahvi, Measurement of microcystin -LR in water samples using improved HPLC method, Glob. J. Health Sci. 7 (2015) 66–70. https://doi.org/10.5539/gjhs.v7n2p66.

[145] R. Teta, G. Della Sala, E. Glukhov, L. Gerwick, W.H. Gerwick, A. Mangoni, V. Costantino, Combined LC-MS/MS and Molecular Networking Approach Reveals New Cyanotoxins from the 2014 Cyanobacterial Bloom in Green Lake, Seattle, Environ. Sci. Technol. 49 (2015) 14301–14310. https://doi.org/10.1021/acs.est.5b04415.

[146] S.K. Zervou, C. Christophoridis, T. Kaloudis, T.M. Triantis, A. Hiskia, New SPE-LC-MS/MS method for simultaneous determination of multi-class cyanobacterial and algal toxins, J. Hazard. Mater. 323 (2017) 56–66. https://doi.org/10.1016/j.jhazmat.2016.07.020.

[147] A. Roy-Lachapelle, S. Vo Duy, G. Munoz, Q.T. Dinh, E. Bahl, D.F. Simon, S. Sauvé, Analysis of multiclass cyanotoxins (microcystins, anabaenopeptins, cylindrospermopsin and anatoxins) in lake waters using on-line SPE liquid chromatography high-resolution Orbitrap mass spectrometry, Anal. Methods. 11 (2019) 5289–5300. https://doi.org/10.1039/c9ay01132c.

[148] X. Ortiz, E. Korenkova, K.J. Jobst, K.A. MacPherson, E.J. Reiner, A high throughput targeted and non-targeted method for the analysis of microcystins and anatoxin-A

using on-line solid phase extraction coupled to liquid chromatography–quadrupole time-of-flight high resolution mass spectrometry, Anal. Bioanal. Chem. 409 (2017) 4959–4969. https://doi.org/10.1007/s00216-017-0437-0.

[149] A.J. Foss, C.O. Miles, I.A. Samdal, K.E. Løvberg, A.L. Wilkins, F. Rise, J.A.H. Jaabæk, P.C. McGowan, M.T. Aubel, Analysis of free and metabolized microcystins in samples following a bird mortality event, Harmful Algae. 80 (2018) 117–129. https://doi.org/10.1016/j.hal.2018.10.006.

[150] A.I. Prieto, R. Guzmán-Guillén, Á. Jos, A.M. Cameán, J.M. de la Rosa, J.A. González-Pérez, Detection of cylindrospermopsin and its decomposition products in raw and cooked fish (Oreochromis niloticus) by analytical pyrolysis (Py-GC/MS), Chemosphere. 244 (2020). https://doi.org/10.1016/j.chemosphere.2019.125469.

[151] C. León, G.A. Peñuela, Detected cyanotoxins by UHPLC MS/MS technique in tropical reservoirs of northeastern Colombia, Toxicon. 167 (2019) 38–48. https://doi.org/10.1016/j.toxicon.2019.06.010.

[152] Y. Yue, B. Zhu, L. Lun, N. Xu, Quantifications of saxitoxin concentrations in bivalves by high performance liquid chromatography-tandem mass spectrometry with the purification of immunoaffinity column, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1147 (2020) 122133. https://doi.org/10.1016/j.jchromb.2020.122133.

[153] J. Li, J. Zhu, Y. Li, T. Huang, Y. Li, <scp>l</scp> -Cysteine-modified magnetic microspheres for extraction and quantification of saxitoxin in rat plasma with liquid chromatography and tandem mass spectrometry, J. Sep. Sci. 43 (2020) 2429–2435. https://doi.org/10.1002/jssc.202000070.

