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Monitoring Approaches for a Toxic Cyanobacterial Bloom

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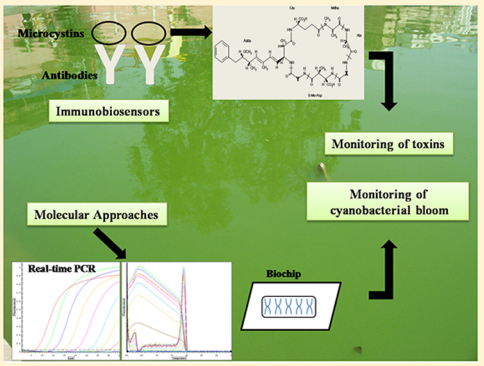
ABSTRACT: Cyanobacterial blooms, dominated by *Microcystis* sp. and associated microcystin variants, have been implicated in illnesses of humans and animals. Little is known regarding the formation of blooms and the presence of cyanotoxin variants in water bodies. Furthermore, the role played by ecological parameters, in regulating *Microcystis* blooms is complicate and diverse. Local authorities responsible for water management are often faced with the challenging task of dealing with cyanobacterial blooms. Therefore, the development of suitable monitoring approaches to characterize cyanobacterial blooms is an important goal. Currently, various biological, biochemical and physicochemical methods/approaches are being used to monitor cyanobacterial blooms and detect microcystins in freshwater bodies. Because these methods can vary as to the information they provide, no single approach seemed to be sufficient to accurately monitor blooms. For example, immunosensors are more suited for monitoring the presence of toxins in clear water bodies while molecular methods are more suited to detect potentially toxic strains. Thus, monitoring approaches should be tailored for specific water bodies using methods based on economic feasibility, speed, sensitivity and field applicability. This review critically evaluates monitoring approaches that are applicable to cyanobacterial blooms, especially those that focus on the presence of *Microcystis*, in freshwater bodies. Further, they were characterized and ranked according to their cost, speed, sensitivity and selectivity. Suggested improvements were offered as well as future research endeavors to accommodate anticipated environmental changes.



INTRODUCTION

Cyanobacteria, formally known as "blue-green algae", are the most ancient life form known to inhabit earth. Aquatic cyanobacteria can occur as planktonic cells or form phototrophic biofilms termed blooms. Cyanobacterial blooms have been implicated in a wide range of social, economic and environmental impacts and are of particular concern for animal and human health. Blooms can alter water quality with the release of toxic and odorous compounds. Amongst the cyanobacteria, the *Microcystis* sp. is the most frequently one encountered in freshwater bodies worldwide and which produces hepatotoxins called microcystins (MCs). MCs are synthesize as a cyclic peptide nonribosomally via a multifunctional modular enzyme complex consisting of both peptide synthetase and polyketide synthase, and which is coded by the *mcy* gene cluster.[1,2] More than 89 MC variants have been described from natural blooms and laboratory cultures of cyanobacteria.[3] MCs inhibit serine-threonine protein phosphatases 1 and 2A in animals including humans, thereby causing damage to the liver as well as nephro- and neuro-toxicity.[4,5] World Health Organization (WHO) has established a drinking water standard for cyanobacteria at 1 μg/L for MC-LR and has developed provisional guidelines as follows: Level 1 (low health risk probability)—20 000 cyanobacterial cells/mL or 10 μg/L chlorophyll *a* (Chl *a*) with dominance of cyanobacteria, Level 2 (moderate possibility of adverse health effects)—100 000 cells/mL or 50 μg/L Chl *a*; and, Level 3 (high health risk probability)—formation of cyanobacterial scums.[6,7] MC-LR, the most commonly occurring MC variant, was evaluated by the International Agency for Research on Cancer of the WHO as a probably carcinogenic for humans and placed into category 2B.[8]

Our knowledge is limited regarding the diverse and complex interactions among physical, chemical and biological variables leading to the proliferation of cyanobacteria and the regulation of MC synthesis at the cellular level. For example, we have observed the prevalence of MC-RR in pond of Varanasi, India, which suggests that dominant variants seem to be geographically specific.[9] Furthermore, difference in MC variants found in tropical and temperate regions make it difficult to determine a common predictable parameter. Lipopolysaccharides (LPS) are an integral component of cyanobacterial cell wall and have been reported to cause irritant and allergic responses in human and animal tissues.[4] The different genera of cyanobacteria that have distinct LPS compositions are largely conserved within that genus.[10] Cyanobacterial LPS are considerably less potent than LPS from pathogenic gram-negative bacteria. Many cyanobacteria including *Microcystis* produce odorous compounds such as geosmin and 2-methyl

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isoborneol (2-MIB).[11] Cyanobacterial species which produce toxins do not necessarily produce taste and odorous compounds.[12] Thus, it seems that the production of these compounds are not biochemically connected to the production of the cyanotoxins.

The lack of definitive correlation between a cyanobacterial bloom formation and MCs production necessitates the need for the development of rapid and more reliable methods for routine monitoring of *Microcystis* as well as MCs and their utilization in environmental sampling. Microscopy, photopigments, and physicochemical analysis are used to detect and monitor toxigenic/nontoxic cyanobacteria and toxins in natural samples. Enumeration of *Microcystis* cells does not indicate toxigenic potential because toxigenic and nontoxic strains can coexist and are morphologically indistinguishable, in natural blooms.[13] The development of a cyanobacterial bloom mainly depends on the available nutrients, thus the environmental monitoring of nutrient concentrations provides valuable information for assessing bloom-forming potential.[14] However, there are deviations from this assumption which makes it difficult to correlate MCs production with nutrients concentrations of a water body.[15]

Cyanobacterial pigments have also been analyzed by spectrophotometric, fluorometric analysis, and remote sensing to provide more reliable information about the extent of a bloom and to assess the status of water bodies.[16−18] MCs are released into the surrounding medium upon lysis of aging cells and do not persist due to degradation. Therefore, monitoring *Microcystis* populations alone, cannot provide a clear picture of potential toxicity in the water body, unless supported with proper quantification of the MCs present. The enzyme-linked immunosorbent assay (ELISA) and the protein phosphatase inhibition assay (PPIA) are employed along with analytical techniques such as high-performance liquid chromatography (HPLC) and liquid chromatography−mass spectrometry (LC-MS) for a more accurate MCs analysis. Chromatographic methods can distinguish between MC variants but often require extensive sample processing while immunological assays are more sensitive for low samples at concentration and are easier to perform for routine screening of environmental samples.[19]

