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**Published in:**
*Trends in Environmental Analytical Chemistry*

**Document Version:**
Peer reviewed version

**Queen's University Belfast - Research Portal:**
*Link to publication record in Queen's University Belfast Research Portal*

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

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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).

![Diagram of cyanobacterial toxins and detection methods](image)

**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>
<thead>
<tr>
<th>Country</th>
<th>Location</th>
<th>Dominant cyanobacteria</th>
<th>Toxin found</th>
<th>Amount of toxins (ng/mL)</th>
<th>Toxin test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>Lake Chaohu</td>
<td><em>M. aeruginosa</em></td>
<td>MCs</td>
<td>1.07</td>
<td>HPLC</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>Hunan Province/ Changsha City</td>
<td><em>Microcystis</em> spp. YFM1</td>
<td>MC-LR,MC-RR,MC-YR</td>
<td>NA</td>
<td>HPLC-ESI-MS</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Hunan Province/ Dong Ting lake</td>
<td><em>Microcystis</em> spp. YFM2</td>
<td>MCs</td>
<td>92.88 μg/10^7 cells</td>
<td>ELISA</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>Beijing City</td>
<td><em>Cylindrospormopsis raciborskii</em></td>
<td>CYNs</td>
<td>NA</td>
<td>PCR and 16s rRNA</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>Lake Taihu</td>
<td><em>Microcystis sp.</em></td>
<td>MC-LR</td>
<td>20-44</td>
<td>ELISA</td>
<td>[28]</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>Dhaka City</td>
<td><em>M. aeruginosa</em></td>
<td>MC-RR, MC-LF</td>
<td>0.25 - 0.22</td>
<td>HPLC/MS</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>Ishakha Lake</td>
<td><em>M. aeruginosa</em></td>
<td>MCs</td>
<td>37.46</td>
<td>ELISA</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Aquaculture pond in Gazipur, Dhaka</td>
<td><em>M. aeruginosa</em></td>
<td>MC-RR, MC-YR, MC-LR</td>
<td>33.2(MC-LR), 9.03 (MC-RR), 5.23 (MC-YR)</td>
<td>HPLC/MS</td>
<td>[31]</td>
</tr>
<tr>
<td>India</td>
<td>Muttukadu backwaters, Chennai, Tamil Nadu</td>
<td><em>M. aeruginosa</em></td>
<td>MCs</td>
<td>NA</td>
<td>Microtox® bioluminescence assay</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td>Manjalar Dam, Theni District, Tamil Nadu</td>
<td><em>M. aeruginosa</em></td>
<td>MC-LR and [D-Asp^3] MC-LR</td>
<td>NA</td>
<td>HPLC-GC/MS</td>
<td>[33]</td>
</tr>
<tr>
<td>Thailand</td>
<td>Khon Kaen, Chanthaburi, Chiang Mai</td>
<td><em>M. aeruginosa</em></td>
<td>MCs</td>
<td>3.62±0.43 (Cold seasons) and 3.10±0.97 (hot)</td>
<td>Microcystin-Adda ELISA kit</td>
<td>[34]</td>
</tr>
<tr>
<td>Country</td>
<td>Location</td>
<td>Water body</td>
<td>Species (Planktolyngbya, Pseudana baena, and Microcystis spp)</td>
<td>CYNs and ATXs (MC-LR, MC-RR, MC-LA, MC-LW, MC-LF)</td>
<td>Analytical Method</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Singapore</td>
<td>Bangkok, and Pathum Thani</td>
<td>Water reservoir</td>
<td><em>Cylindrospermopsis, Planktolyngbya, Pseudana baena, and Microcystis spp</em></td>
<td>0.4 (CYNs) and 0.1 (ATXs)</td>
<td>LC-MS/MS</td>
<td>[35]</td>
</tr>
<tr>
<td>Turkey</td>
<td>Lake Kovada</td>
<td><em>M. aeruginosa</em>, Synechococcus sp., <em>Phormidium limosum</em>, <em>Phormidium formosa</em> and <em>Planktothrix limnetica</em></td>
<td>MC-LR, MC-RR, MC-LA, MC-LW, MC-LF</td>
<td>98.9 (MC-LW) 0.5 (MC-LR)</td>
<td>ELISA and HPLC</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>Lake Uluabat</td>
<td><em>Microcystis spp.</em></td>
<td></td>
<td>0.2–330</td>