[154] X. min Xu, B. fen Huang, J. jiao Xu, Z. xuan Cai, J. Zhang, Q. Chen, J.L. Han, Fast and quantitative determination of saxitoxin and neosaxitoxin in urine by ultra performance liquid chromatography-triple quadrupole mass spectrometry based on the cleanup of solid phase extraction with hydrophilic interaction mechanism, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1072 (2018) 267–272. https://doi.org/10.1016/j.jchromb.2017.11.032.

[155] W.A. Bragg, A. Garrett, E.I. Hamelin, R.M. Coleman, K. Campbell, C.T. Elliott, R.C. Johnson, Quantitation of saxitoxin in human urine using immunocapture extraction and LC-MS, Futur. Sci. 10 (2018) 229–239. https://doi.org/10.4155/bio-2017-0156.

[156] Y. Zhang, J.K. Whalen, S. Vo Duy, G. Munoz, B.R. Husk, S. Sauvé, Improved extraction of multiclass cyanotoxins from soil and sensitive quantification with on-line purification liquid chromatography tandem mass spectrometry, Talanta. 216 (2020)

120923. https://doi.org/10.1016/j.talanta.2020.120923.

[157] D. Filatova, O. Núñez, M. Farré, Ultra-Trace Analysis of Cyanotoxins by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry, Toxins (Basel). 12 (2020) 247. https://doi.org/10.3390/toxins12040247.

[158] N.H. Tran, Y. Li, M. Reinhard, K.C. Goh, N.H.B. Sukarji, L. You, Y. He, K.Y.H. Gin, Quantification of cylindrospermopsin, anatoxin-a and homoanatoxin-a in cyanobacterial bloom freshwater using direct injection/SPE coupled with UPLC-MS/MS, Sci. Total Environ. 731 (2020). https://doi.org/10.1016/j.scitotenv.2020.139014.

[159] C. Lihui, J. Yuzhu, Z. Bin, P. Qiuren, C. Qinqin, C. Weipeng, Determination of cylindrospermopsin, nodularin and microcystins in freshwater fish by dispersive solid phase extraction- liquid chromatography-tandem mass spectrometry, Chinese J. Chromatogr. (Se Pu). 37 (2020) 723–728. https://doi.org/10.3724/SP.J.1123.2019.01022.

[160] D.S.W. Palagama, R.E. West, D. Isailovic, Improved solid-phase extraction protocol and sensitive quantification of six microcystins in water using an HPLC-orbitrap mass spectrometry system, Anal. Methods. 9 (2017) 2021–2030. https://doi.org/10.1039/c6ay03459d.

[161] Z.Y. Qian, Z.G. Li, J. Ma, T. ting Gong, Q.M. Xian, Analysis of trace microcystins in vegetables using matrix solid-phase dispersion followed by high performance liquid chromatography triple-quadrupole mass spectrometry detection, Talanta. 173 (2017) 101–106. https://doi.org/10.1016/j.talanta.2017.05.079.

[162] M. Manubolu, J. Lee, K.M. Riedl, Z.X. Kua, L.P. Collart, S.A. Ludsin, Optimization of extraction methods for quantification of microcystin-LR and microcystin-RR in fish, vegetable, and soil matrices using UPLC–MS/MS, Harmful Algae. 76 (2018) 47–57. https://doi.org/10.1016/j.hal.2018.04.009.

[163] R.E. Wharton, G. Ojeda-Torres, B. Cunningham, M.C. Feyereisen, K.L. Hill, N.L. Abbott, C. Seymour, D. Hill, J. Lang, E.I. Hamelin, R.C. Johnson, Quantification of Microcystin-LR in Human Urine by Immunocapture Liquid Chromatography Tandem Mass Spectrometry, Chem. Res. Toxicol. 31 (2018) 898–903. https://doi.org/10.1021/acs.chemrestox.8b00126.