The gene cluster involved in MC production has been fully sequenced from *Microcystis*,[20] leading to the development of molecular methods to monitor MC producers in environmental samples. Thus a toxic population at low density can be detected using conventional[21] and real-time PCR[22] as an early warning tool. Molecular techniques are more advantageous owing to their ability to provide the taxonomic resolution from the genus to the genotype level.[23] Molecular techniques can also be combined with biochemical assays to provide a powerful monitoring approach for environmental samples. Because toxic and nontoxic *Microcystis* blooms occur under differing environmental conditions and geographical characteristics of a water body, monitoring techniques must be tailored for each specific targeted water body. To develop a model that simulates the magnitude and timing of blooms it is also necessary to determine those key factors which govern the dynamics of a cyanobacterial bloom formation. Current biological and physicochemical methods and their advantages and limitations for monitoring *Microcystis* and MCs are presented in Figure 1. Measured values or data obtained from the integration of appropriate biological and physicochemical methodologies not only document the status of a toxic cyanobacterial bloom but also provide scientifically supported input for the control and mitigation of these blooms.

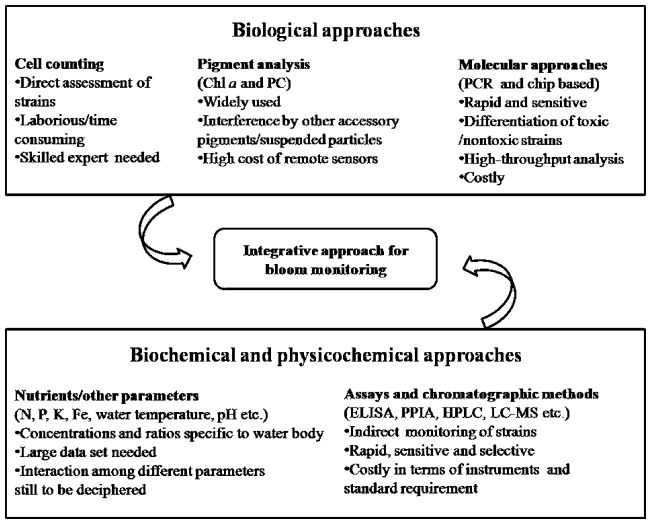

Figure 1. Summary of monitoring approaches for cyanobacterial bloom.

BIOLOGICAL METHODS

Cell Counting. Generally, microscopic identification and cell counting are the basic techniques for monitoring a cyanobacterial bloom. Several systematic methods for counting cyanobacteria cells have been documented by Lawton et al.[14] Blooms dominated by colonies of *Microcystis* sp., consist of a large number of cells, which can interfere with the accurate estimation of cell number. Reynolds and Jaworski[24] compared alkaline hydrolysis and sonication of suspensions of *Microcystis* colonies to facilitate the estimation of cell numbers. Colonies were not destroyed but cells were incompletely dispersed in the suspension. Heating suspensions of *Microcystis* cultures at 80 °C promoted separation of cells by vortexing.[25] These methods were also compared along with sonication and TiO_2 treatments for making single cell suspension of *Microcystis* colonies.[26] In both of the studies, boiling/heating along with vortexing was found to be the most suitable method for generating free cells from colonies. Sonication is a simple and quick method but may lead to damage of cells, resulting in an underestimation of cell numbers. Flow cytometry has also been applied for the establishment of cell count of *Microcystis* sp.[27−29] Flow cytometry, in addition to microscopic analysis, can be used for monitoring blooms but this technique does not provide detailed information on species composition.[29,30] Cell counting helps in the direct assessment of those organisms present in a cyanobacterial bloom without the requirement for elaborate equipment; however, it is very laborious, time-consuming and requires a skilled analysts for species identification.

Estimation of Pigments (Chlorophyll *a* and Phycocyanin). *Spectrophotometric and Fluorometric Analysis.* The photosynthetic pigment Chl *a* is useful for the estimation of the total phytoplankton population as it contributes 0.5−1% of the ash-free dry weight.[14] Spectrophotometric[16,31] and fluorometric analysis[17] are the preferred methods for the determination of Chl *a*, both in the field and in the laboratory. Chl *a* is usually extracted from the target organism using an organic solvent such as methanol or acetone.[32]

Hilton et al.[33] compared fluorescence excitation at 680 nm from 30 cultures of freshwater algae for the differentiation of algal species. The ratio of the fluorescence intensity at two wavelength bands, 360−390 and 510−580, relative to the intensity in the range 435−460 nm was found to be the most discriminating. However, the presence of other accessory pigments and degradation products may interfere with its determination. As a consequence, submersible probes using in vivo fluorescence with specific excitation spectra have been developed for monitoring phytoplankton in various aquatic environments throughout the world (Table 1).[34−37] A submersible probe (FluoroProbe), containing five light emitting diodes, was developed for the estimation of total Chl *a*, making it suitable for monitoring variable phytoplankton compositions.[38] Most cyanobacterial monitoring is conducted by conventional laboratory methods such as taxonomic analysis, phytoplanktonic pigment extractions, and analysis of toxins in the water samples. Therefore, the incorporation of an online probe using in vivo fluorescence to monitor phytoplankton in real time has an obvious advantage over conventional methods.[36,37]