<td>LC-MS/MS, LC-UV-MS, LC-HRMS and ELISA</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td>Kucukcekmece Lagoon</td>
<td><em>Microcystis spp.</em></td>
<td>MC-YR and MC-LR</td>
<td>0.06 -24.2</td>
<td>HPLC-PDA</td>
<td>[38]</td>
</tr>
<tr>
<td>Iran</td>
<td>Anzali wetland</td>
<td><em>Anabaena</em></td>
<td>MC-LR</td>
<td>0.18–3.02</td>
<td>HPLC-UV</td>
<td>[39]</td>
</tr>
<tr>
<td>Japan</td>
<td>Artificial ponds in Okinawa</td>
<td><em>M. aeruginosa</em></td>
<td>MC-LR, MC-RR, MC-LA, MC-LF, MC-YR, MC-LR</td>
<td>NA</td>
<td>^H NMR spectrometry, LC-MS</td>
<td>[40]</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Mekong Delta</td>
<td><em>M. aeruginosa</em></td>
<td>MC-LR and MC-RR, MC-dmLR, MC-LW, and MC-LF</td>
<td>11,039</td>
<td>LC-MS</td>
<td>[41]</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Structure</th>
<th>MCs</th>
<th>NODs</th>
<th>STXs</th>
<th>ATXs</th>
<th>CYNs</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="image">Image</a></td>
<td>Anabaena, Aphanocapsa, Microcystis, Nostoc, Oscillatoria, Planktothrix</td>
<td>Nodularia spumigena</td>
<td>Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya</td>
<td>Anabaena, Aphanizomenon, Oscillatoria, Planktothrix</td>
<td>Aphanizomenon, Cylindrospermopsis, Umezakia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Producing organisms</th>
<th>MCs</th>
<th>NODs</th>
<th>STXs</th>
<th>ATXs</th>
<th>CYNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Drinking water, Irrigation water</td>
<td>Drinking water, Irrigation water</td>
<td>Drinking water, Irrigation water</td>
<td>Drinking water, Irrigation water</td>
<td>Drinking water, Irrigation water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Short-term health effects</th>
<th>MCs</th>
<th>NODs</th>
<th>STXs</th>
<th>ATXs</th>
<th>CYNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal liver inflammation, leading to death, Pneumonia dermatitis</td>
<td>Similar to MCs</td>
<td>Tingling, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death</td>
<td>Tingling, numbness, drowsiness, incoherent speech, respiratory paralysis leading to death</td>
<td>Gastrointestinal liver inflammation, Pneumonia dermatitis</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Long-term health effects</th>
<th>MCs</th>
<th>NODs</th>
<th>STXs</th>
<th>ATXs</th>
<th>CYNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour promoter, liver failure leading to death</td>
<td>Similar to MCs</td>
<td>Unknown</td>
<td>Cardiac arrhythmia leading to death</td>
<td>Malaise, liver failure leading to death</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Provisional guideline values (µg/mL)</th>
<th>MCs</th>
<th>NODs</th>
<th>STXs</th>
<th>ATXs</th>
<th>CYNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Drinking water, life time)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 (Drinking water, Short term exposure)</td>
<td>24 (Recreational)</td>
<td>1 (Drinking water)</td>
<td>3 (Drinking water)</td>
<td>30 (Drinking water)</td>
<td>0.7 (Drinking water, life time exposure)</td>
</tr>
<tr>
<td>30 (Recreational)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 (Recreational)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The structures refer to microcystin-LR, nodularin, saxitoxin, anatoxin-a and cylindropermopsin respectively. MCs: microcystins; NOD: nodularins; CYNs: cylindropermopsin; 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, _rpoC1_, and _cpcBA-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 level 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 *rpoC1* and *cyrJ* 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 cyanobacteria.