[164] D.S.W. Palagama, D. Baliu-Rodriguez, A. Lad, B.S. Levison, D.J. Kennedy, S.T. Haller, J. Westrick, K. Hensley, D. Isailovic, Development and applications of solid-phase extraction and liquid chromatography-mass spectrometry methods for

quantification of microcystins in urine, plasma, and serum, J. Chromatogr. A. 1573 (2018) 66–77. https://doi.org/10.1016/j.chroma.2018.08.023.

[165] D. Vudathala, S. Smith, L. Khoo, D.D. Kuhn, M.E. Mainous, J. Steadman, L. Murphy, Analysis of microcystin-LR and nodularin using triple quad liquid chromatography-tandem mass spectrometry and histopathology in experimental fish, Toxicon. 138 (2017) 82–88. https://doi.org/10.1016/j.toxicon.2017.08.005.

[166] L. Díez-Quijada Jiménez, R. Guzmán-Guillén, G.M. Cătunescu, A. Campos, V. Vasconcelos, Á. Jos, A.M. Cameán, A new method for the simultaneous determination of cyanotoxins (Microcystins and Cylindrospermopsin) in mussels using SPE-UPLC-MS/MS, Environ. Res. 185 (2020) 109284. https://doi.org/10.1016/j.envres.2020.109284.

[167] P.B. Fayad, A. Roy-Lachapelle, S.V. Duy, M. Prévost, S. Sauvé, On-line solid-phase extraction coupled to liquid chromatography tandem mass spectrometry for the analysis of cyanotoxins in algal blooms, Toxicon. 108 (2015) 167–175. https://doi.org/10.1016/j.toxicon.2015.10.010.

[168] L. Díez-Quijada, R. Guzmán-Guillén, A. Prieto Ortega, M. Llana-Ruíz-Cabello, A. Campos, V. Vasconcelos, Á. Jos, A. Cameán, New Method for Simultaneous Determination of Microcystins and Cylindrospermopsin in Vegetable Matrices by SPE-UPLC-MS/MS, Toxins (Basel). 10 (2018) 406. https://doi.org/10.3390/toxins10100406.

[169] P.K. Kulabhusan, J.M. Rajwade, V. Sugumar, G. Taju, A.S. Sahul Hameed, K.M. Paknikar, Field-Usable Lateral Flow Immunoassay for the Rapid Detection of White Spot Syndrome Virus (WSSV), PLoS One. 12 (2017) e0169012. https://doi.org/10.1371/journal.pone.0169012.

[170] S. Melnik, A.C. Neumann, R. Karongo, S. Dirndorfer, M. Stübler, V. Ibl, R. Niessner, D. Knopp, E. Stoger, Cloning and plant-based production of antibody MC10E7 for a lateral flow immunoassay to detect [4-arginine]microcystin in freshwater, Plant Biotechnol. J. 16 (2018) 27–38. https://doi.org/10.1111/pbi.12746.

[171] L.A. Lawton, H. Chambers, C. Edwards, A.A. Nwaopara, M. Healy, Rapid detection of microcystins in cells and water, Toxicon. 55 (2010) 973–978. https://doi.org/10.1016/j.toxicon.2009.05.030.

[172] Y. Liu, J. Ji, F. Cui, J. Sun, H. Wu, F. Pi, Y. Zhang, X. Sun, Development of a two-step immunochromatographic assay for microcystin-LR based on fluorescent microspheres, Food Control. 95 (2019) 34–40.

https://doi.org/10.1016/j.foodcont.2018.07.036.

[173] S. Akter, T. Kustila, J. Leivo, G. Muralitharan, M. Vehniäinen, U. Lamminmäki, Noncompetitive chromogenic lateral-flow immunoassay for simultaneous detection of microcystins and nodularin, Biosensors. 9 (2019). https://doi.org/10.3390/bios9020079.

[174] J. Li, C. Wang, X. Yu, H. Lin, C. Hui, L. Shuai, S. Zhang, Rapid detection of Cyanobacteria by recombinase polymerase amplification combined with lateral flow strips, Water Sci. Technol. Water Supply. 19 (2019) 1181–1186. https://doi.org/10.2166/ws.2018.174.