Phycocyanin (PC) is a cyanobacterial pigment whose specific fluorescence is a function of cyanobacterial biomass. Dominance of cyanobacterial blooms (mostly toxic) in certain freshwater aquatic bodies necessitates PC measurement as a practical approach over that of Chl *a*. There are studies reporting positive correlations between PC fluorescence with other parameters of cyanobacterial biomass.[39,46−48] Asai et al.[49] used a two-channel fluorometric sensor with excitation wavelengths at 620 nm for detecting PC of the cyanobacteria and 440 nm for subtracting the interference caused by eukaryotic algae present in the sample. The sensor was equipped with an ultrasonicator which allowed for the detection of *Microcystis aeruginosa* in the bloom. A three-stage alert system was proposed to monitor *Microcystis* bloom in Korean lakes[50] and was based on PC levels of 0.1 (caution), 30 (warning), and 700 μg/L (outbreak), respectively. Other examples for PC probe as a monitoring tool are reported in Table 1.[41−45] In vivo PC fluorescence measurements using field probes can provide early detection of cyanobacteria in drinking water intakes.[43] Bastien et al.[43] used a YSI 6600 probe which transforms the fluorescence of PC into an equivalent cell density of *M. aeruginosa*. The TriOS microFlublue probe can convert the measured concentration of PC to density equivalent or biomass to evaluate the risk associated with a cyanobacterial bloom.[42] These probes can be used as a rapid tool for locating the cyanobacterial bloom but pigment contents (Chl *a* or PC) may vary with species and metabolic state of the cells. Other factors that are of concern when using fluorometric sensors are the location and cyanobacterial compositions of the bloom and the presence of suspended particles in the water which can cause interference with the estimation of pigment concentrations, making recalibration of individual probes necessary. These probes also possess other limitations such as light source, turbidity of water, etc.[45]

Remote Sensing. Monitoring and/or forecasting of a cyanobacterial bloom involving remote sensing can provide needed spatial and temporal coverage. The use of remote sensing to predict cyanobacterial and other intense phytoplankton blooms, was extensively reviewed by Kutser.[51] The signals obtained in remote sensing might be confusing.[52] Dierssen et al.[52] have shown that the red color of phytoplankton bloom is caused by human visual system,

Table 1. Submersible Sensors Using In Vivo Fluorescence for Monitoring Phytoplankton and Cyanobacteria

pigment analysis	probe used	excitation and emission wavelength(s) (nm)	location	references
chlorophyll and other pigments (Phytoplankton)	FluoroProbe, bbe-Moldaenke	450, 525, 570, 590, 610	five stations at Schlei Baltic sea, Germany;	34;
			five Czech reservoirs;	38
			Vír reservoir; fifty lakes and reservoirs, Ile-de-France region (IDF)	39;
				40
	algae online analyzer (AOA)	450, 525, 570, 590, 610 and 690	Sulejow reservoir, Poland dominated by *M. aeruginosa*;	36;
			phytoplankton communities from two Southeastern United States estuaries, North Inlet, South Carolina and the Neuse river estuary	37
	phytosensor	430 ± 30, 540 ± 15, 625 ± 20 and 690	bloom in the Cahaya Bahru reservoir, Malaysia	35
phycocyanin (cyanobacteria)	fluorometer 10-AU, Turner	620 and 650	cyanobacterial bloom in the Baltic sea	41
	TriOS micro Flu−blue	620 and 655	freshwater bodies of western France	42
	YSI 6600 V2−4 multiprobe and TriOS	590 and 660	*M. aeruginosa* cultures and field samples from lakes of Québec, Canada; microcystin producing *Microcystis* and *Anabaena* sp. in drinking water treatment plant; monitoring of Yamaska river and Lake Champlain,Québec, Canada	43;
				44;
				45

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namely by spectral response curves of the red and green receptors. They have also concluded that confusion arose regarding the type of bloom prevailing because almost any phytoplankton species present in high concentration can color the water red. This color also depends on the amount of dissolved organic matter or suspended sediments. Occasionally, the presence of low level of toxic algal species may not cause discoloration of water.[53] Therefore, dependency on colors only, questions the accuracy of remote sensing in forecasting cyanobacterial bloom. Pigment dependent spectral signatures of cyanobacterial blooms cannot distinguish a bloom-less shallow water area from bloom-containing deep water area.[54,55]

Several types of algorithms have been developed for use in remote sensing strategies. Vincent et al.[56] developed algorithms for the Landsat Thematic Mapper (TM) to detect cyanobacterial blooms in Lake Erie, usually dominated by *Microcystis* sp. They used six reflective infrared (IR) spectral bands to detect levels of PC in the lake. A cyanobacterial bloom can develop within a short period of time but the Landsat sensor lacks the temporal frequency required for monitoring such an event. Remote sensing can also be used for quantitative mapping of PCs.[18,57] It is difficult to separate cyanobacterial bloom from other phytoplankton, if the Chl *a* level is below a threshold limit (8−10 mg/m^3). This limits its use in early warning systems.[58] A semianalytical ratio algorithm using the 709 and 620 nm bands of MERIS (Medium Resolution Imaging Spectrometer) was employed for quantitative mapping of cyanobacterial PC.[18,57] This method gave a suitable spatial resolution and signal-to-noise ratio. However, an exact estimation of cyanobacterial PC was affected because of environmental dependent variations in PC content of different species as well as interference of Chl *a* at 620 nm. MERIS has higher spectral resolution in the red portion of the electromagnetic spectrum and was used for differentiating cyanobacterial blooms from other phytoplankton blooms.[59] Spectral shape (SS) at 681 nm, that is, fluorescent line height (FLH), was used to detect Chl *a*. *Microcystis* sp. scatters light owing to the presence of gas vacuoles, resulting in a lower fluorescence signal than other phytoplankton.[60] Strong and weak fluorescence at 681 nm were indicative of algae or algae with *Microcystis* population. This observation has been the basis for identification of *M. aeruginosa* from other species of phytoplankton. Shape algorithms could characterize the *Microcystis* bloom in western Lake Erie during the summer and autumn of 2008.[61] MERIS sensor on board the European Space Agency's ENVISAT was also used to monitor blooms dominated by *Microcystis* sp. in Zeekoevlei Lake situated on the Cape Flats, Cape Town, South Africa[62] and Tajo River, Spain.[63] Moderate resolution imaging spectroradiometer (MODIS) band 1 (620−670 nm) was used in the Baltic Sea for mapping the biomass of cyanobacterial blooms,[51] whereas band 2 (841−876 nm) separated dense subsurface cyanobacterial blooms from surface scum.[64] Even with good spatial resolution, single band data from MODIS were inadequate to separate algal blooms from plumes of turbid water.