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 electrochemiluminescent (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 Fe₃O₄ nanoclusters/polydopamine/gold nanoparticles (Fe₃O₄@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.

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 amperometrically 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

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Matrix</th>
<th>Recognition element</th>
<th>Sensor platform</th>
<th>Linear range (ng/mL)</th>
<th>LOD (ng/mL)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>mAb</td>
<td>MoS2/AuNCs and Au@PtNPs</td>
<td>0.001–1000</td>
<td>0.0003</td>
<td>[119]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>Abs</td>
<td>MGNNP-PEI and GCE</td>
<td>0.1-500</td>
<td>0.053</td>
<td>[120]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>Abs</td>
<td>AuNP/PPyMS and Electrochemical stripping signal</td>
<td>0.0002 - 50</td>
<td>0.0001</td>
<td>[121]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Tap Water</td>
<td>Abs</td>
<td>SiO2@MSN and electro-chemical</td>
<td>0.0005-25</td>
<td>0.0003</td>
<td>[122]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Lake water</td>
<td>Abs</td>
<td>CdTe quantum dots and fluorescent immunosensor</td>
<td>0.0001-3</td>
<td>0.00004</td>
<td>[123]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>Abs</td>
<td>3D graphene based electrode</td>
<td>0.05-20</td>
<td>0.05</td>
<td>[124]</td>
</tr>
<tr>
<td>STXs</td>
<td>Water/Shellfish</td>
<td>Abs</td>
<td>Cu-Graphene and Potentiometric</td>
<td>1-1000</td>
<td>1</td>
<td>[125]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>mAbs</td>
<td>Au-GCE</td>
<td>0.01-100</td>
<td>0.004</td>
<td>[126]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>mAbs</td>
<td>Graphene film</td>
<td>0.005-10</td>
<td>0.0023</td>
<td>[127]</td>
</tr>
<tr>
<td>STXs</td>
<td>Sea water</td>
<td>Abs</td>
<td>Antibody-Magnetic beads(recognition) and g-C3N4-PdNPs for oxidation</td>
<td>0.02-0.4</td>
<td>0.0012</td>
<td>[128]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Lake water</td>
<td>Abs</td>
<td>GH@PDA and and mesoporous carbon sphere enzyme label</td>
<td>0.01-10</td>
<td>0.0097</td>
<td>[129]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Reservoir water</td>
<td>Abs</td>
<td>SPCEs and SCEA</td>
<td>0.001–100</td>
<td>0.00011</td>
<td>[130]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Lake water</td>
<td>Abs</td>
<td>GNS@SiO2 and SERS</td>
<td>0.01 -100</td>
<td>0.014</td>
<td>[131]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Water</td>
<td>Abs</td>
<td>AuNPs@MOF and GCE, competitive immunosensor</td>
<td>0.05 –75000</td>
<td>0.02</td>
<td>[132]</td>
</tr>
</tbody>
</table>
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
solid phase extractions (SPE) 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. 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. 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). 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.

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. 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. 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. 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