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

[176] M. Sharafeldin, J.J. Davis, Point of Care Sensors for Infectious Pathogens, Anal. Chem. 93 (2021) 184–197. https://doi.org/10.1021/acs.analchem.0c04677.

[177] P. Kumar Kulabhusan, B. Hussain, M. Yüce, Current Perspectives on Aptamers as Diagnostic Tools and Therapeutic Agents, Pharmaceutics. 12 (2020) 646. https://doi.org/10.3390/pharmaceutics12070646.

[178] M. Witt, J.-G. Walter, F. Stahl, Aptamer Microarrays—Current Status and Future Prospects, Microarrays. 4 (2015) 115–132. https://doi.org/10.3390/microarrays4020115.

[179] C. Nakamura, T. Kobayashi, M. Miyake, M. Shirai, J. Miyake, Usage of a DNA aptamer as a ligand targeting microcystin, Mol. Cryst. Liq. Cryst. Sci. Technol. Sect. A Mol. Cryst. Liq. Cryst. 371 (2001) 369–374. https://doi.org/10.1080/10587250108024762.

[180] A. Ng, R. Chinnappan, S. Eissa, H. Liu, C. Tlili, M. Zourob, Selection, characterization, and biosensing application of high affinity congener-specific microcystin-targeting aptamers, Environ. Sci. Technol. 46 (2012) 10697–10703. https://doi.org/10.1021/es301686k.

[181] X. Li, R. Cheng, H. Shi, B. Tang, H. Xiao, G. Zhao, A simple highly sensitive and selective aptamer-based colorimetric sensor for environmental toxins microcystin-LR in water samples, J. Hazard. Mater. 304 (2016) 474–480. https://doi.org/10.1016/j.jhazmat.2015.11.016.

[182] S.M. Taghdisi, N.M. Danesh, M. Ramezani, N. Ghows, S.A. Mousavi Shaegh, K. Abnous, A novel fluorescent aptasensor for ultrasensitive detection of microcystin-LR

based on single-walled carbon nanotubes and dapoxyl, Talanta. 166 (2017) 187–192. https://doi.org/10.1016/j.talanta.2017.01.053.

[183] E.H. Lee, A. Son, Fluorescence resonance energy transfer based quantum dot-Aptasensor for the selective detection of microcystin-LR in eutrophic water, Chem. Eng. J. 359 (2019) 1493–1501. https://doi.org/10.1016/j.cej.2018.11.027.

[184] E. Bertone, M.A. Burford, D.P. Hamilton, Fluorescence probes for real-time remote cyanobacteria monitoring: A review of challenges and opportunities, Water Res. 141 (2018) 152–162. https://doi.org/10.1016/j.watres.2018.05.001.

[185] D. Masias, K. Gómez, C. Contreras, L. Gaete, C. García, Rapid screening fluorescence method applied to detection and quantitation of paralytic shellfish toxins in invertebrate marine vectors, Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 36 (2019) 1118–1137. https://doi.org/10.1080/19440049.2019.1615645.

[186] R. Yang, D. Song, S. Fang, Y. Liu, X. Zhou, F. Long, A. Zhu, Development of novel portable and reusable fiber optical chemiluminescent biosensor and its application for sensitive detection of microcystin-LR, Biosens. Bioelectron. 121 (2018) 27–33. https://doi.org/10.1016/j.bios.2018.08.062.

[187] J. Yao, L. Li, P. Li, M. Yang, Quantum dots: From fluorescence to chemiluminescence, bioluminescence, electrochemiluminescence, and electrochemistry, Nanoscale. 9 (2017) 13364–13383. https://doi.org/10.1039/c7nr05233b.

[188] R. Elshafey, M. Siaj, M. Zourob, DNA aptamers selection and characterization for development of label-free impedimetric aptasensor for neurotoxin anatoxin-a, Biosens. Bioelectron. 68 (2015) 295–302. https://doi.org/10.1016/j.bios.2015.01.002.