Airborne sensors have also been used by many investigators for mapping cyanobacterial blooms. Hunter et al.[65] used time-series airborne remote sensing to monitor the spatial distribution of potentially toxic *M. aeruginosa* in the shallow eutrophic waters of Barton Broad, U.K. Compact Airborne Spectrographic Imager (CASI-2) was operated in spatial mode using the bands centered at 620 and 670 nm to target the absorption features associated with cyanobacterial PC and Chl *a*, respectively. They used semiempirical water-leaving radiance algorithms for the quantification of Chl *a* and PC and applied to the CASI-2 imagery for mapping of the *M. aeruginosa* bloom. Data from the CASI-2 were also used to monitor seasonal changes in the pigments of shallow lakes in the U.K.[66] Along with CASI, Airborne Imaging Spectroradiometer for Applications (AISA) Eagle and Hawk data were collected over Esthwaite Water. These are tandem hyperspectral instruments with 244 and 240 contiguous bands distributed across the 400−970 nm and 1000−2400 nm spectral range, respectively.[67] Their spectral and spatial resolution made them very effective tools for the mapping of blooms in the small or medium lakes.[51] High-resolution time-series data from airborne sensors can be more easily acquired in comparison to satellite sensors.[65] However, remote sensing is costly which interferes with the frequent data acquisition.[51] Forecasting cyanobacterial bloom using remote sensing devices suffers from high cost, dependency on meteorological conditions with long monitoring intervals, which limits their use for routine monitoring.

Molecular Techniques. *PCR-Based Methods.* The uncertain distribution of toxic and nontoxic strains within most genera prevents an accurate diagnosis of bloom samples and fails to indicate the toxigenic potential of the cyanobacterial population. Molecular-based techniques offer several advantages over other conventional monitoring methods. Toxic and nontoxic strains can be detected rapidly. Real-time PCR is useful for quantitative analysis of cyanobacterial strains and variations in community dynamics of cyanobacteria can be evaluated by denaturing gradient gel electrophoresis analysis (DGGE). DNA chip/microarray, using gene specific oligonucleotide probes, is a technique suitable for high-throughput analysis and to study cyanobacterial community composition (Figure 2). Various genes for example, 16S rRNA, internal transcribed spacer (ITS) and phycocyanin intergenic spacer (PC-IGS) have already been employed for characterizing toxic and nontoxic *Microcystis* colonies in natural populations.[21,68−70] Janse et al.[71] used the rRNA-ITS DGGE approach for the identification of toxic and nontoxic *Microcystis* genotypes in single isolated colonies as well as in natural samples. The genetic variations in the phycocyanin operon have enabled the intrageneric delineation of toxic cyanobacterial strains along with the detection of cyanobacterial population in the presence of other organisms.[72] Because of the uneven distribution of toxic and nontoxic cyanobacterial strains was observed within the PC-IGS and 16S rRNA trees.[21] The PC-IGS region was

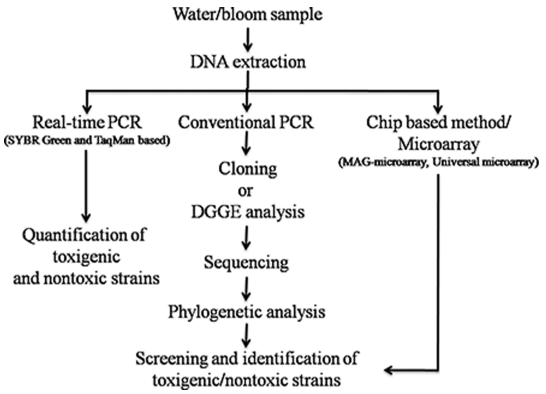

Figure 2. Molecular approaches for identification and monitoring of cyanobacterial strains.

Table 2. Molecular Based Methods for the Analysis and Monitoring of Nontoxic and Potentially Toxic *Microcystis* sp

method	target genes	location/sample type/application	references
conventional PCR	16S rRNA	*Microcystis* sp. cultures; 37 *Microcystis* sp. cultures as well as several field samples from Australia	68 21,
	PC-IGS	environmental samples—Malpas Dam, Australia; Daechung Reservoir, Korea	69 70;
	mcyA (NMT domain)	*Microcystis* cultures as well as several field samples	21
	mcyA (condensation domain)/*mcyA*	24 *Microcystis* strains, samples from Lake Wannsee, Germany; 322 *Microcystis* colonies Mikata, Japan	73; 75; 76;
	mcyB (A1 domain)	*Microcystis* colonies from Lake Wannsee, Germany; *Microcystis* collected from 9 countries and 13 water bodies	77; 75
	mcyB	30 *Microcystis* strains and more than 200 bloom samples from Chinese lakes; samples from Lake Oneida, U.S.; Lake Ontario; Grangent reservoir, France	78; 79 80; 81
	mcyD	cultures of *Microcystis* and other microcystin producing strains; samples from Lake Oneida, USA	82; 79
	mcyE	potential MC-producing *Microcystis* existed in 70% of the lake samples in 70 Finnish lakes	13
multiplex PCR	*mcyA,B,C,D,E,G*	9 *Microcystis* strains and 8 field colonies from Valmayor and Pinilla dams, Spain; *Microcystis* cultures and bloom samples from 7 ponds in Varanasi, India	83; 84
real-time PCR	16S rRNA	quantification of total cyanobacteria and total *Microcystis* sp. in western Lake Erie samples; water samples from Hirosawa-no-ike fish pond;bloom samples from San Francisco Estuary (SFE)	85; 86; 87
	PC-IGS	quantification of total *Microcystis*	22;
		population in Lake Wannsee, Germany; Daechung Reservoir, Korea; Lake Taihu, China	88; 89
	mcyA	quantification of toxigenic *Microcystis* in Hirosawa-no-ike fish pond (*mcy A*/16S rRNA ranged from 0.7 to 41%; Durgakund Pond, India (toxigenic *Microcystis* sp. to that of total *Microcystis* sp. ranged from 0% to 14%)	86; 9
	mcyB	*mcy* genotype proportion ranged from 1 to 38% in Lake Wannsee, Germany	22
	mcyD	quantification of toxic *Microcystis* and other toxin producers in Lake Wannsee, Germany; ratio of toxic *Microcystis* among total *Microcystis* ranged from 0.01to 27% in San Francisco Estuary; ratio of *Microcystis mcyD* to *Microcystis* PC-IGS ranged from 3.8% to 41.1% and 10.3% to 65.8% during 2009 and 2010 blooms	85; 87 89
	mcyE	quantification of *mcyE* copy numbers in Lake Tuusulanjärvi and Lake Hiidenvesi, Finland. *Microcystis* was dominant in Lake Tuusulanjärvi	90;
	mcyJ	quantitative analysis of potentially toxic *Microcystis* sp. in samples from Daechung Reservoir, Korea. The ratio of *mcyJ* genes to *cpcBA* genes was 68.3% in August	88
chip based methods	16SrRNA	10 probes were established to determine the relative abundances of various cyanobacteria including *Microcystis* in the selected lakes; detection of 19 cyanobacterial groups and environmental samples	91 92
	mcyE	genus-specific probe pairs were designed for the detection of the *mcyE* and *ndaF* of five hepatotoxin-producing cyanobacterial genera including *Microcystis*; the chip assay successfully detected the presence and expression of *mcyE* genes with RNA samples extracted from cyanobacterial strains and environmental water samples. Both microcystin-producing *Anabaena* and *Microcystis* were identified in Lake Tuusulanjärvi samples	93; 94