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Matrix</th>
<th>Sample preparation method</th>
<th>Recovery (%)</th>
<th>Instrumental approach</th>
<th>LOQ</th>
<th>LOD</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC-LR, MC-RR, MC-YR, MC-LW, MC-LF, NODs, and CYNs</td>
<td>Water</td>
<td>SPE</td>
<td>64-115</td>
<td>UHPLC MS/MS; RP18 column</td>
<td>0.046 µg/L - 0.053 µg/L</td>
<td>0.05 µg/L</td>
<td>[151]</td>
</tr>
<tr>
<td>STXs</td>
<td>Aquatic species</td>
<td>IAC and SPE</td>
<td>79.3-102.9</td>
<td>LC-MS/MS/TSK-Gelamide column</td>
<td>0.4 µg/kg</td>
<td>0.1 µg/kg</td>
<td>[152]</td>
</tr>
<tr>
<td>STXs</td>
<td>Rat plasma sample</td>
<td>magnetic solid-phase extraction (Fe₃O₄/ l-Cys MSPE)</td>
<td>NA</td>
<td>LC–MS/MS</td>
<td>5 ng/mL</td>
<td>0.5 ng/mL</td>
<td>[153]</td>
</tr>
<tr>
<td>STXs and Neo-STXs</td>
<td>Urine</td>
<td>SPE -HILIC</td>
<td>81.5–117 (STXs) 89.0–118 (Neo-STXs)</td>
<td>LC–MS/MS</td>
<td>0.5 ng/mL (STX) and 2 ng/mL for (Neo-STXs)</td>
<td>0.2 ng/mL(STX) and 1 ng/mL (Neo-STXs)</td>
<td>[154]</td>
</tr>
<tr>
<td>STXs</td>
<td>Human urine</td>
<td>Immuno-magnetic</td>
<td>80</td>
<td>LC-MS</td>
<td>1.00–100 ng/ml</td>
<td>NA</td>
<td>[155]</td>
</tr>
<tr>
<td>MCs, CYNs, and ATXs</td>
<td>Soil</td>
<td>Online SPE</td>
<td>60 - &gt;90</td>
<td>UHPLC-tandem mass spectrometry</td>
<td>NA</td>
<td>0.001–0.3 ng/g</td>
<td>[156]</td>
</tr>
<tr>
<td>CYNs, ATX-a, NODs and MC-LR, MC-RR, MC-YR, MC-LA, MC-LY, MC-LW, MC-LF</td>
<td>Water</td>
<td>SPE using a polymeric cartridge and a graphitized non-porous carbon cartridge</td>
<td>66.6–87.3</td>
<td>UHPLC-HRMS</td>
<td>1–50 µg/L</td>
<td>4 and 150 pg/L</td>
<td>[157]</td>
</tr>
<tr>
<td>ATX-a, CYN, and HATX-a</td>
<td>Freshwater</td>
<td>SPE</td>
<td>73–97</td>
<td>UPLC- MS/MS</td>
<td>NA</td>
<td>0.6 to 15 ng/L</td>
<td>[158]</td>
</tr>
<tr>
<td>CYN, NOD, MC-RR, MC-</td>
<td>Fish</td>
<td>SPE</td>
<td>62.3-101.2</td>
<td>DSPE-LC-MS/MS</td>
<td>NA</td>
<td>5-10 µg/kg</td>
<td>[159]</td>
</tr>
<tr>
<td>YR, and MC-LR</td>
<td>tissues</td>
<td>SPE</td>
<td>HPLC-Orbitrap MS</td>
<td>NA</td>
<td>1 ng/ L</td>
<td>[160]</td>
<td></td>
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<tr>
<td>MC-LR, MC-YR, MC-RR, MC-LA, MC-LW, and MC-LF</td>
<td>Water</td>
<td>SPE</td>
<td>97.1- 100.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC-LR, MC-RR, MC-YR, MC-LW, MC-LF</td>
<td>Vegetables</td>
<td>MSPD</td>
<td>1.9- 96.5</td>
<td>HPLC-MS</td>
<td>NA</td>
<td>13 μg/kg</td>
<td>[161]</td>
</tr>
<tr>
<td>MC-RR, MC-LR</td>
<td>Fish, Plant and soil sample</td>
<td>SPE</td>
<td>94-98</td>
<td>UPLC-MS/MS</td>
<td>NA</td>
<td>0.026 μg/g</td>
<td>[162]</td>
</tr>
<tr>
<td>MC-LR</td>
<td>Human urine</td>
<td>Immunocapture</td>
<td>100</td>
<td>LC–MS/MS</td>
<td>NA</td>
<td>0.00455 ng/mL</td>
<td>[163]</td>
</tr>
<tr>
<td>MC-LR, MC-RR, MC-LA, MC-LF, MC-LW, and MC-YR</td>
<td>Mouse urine, Mouse plasma, and Human serum</td>
<td>SPE</td>
<td>90.4 to 104.3</td>
<td>HPLC-orbitrap-MS And UHPLC-QqQ-MS/MS</td>
<td>0.13 μg/L (MC-LR, RR,YR) ~0.50 μg/L (MC-LA, LF, and LW)</td>
<td>NA</td>
<td>[164]</td>
</tr>
<tr>
<td>MC-LR and NODs</td>
<td>Fish (Liver and Plasma)</td>
<td>SPE</td>
<td>NA</td>
<td>LC/MS/MS</td>
<td>NA</td>
<td>25 ng/g</td>
<td>[165]</td>
</tr>
<tr>
<td>MCs-LR, MC-RR, MC-YRand CYNs</td>
<td>Mussels</td>
<td>SPE</td>
<td>70.37–114.03</td>
<td>UPLC-MS/MS</td>
<td>0.23- 0.40 ng/g</td>
<td>0.01-0.39 ng/g</td>
<td>[166]</td>
</tr>
<tr>
<td>MC-RR, MC-YR, MC-LR, MC-LY, MC-LW and MC-LF, ANA-a and CYN</td>
<td>Water</td>
<td>SPE</td>
<td>91–101</td>
<td>LC-HESI-MS/MS</td>
<td>NA</td>
<td>0.01–0.02 μg/L</td>
<td>[167]</td>
</tr>
<tr>
<td>MC-LR, MC-RR, MC-YR and CYN</td>
<td>Vegetables</td>
<td>SPE</td>
<td>41–93</td>
<td>UPLC-MS/MS</td>
<td>5–50 ng/g</td>
<td>0.06–0.42 ng/g</td>
<td>[168]</td>
</tr>
</tbody>
</table>