[189] R. Elshafey, M. Siaj, M. Zourob, In vitro selection, characterization, and biosensing application of high-affinity cylindrospermopsin-targeting aptamers, Anal. Chem. 86 (2014) 9196–9203. https://doi.org/10.1021/ac502157g.

[190] B. Liu, X. Li, S. Liu, X. Hun, A chemiluminescence aptasensor based on Cu/Co nanorods for microcystin-RR detection, Microchem. J. 145 (2019) 648–654. https://doi.org/10.1016/j.microc.2018.11.031.

[191] D. He, Z. Wu, B. Cui, Z. Jin, A novel SERS-based aptasensor for ultrasensitive sensing of microcystin-LR, Food Chem. 278 (2019) 197–202. https://doi.org/10.1016/j.foodchem.2018.11.071.

[192] M. Li, H. Lin, S.K. Paidi, N. Mesyngier, S. Preheim, I. Barman, A Fluorescence and Surface-Enhanced Raman Spectroscopic Dual-Modal Aptasensor for Sensitive

Detection of Cyanotoxins, ACS Sensors. 5 (2020) 1419–1426. https://doi.org/10.1021/acssensors.0c00307.

[193] G. Zhang, C. Li, S. Wu, Q. Zhang, Label-free aptamer-based detection of microcystin-LR using a microcantilever array biosensor, Sensors Actuators, B Chem. 260 (2018) 42–47. https://doi.org/10.1016/j.snb.2017.12.112.

[194] M. Bilibana, A. Williams, C. Rassie, C. Sunday, H. Makelane, L. Wilson, N. Ntshongontshi, A. Jijana, M. Masikini, P. Baker, E. Iwuoha, Electrochemical Aptatoxisensor Responses on Nanocomposites Containing Electro-Deposited Silver Nanoparticles on Poly(Propyleneimine) Dendrimer for the Detection of Microcystin-LR in Freshwater, Sensors. 16 (2016) 1901. https://doi.org/10.3390/s16111901.

[195] R. Chinnappan, R. AlZabn, K.M. Abu-Salah, M. Zourob, An aptamer based fluorometric microcystin-LR assay using DNA strand-based competitive displacement, Microchim. Acta. 186 (2019). https://doi.org/10.1007/s00604-019-3504-8.

[196] P. Wu, S. Li, X. Ye, B. Ning, J. Bai, Y. Peng, L. Li, T. Han, H. Zhou, Z. Gao, P. Ding, Cu/Au/Pt trimetallic nanoparticles coated with DNA hydrogel as target-responsive and signal-amplification material for sensitive detection of microcystin-LR, Anal. Chim. Acta. 1134 (2020) 96–105. https://doi.org/10.1016/j.aca.2020.08.004.

[197] Y. Zhang, Z. Zhu, X. Teng, Y. Lai, S. Pu, P. Pang, H. Wang, C. Yang, C.J. Barrow, W. Yang, Enzyme-free fluorescent detection of microcystin-LR using hairpin DNA-templated copper nanoclusters as signal indicator, Talanta. 202 (2019) 279–284. https://doi.org/10.1016/j.talanta.2019.05.013.

[198] J. Lv, S. Zhao, S. Wu, Z. Wang, Upconversion nanoparticles grafted molybdenum disulfide nanosheets platform for microcystin-LR sensing, Biosens. Bioelectron. 90 (2017) 203–209. https://doi.org/10.1016/j.bios.2016.09.110.

[199] Z. Zhao, H. Chen, L. Ma, D. Liu, Z. Wang, A label-free electrochemical impedance aptasensor for cylindrospermopsin detection based on thionine-graphene nanocomposites, Analyst. 140 (2015) 5570–5577. https://doi.org/10.1039/c5an00704f.