used to identify potentially toxic cyanobacterial species in environmental samples.[69] In another study, PCR products generated by primers for the *mcyA* gene were digested with restriction enzymes to identify the producer genera based on the differences in resulting bands.[73] A multiplex PCR targeting 16S rRNA and *mcyA* gene, demonstrated the presence of toxic *Microcystis* cells in dietary supplements produced from *Aphanizomenon flos-aquae* (a freshwater species of cyanobacteria).[74] Conventional and the multiplex PCR approach based on amplification of various *mcy* genes has already been used for the early detection of toxic *Microcystis* blooms in various water bodies (Table 2). Mutations in the *mcy* gene cluster may hamper the production of MC leading to overestimation of the potential toxin producers within the bloom. This idea was strengthened by reports of the presence of toxic genotypes of *Microcystis* strains which were unable to produce MCs.[21,95,96] Therefore, new primers/probes must be designed and tested for assessing the presence of new strains within the blooms.[23]

Real-Time PCR Based Methods. SYBR Green and TaqMan based quantitative real-time PCR using specific primers/probes have been used for rapid monitoring of total cyanobacteria and nontoxic/toxigenic *Microcystis* sp. in freshwater bodies using specific primers/probes (Table 2).[9,22,85−90] A Real-time PCR assay has been developed with a detection limit of 8.8 cells per reaction.[97] Rinta-Kanto et al.[85] detected 5000 gene copies/L in lake water samples. Seasonal variations in concentrations of *mcyA* and 16S rRNA genes were investigated in a hypereutrophic fish pond.[86] Whole cell real-time PCR with a sensitivity of 1000 cells/mL was developed for the detection and quantification of hepatotoxigenic cyanobacteria in bloom samples.[98] The sensitivity of real-time PCR and melting curve analysis of target genes simplify the detection, quantification, and genotyping. Quantification of toxic/nontoxic cyanobacteria can be linked with various environmental parameters along with the levels of MC variants, giving new insights into the regulating factors involved in bloom upsurge in different regions of the world.[99] However, real-time PCR has limitations,

as the amplification efficiency decreases with increase in the length of the amplification product and optimization becomes difficult and time-consuming.

Chip-Based Methods. Microarray can be used to detect sequence variations and monitor gene expression levels on a genomic scale.[100] These are based on hybridization of labeled targets[100] or polymerase extension of arrayed primers.[101] Thus, microarray has emerged as an interesting tool for high throughput analysis and large scale screening and identification of potentially toxic cyanobacteria in bloom samples (Table 2).[91−94] Various modifications have been incorporated in this technique by many workers to monitor toxic cyanobacteria. Rudi et al.[91] developed a microarray based on specific 16S rDNA oligonucleotide probes. These probes were hybridized to their respective complements spotted onto a DNA array. Cyanobacterial genera like *Phormidium*, *Microcystis*, *Planktothrix*, *Anabaena*, *Aphanizomenon*, and *Nostoc* were identified using such techniques in eight Norwegian lakes. Qualitative results were obtained regarding the presence or absence of the various cyanobacterial genera. A new technology, MAG-microarray, was developed by combining microarray with magnetic-capture hybridization technique. This technique used bacterial magnetic particles (BMPs) and identified and discriminated five cyanobacterial genera.[102] A universal microarray was developed in the framework of the European Union project MIDI-CHIP,[92] based on the DNA microchip, developed by Gerry et al.[103] Here the polymerase chain reaction/ligase detection reaction (PCR/LDR) was combined with zip-code hybridization. Zip-codes are thermodynamically similar and have no homology to either the target sequence or to other sequences in the genome. This method required two probes, a discriminatory probe and a common probe, specific for each target sequence. A fluorescent label is coupled to one of the probes, while a complementary zip code to the other. Such arrays detected 19 major cyanobacteria groups using 16S rRNA gene probes and were validated with known strains and environmental samples.[92] The optimization of hybridization becomes difficult for large sets of different probes. This method was further developed for the detection of microcystin (*mcyE*)/nodularin (*ndaF*) genes of five hepatotoxin-producing cyanobacteria[93] and produced a positive signal even from 1 to 5 fmol of PCR product. The chip and quantitative real-time PCR (qPCR) assays were also optimized to study the expression of microcystin biosynthesizing genes (*mcy*) with RNA samples extracted from cyanobacterial cultures and Lake Tuusulanjärvi samples.[94] Thus, microarray can be used for the rapid identification of cyanobacterial groups undetectable or present in low quantities, making it suitable for monitoring toxic as well as nontoxic strains in large number of environmental samples.