NA: Not available; IAC: Immuno-affinity 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\textsubscript{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 LFIA 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].
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>
<thead>
<tr>
<th>Source</th>
<th>Cyanotoxins</th>
<th>Sequence of the aptamer (5’-3’)</th>
<th>Sensing system</th>
<th>Working range (ng/mL)</th>
<th>LOD (ng/mL)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>MC-LR</td>
<td>GGC GCC AAA CAG GAC CACCAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC</td>
<td>Microcantilever array</td>
<td>1–500</td>
<td>50</td>
<td>[193]</td>
</tr>
<tr>
<td>Water</td>
<td>MC-LR</td>
<td>GGCGCCAAAACAGGACCACCATG ACAAATTACCCATACCACCTCATTATGCCCATCTC CGC</td>
<td>Glassy carbon electrode and CV</td>
<td>0.1-1.1</td>
<td>0.04</td>
<td>[194]</td>
</tr>
<tr>
<td>Spiked water</td>
<td>MC-LR</td>
<td>GGCGCCAAAACAGGACCACCATG ACAAATTACCCATACCACCTCATTATGCCCATCTC CGC</td>
<td>Competitive displacement assay and molecular beacon</td>
<td>0.4-199</td>
<td>0.004</td>
<td>[195]</td>
</tr>
<tr>
<td>Water</td>
<td>MC-LR</td>
<td>GGC GCC AAA CAG GAC CAC CAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC</td>
<td>Cu/Au/Pt trimetallic nanoparticles/colorimetric platform</td>
<td>0.004-10</td>
<td>0.003</td>
<td>[196]</td>
</tr>
<tr>
<td>Spiked water</td>
<td>MC-LR</td>
<td>GGC GCC AAA CAG GAC CAC CAT GAC AAT TAC CCA TAC CAC CTC ATT ATG CCC CAT CTC CGC</td>
<td>Enzyme-free fluorescent detection</td>
<td>0.005 -1200</td>
<td>0.00003</td>
<td>[197]</td>
</tr>
<tr>
<td>Water</td>
<td>MC-LR</td>
<td>GGCGCCAAAACAGGACCACCATG ACAAATTACCCATACCACCTCATTATGCCCATCTC CGC</td>
<td>Fluorescent nanoparticles</td>
<td>0.01–50</td>
<td>0.002</td>
<td>[198]</td>
</tr>
<tr>
<td>Water</td>
<td>CYNs</td>
<td>ATCAGGCAAAACCGATGGTCCGGCACCACCTAAAACACCAGGCCACCACCACACACCCCGCG</td>
<td>thionine–graphene nanocomposite modified GCE/[Fe(CN)]₆⁴⁻/₃</td>
<td>0.39-78</td>
<td>0.117</td>
<td>[199]</td>
</tr>
<tr>
<td>Tap water</td>
<td>CYNs</td>
<td>GGCATCAGGCAAAACCGATGGTCCGGCACCACCTAAAACACCAGGCCACCACCACACACACCGCG</td>
<td>GO and Fluorescent sensing</td>
<td>NA</td>
<td>0.007</td>
<td>[200]</td>
</tr>
<tr>
<td>Water</td>
<td>NOD-R</td>
<td>Label-free BLI</td>
<td>0.033-0.1</td>
<td>0.13</td>
<td>[201]</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
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<td>----------------------</td>
<td>-----------</td>
<td>------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>Water STXs</td>
<td>GGTATTGAGGGTCCATCCCGTGAAAC ATGTTCAATTGGGCGCACTCCGCTTTTCTGTA GTAGATGGCTCTAACTCTCCTCT</td>
<td>AuNPs based colorimetric sensing</td>
<td>0.000002-29.9</td>
<td>0.000002</td>
<td>[202]</td>
<td></td>
</tr>
<tr>
<td>Spiked shellfish, ribbon fish and water STXs</td>
<td>AACCTTTGTCGGGCAAGG TAGGT</td>
<td>Optical BLI</td>
<td>10 - 2000</td>
<td>0.5</td>
<td>[203]</td>
<td></td>
</tr>
<tr>
<td>Blue mussels and Asia green mussels and water STXs</td>
<td>GGTATTGAGGGTCCATCCCGTGAAAC ATGTTCAATTGGGCGCACTCCGCTTTTCTGTA GTAGATGGCTCTAACTCTCCTCT</td>
<td>Gold electrode modified with MWCNT/Amperometric</td>
<td>0.26-8.9</td>
<td>0.11</td>
<td>[204,205]</td>
<td></td>
</tr>
</tbody>
</table>

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 phycoeyanin 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.
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