[200] R. Chinnappan, R. AlZabn, A.K. Fataftah, A. Alhoshani, M. Zourob, Probing high-affinity aptamer binding region and development of aptasensor platform for the detection of cylindrospermopsin, Anal. Bioanal. Chem. 412 (2020) 4691–4701. https://doi.org/10.1007/s00216-020-02723-4.

[201] S. Ouyang, B. Hu, R. Zhou, D. Liu, D. Peng, Z. Li, Z. Li, B. Jiao, L. Wang, Rapid and sensitive detection of nodularin-R in water by a label-free BLI aptasensor, Analyst. 143 (2018) 4316–4322. https://doi.org/10.1039/c8an00567b.

[202] L. Qiang, Y. Zhang, X. Guo, Y. Gao, Y. Han, J. Sun, L. Han, A rapid and ultrasensitive colorimetric biosensor based on aptamer functionalized Au nanoparticles for detection of saxitoxin, RSC Adv. 10 (2020) 15293–15298. https://doi.org/10.1039/d0ra01231a.

[203] S. Gao, X. Zheng, J. Wu, A biolayer interferometry-based competitive biosensor for rapid and sensitive detection of saxitoxin, Sensors Actuators, B Chem. 246 (2017) 169–174. https://doi.org/10.1016/j.snb.2017.02.078.

[204] S.M. Handy, B.J. Yakes, J.A. DeGrasse, K. Campbell, C.T. Elliott, K.M. Kanyuck, S.L. DeGrasse, First report of the use of a saxitoxin-protein conjugate to develop a DNA aptamer to a small molecule toxin, Toxicon. 61 (2013) 30–37. https://doi.org/10.1016/j.toxicon.2012.10.015.

[205] L. Hou, L. Jiang, Y. Song, Y. Ding, J. Zhang, X. Wu, D. Tang, Amperometric aptasensor for saxitoxin using a gold electrode modified with carbon nanotubes on a self-assembled monolayer, and methylene blue as an electrochemical indicator probe, Microchim. Acta. 183 (2016) 1971–1980. https://doi.org/10.1007/s00604-016-1836-1.

[206] X. Guo, F. Wen, N. Zheng, M. Saive, M.L. Fauconnier, J. Wang, Aptamer-Based Biosensor for Detection of Mycotoxins, Front. Chem. 8 (2020) 195. https://doi.org/10.3389/fchem.2020.00195.

[207] I. Cunha, R. Biltes, M.G.F. Sales, V. Vasconcelos, Aptamer-based biosensors to detect aquatic phycotoxins and cyanotoxins, Sensors (Switzerland). 18 (2018). https://doi.org/10.3390/s18072367.

[208] S. Pouria, A. De Andrade, J. Barbosa, R.L. Cavalcanti, V.T.S. Barreto, C.J. Ward, W. Preiser, G.K. Poon, G.H. Neild, G.A. Codd, Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil, Lancet. 352 (1998) 21–26. https://doi.org/10.1016/S0140-6736(97)12285-1.

[209] M.M. Wijewickrama, P.M. Manage, Accumulation of microcystin-LR in grains of two rice varieties (Oryza sativa L.) and a leafy vegetable, ipomoea aquatica, Toxins (Basel). 11 (2019). https://doi.org/10.3390/toxins11080432.

[210] N.M. Flores, T.R. Miller, J.D. Stockwell, A Global Analysis of the Relationship between Concentrations of Microcystins in Water and Fish, Front. Mar. Sci. 5 (2018) 30. https://doi.org/10.3389/fmars.2018.00030.

[211] D. Mackay, A.K.D. Celsie, J.A. Arnot, D.E. Powell, Processes influencing chemical biomagnification and trophic magnification factors in aquatic ecosystems: Implications for chemical hazard and risk assessment, Chemosphere. 154 (2016) 99–108.

https://doi.org/10.1016/j.chemosphere.2016.03.048.