The advantages and disadvantages of the Biological Methods previously discussed are summarized as follows. Chl *a* content represents total algal biomass therefore it is not suitable for monitoring cyanobacteria. PC is mainly associated with cyanobacteria and can be used for their detection. Data associated with contaminated water can be used in advance to anticipate the health risk by comparing the PC or Chl *a* levels with that of WHO levels. Such assessment provides time to analyze cyanotoxins and ensures that immediate actions can be implemented by water body manager without waiting for the results of other analysis. Traditional approaches like microscopic identification of phytoplankton and cell counting may take 2−5 days to produce results as samples must be transported to laboratories where trained personnel perform the analyses. Other approaches such as remote sensing can provide the spatial and temporal coverage needed. Probes are easily applicable in the field, can monitor blooms daily, and provide instantaneous information on cyanobacterial biomass. Remote sensing technology is useful for mapping the development and extent of a bloom in case of vast coastal areas or larger lakes, but not suitable for rapid detection of blooms in small ponds, lakes and drinking water supplies. As far as molecular methods are concerned, high cost is involved in their use but, they are useful for early detection of potentially toxic organisms. Real time PCR and chip-based methods are rapid and may take less than 30 min to quantify the potentially toxic genotypes.

Biochemical and Physicochemical Methods. *Nutrients and Other Parameters (Water Temperature, pH, Salinity, Etc.).* Nutrients and other variable parameters such as water temperature, pH, salinity, etc. can regulate the growth of cyanobacteria.[104,105] Therefore, these parameters are considered valuable in assessing the potential for future bloom development. Cyanobacterial cells can store surplus phosphate in polyphosphate bodies.[106] Nitrogen also can be stored in cyanophycin granules and phycobiliproteins.[107] Marked variations in the amount of cyanophycin in a natural population of *Microcystis flos-aquae* have been reported.[108] Stewart[108] observed low level of this polymer in the cyanobacteria during a bloom, whereas relatively higher levels occurred in pre- and postbloom. Investigations of the events leading to cyanophycin accumulation and utilization in such phases of blooms, are needed to confirm the role of this compound in the nitrogen economy of the cell.[107] Therefore, Lawton et al.[14] suggested that total phosphorus (TP) and total nitrogen (TN) of the aquatic bodies may be used to forecast cyanobacterial bloom development. Many studies found that low N:P ratios favor cyanobacterial growth in nutrient-rich environments, as some cyanobacteria can fix elemental N_2.[109−112] Smith[110] reported the occurrence of a cyanobacterial bloom at a N:P ratio (on weight basis) of less than 29:1, and later modified as 22:1.[113] The concentrations of N and P as well as their ratios were reported to be reliable indicators of blooms in the Daechung reservoir dominated by *Microcystis* sp.[47] There was an increase in the annual average of cyanobacterial proportion with a decrease in TN:TP based on long-term data set analysis from Lake Okeechobee, USA.[114] However, the opposite relationship was recorded between monthly average TN:TP and cyanobacterial population in this lake.[113] McCarthy et al.[115] also reported that N:P ratio was useful for predicting *Microcystis* dominance in Taihu lake (China) but dissolved inorganic nitrogen (DIN) and TN or NH_4^+:NO_x were better indicators for predicting phytoplankton community structure. Monitoring of TP was reported to be a key factor to predict cyanobacterial bloom and phytoplankton biomass.[116] This report was strengthened because the cyanobacterial blooms were found to be more strongly correlated with TP, TN or biomass itself rather than the TN:TP ratio, with the presence of TP above 70 μg/L favoring the dominance of cyanobacteria.[117]

Subsequent studies in forecasting of blooms emphasized the importance of other parameters such as water temperature, salinity, pH, etc. Variations in TP along with salinity and water temperature were taken into account for predicting variations in Chl *a* and cyanobacteria.[105] Increasing nutrient concentrations and variations in the N:P ratios also affected the recruitment and abundance of *Microcystis* in Lake Krankesjön, Southern

Table 3. Comparison of Analytical Methods for the Detection of Cyanotoxins[a]

methods	detection limits	cost	application/comments	references
ELISA	0.05 μg/L	high	rapid, sensitive, low level of expertise required, limited shelf life, may underestimate toxin concentration	125 126,
PPIA-colorimetric-radiolabeled	0.25 μg/L		selective, most sensitive, laborious,	126,
	subpicogram	high	no identification of toxins	127
HPLC-UV/PDA/MS	0.02 μg/L	high	correct identification of toxins with some uncertainties, moderate level of expertise needed	128
LC-MS	0.02−0.0026 μg/L	very high	a number of different interfaces, highly specific, mass confirmation, no need for sample preparation	129,
LC-MS/MS				130
MALDI-TOF	0.1 μg/L	very high	selective, sensitive, mass measurements severe matrix effects	131,
SELDI-TOF	2.5 pg/2 μL	very high		132
GC-MS	0.0043 μg/L	high	characteristic ion spectra, specific	133
NMR		very high	characterization of toxins, needs mg quantities, expert interpretation	134
CE-MS		high	poor sensitivity, needs further development	135

[a]ELISA: Enzyme-linked immunosorbent assay; PPIA: Protein phosphatase inhibition assay; HPLC: High-performance liquid chromatography; LC-MS: Liquid chromatography-mass spectrometry; MALDI-TOF: Matrix-assisted laser desorption/ionization time-of-flight; GC-MS: Gas chromatography-mass spectrometry; CE: Capillary electrophoresis; UV: ultraviolet; PDA: photo-diode array.

Table 4. Biochemical and Physicochemical Methods Used for the Analysis of Microcystin Variants for Indirect Monitoring of Water Bodies

methods	location	references
enzyme-linked immunosorbent assay (ELISA)	monitoring of drinking water and surface water from Seine and Clain Rivers and reservoirs, France; water samples including Miyun reservoir, Beijing China	136; 137
protein phosphatase inhibition assay (PPIA)	water and algal samples from Daechung reservoir, South Korea; bloom samples from fresh water lakes and reservoirs in England and South-Africa	138; 139
high-performance liquid chromatography (HPLC)	water samples from Lake Euam, Korea; Samples from Lake Constance, Canada	140; 141
liquid chromatography−mass spectro-metry (LC-MS) LC-MS/MS	*M. aeruginosa* from Guadiana River, Portugal; microcystins in water samples of Taihu Lake, China *M. aeruginosa* and *M. wesenbergii* bloom samples from Sulejow reservoir, Poland; Monitoring of Qiantang River, China	142; 143 144; 145
matrix-assisted laser desorption/ionisation time-of-flight(MALDI-TOF)	322 colonies of *Microcystis* morphospecies from 13 water bodies in 9 European countries; 26 strains of *M. aeruginosa* from the Tamega River, Portugal; monitoring of water samples and dietary supplements	75; 146; 131
gas chromatography−mass spectrometry (GC-MS)	samples from Lake Kasumigaura, Japan; Ayutaya, Thailand and Taifu Lake, China	147
capillary electrophoresis (CE)	samples from Brno reservoir (Czech Republic); samples from Hungarian lakes and surface water bodies	135; 148

Sweden.[118] The recruitment rate of *Microcystis* was found to be the highest with low N:P ratios, which might have induced blooms in the lake. Ahn et al.[119] analyzed K and Fe and proposed K:Fe ratio as a new parameter for predicting a bloom in the Daechung Reservoir that was dominated by *Microcystis* sp. In a comparison, ratio of K and Fe to a threshold limit of 200 would reflect the same type of bloom as other parameters predicted. This threshold limit (200) equated to a cyanobacterial concentration of 20 000 cells/mL[120] and PC concentration of 20 pM.[47] Along with the nutrients, other environmental factors like high water temperature,[111] low light intensity,[121,122] high pH and low CO_2[104,123,124] may favor cyanobacterial bloom formation. Therefore, nutrient concentrations alone cannot be used as a sole parameter for predicting a cyanobacterial bloom. This necessitates an integrated approach in the development of a holistic model to forecast the bloom.[105] In addition to nutrients and other environmental parameters, toxigenic *Microcystis* sp. can also be monitored using various biochemical and physicochemical techniques that are helpful in the identification and quantification of low concentrations of MCs. Various physicochemical analyses are available for the rapid screening of a large number of samples, the regular monitoring of sites where the toxin patterns are well established, and the monitoring of new toxic cyanobacterial metabolites. However, cost, selectivity and sensitivity of these techniques, which are summarized in Table 3, are important criteria for obtaining reliable information. The various physicochemical techniques that have been used for the monitoring of toxins in various water bodies of different countries are reviewed in Table 4.

Enzyme-Linked Immunosorbent Assay (ELISA). The enzyme-linked immunosorbent assay (ELISA) has been developed using either monoclonal[123,147,148] or polyclonal[137,126,151] antibodies against cyanotoxins like MCs. Antibodies have been generated with variable cross-reactivities against one of the MC variants (MC-LR) and successfully used to determine the MC content of environmental samples.[152] A commercial kit (EnviroGard kit) was evaluated for determining MCs in water samples.[136] Other commercial kits are also available and useful as screening tools but susceptible to

interferences that limits their use for quantitative analyses. An indirect competitive ELISA derived from polyclonal antibodies, resulted in good cross-reactivity against a range of purified MC variants.[151] A direct competitive enzyme-linked immunosorbent assay (dc-ELISA) to detect MCs in water samples is available and demonstrates good cross-reactivity with other MC variants.[137] The presence of more than 89 MC variants has been recorded to date therefore, cross-reactivity of the antibodies might underestimate some variants. Lower concentrations of hydrophobic MC variants have been reported, when analyzed with ELISA as compared to other methods.[153] ELISAs are highly sensitive and specific and require minimum sample processing for rapid monitoring and detection of MC concentrations that are within the levels set by WHO (Table 4).[6]

Protein Phosphatase Inhibition Assay (PPIA). Inhibition of eukaryotic protein phosphatases 1 and 2A is an established assay as an indicator of toxin concentration. Measurements are made using (a) radioisotopic techniques based on ^{32}P radiolabeled substrates[154−156] and (b) colorimetric assays based on substrates such as *p*-nitrophenyl phosphate.[126,127,153,157,158] The former method is dependent on radiolabeled proteins and therefore, not suitable for routine monitoring in the present context. A colorimetric phosphatase inhibition assay was developed for determining peptide hepatotoxins using phosvitin as the substrate and protein phosphatases 1 as the dephosphorylating enzyme for determining peptide hepatotoxins.[159] A single cyanobacterial hepatotoxins specific assay was developed by combining immunoassay with a colorimetric protein phosphatase inhibition.[139] Immunoextraction followed by the phosphatase inhibition test was also used for a rapid on-site monitoring of the toxicity of MC producing bloom.[127] These assays are cost-effective and can be used for the routine screening of environmental samples but are not specific and may respond to other protein phosphatase inhibitors (tautomycin and okadaic acid).

High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography−Mass Spectrometry (LC-MS). HPLC coupled with a photodiode-array (PDA) detector for UV absorbance is the most commonly used technique for analyzing MCs at 238 nm.[141,160−163] Usually, a range of 200−300 nm is useful for the identification of MCs with absorption maxima between 222 and 238 nm.[164] Identification, based on UV absorbance alone was not sufficient because other compounds with similar absorbance spectra might lead to inaccuracy.[165] It was also observed that the diode-array detector lacked specificity for MCs and was prone to interferences from other analytes.

Lack of standards for many MC variants makes identification difficult, therefore results are generally expressed as MC-LR equivalents.[166] Removal of impurities from the samples is necessary and cartridges such as octadecyl silanized silica gel (ODS), based on hydrophobic interactions are used for cleanup.[167−169] A more polar cyanocartridge was used to increase the adsorbing power for MCs and for better recoveries and chromatograms.[140] Different conventional C-18 and C-16 amide columns have been used previously used for separating MCs.[129] Decrease in toxin yields with the use of C-18 cartridges has also revealed limitations.[153] Rapala et al.[153] recovered several MC variants using solid phase extraction with Oasis HLB cartridges based on the cartridge's hydrophobic-lipophilic balance. Immunoaffinity columns offered superior cleanup and selective extraction of MCs in environmental samples with trace amounts of MCs.[169−171] Various columns used for preconcentration and cleanup of MCs have been exhaustively reviewed by Trojanowicz.[172] The proper selection of mobile phase (methanol based) led to better resolution of the MC-LR and -YR variants.[128] Column techniques used for the extraction and cleanup of MCs are time-consuming and require skilled personnel. A highly sensitive and selective method was developed for the analysis of MCs by coupling of postcolumn immunological or enzyme-inhibition assays to liquid chromatography.[173]

LC-MS offers potential for high throughput analysis of MCs in water samples (Table 3 and 4). Samples are ionized to generate charged molecules and identified by m/z measurements.[174] LC-MS with a 2.8 min run-time has been described but resolution was low.[175] Triple quadrupole is the most commonly used MS analyzer in quantitative determination of MCs.[176,177] LC with MS using frit-fast atom bombardment technique[178] and electrospray ionization (ESI-MS),[142,143] was used for the accurate determination of MCs in natural blooms. Quantification by MS is sometimes prone to errors due to variable ionization of different analytes; however, these errors may be eliminated by the use of isotope-labeled internal standards.[176]

Tandem mass spectrometry (MS/MS) is more specific and useful in the identification of unknown toxins in environmental samples.[179] A fragment ion (m/z 135), derived from the side chain ADDA ((2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid) was used for the confirmed identification of MCs.[166,180,181] Ion traps or MS/MS are suitable for fast scans over a wider scan range with the capacity to perform exact mass measurements of the compounds based on molecular formula. MCs in surface water were determined by MS/MS with an electrospray ion source (ESI).[145] Neffling et al.[144] used LC coupled to ultra ion trap MS with an ESI to compare eight reversed phase columns. They suggest the use of small particle size, fused core particles or monolithic silica columns for routine MC analysis (Table 4). Higher flow rates can be achieved with ESI-MS with limits of detection somewhat better than that of triple quadrupole systems.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF). MALDI-TOF MS, a rapid, selective and sensitive technique, with high resolution, allowed exact mass measurements and detection of compounds, based on molecular formula. This technique provided high-throughput and multiplexed analyses and has been employed for identification of MC variants.[146,182,183] Individual colonies of *Microcystis* sp. from nine different European countries were analyzed using MALDI-TOF MS equipped with a reflectron, postsource decay (PSD) and collision-induced dissociation (CID) options.[75] This analysis revealed the presence of eight different MC variants. Ultrahigh performance liquid chromatography (UPLC) was coupled to orthogonal acceleration TOF MS for the detection and quantification of MCs.[131] The limit of detection was reported to be 0.1 μg/L in water samples without cleanup (Table 3). In another approach, MCs were captured on a hydrophobic chip and subsequently ionised by surface-enhanced laser desorption ionization time-of-flight MS (SELDI-TOF-MS), enabling determination of 2.5 pg MC-LR in 2 μL water.[132] However, severe matrix effects were experienced making it difficult to monitor the characteristic m/z 135 of MCs due to the high background interference.

Gas Chromatography–Mass Spectrometry (GC-MS). The GC-MS approach has been used to monitor MCs in water bodies containing blooms. A method, based on the quantitation of 2-methyl-3-methoxy-4-phenylbutyricacid (MMPB) as an oxidation product of MCs by GC with a flame ionization detector, was developed by Sano et al.[133] MMPB was analyzed directly, without derivatization using selected ion monitoring.[134] Later, a new and high-sensitivity method, for determining MC content (pmol range) by GC with a flame ionization detector gas chromatography/chemical ionization mass spectrometry (GC/CI-MS), was developed using erythro-2-methyl-3-(methoxy-d_3)-4-phenylbutyric acid (MMPB-d_3) as the internal standard.[147] These methods are suitable for complex sample matrixes but time-consuming. It is not recommended for monitoring water samples because the technique is unable to distinguish MC variants.

Nuclear Magnetic Resonance (NMR). Two-dimensional NMR (2D NMR) has been useful for structural determination of MCs. Thus, a nondestructive method using a combination of 2D NMR techniques was developed for structural determination of MCs.[184] In spite of its usefulness for the characterization and identification of MCs, it was costlier than other analytical methods and required large amount of pure samples and an expert interpretation.[134] Therefore, routine monitoring is not suggested by this method.

Capillary Electrophoresis (CE). This technique is based on separation of charged molecules in a buffer solution under the influence of a strong electric field. Two modes have been used, namely capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) with different detection methods. MEKC mode for the analysis of MCs followed by MS detection was used.[185] Capillary electrochromatography (CEC) with reversed-phase monolithic capillary columns was used for the analysis of MCs in *M. aeruginosa* blooms collected from Brno reservoir, Czech Republic.[135] Both CZE and MEKC methods were developed for the simultaneous determination of MC variants in bloom samples having *M. aeruginosa* that were collected from Hungarian lakes and surface water bodies.[148] However, these methods demonstrated poor sensitivity compared to HPLC and therefore are not recommended for routine monitoring of environmental samples (Table 3).

The selection of various analytical methods for monitoring cyanobacterial blooms have to be evaluated against different parameters such as speed, cost effectiveness, sensitivity and selectivity (Figure 3). Cell counting and pigment monitoring are less costly and useful for initial screening of bloom samples. Conventional PCR is easy and less costly as compared to SYBR Green and TaqMan based real-time PCR and chip-based methods. These molecular tools are selective and suitable for monitoring key toxic species. It is necessary to process water before recommending for drinking purpose. Therefore, several conventional and advanced treatment options are useful for the removal of cyanobacterial cells and cell-bound toxins. Coagulation and filtration are effective in the removal of cells while dissolved air flotation has been shown to be effective in the removal of *Microcystis*.[186] The recommended way is to remove the intact cells, as the removal of dissolved toxins by conventional methods become more difficult. Powdered activated carbon (PAC) and granular activated carbon (GAC) are used to adsorb toxins present in source water. The effectiveness of the adsorption is influenced by water quality, concentration and type of toxins as well as the presence of natural organic matter.[186] Disinfection processes like chlorination is effective for the destruction of released MCs but its efficiency depends on pH, chlorine dose and oxidant nature and should not be relied upon as the sole barrier to contamination.[187] ELISA and PPIA methods are easy, fast and suitable for rapid monitoring of total MCs in raw and treated water with a detection limit of 0.05 and 0.25 μg/L (Table 3). Although the initial capital expenditure to establish most of the chromatographic techniques is high, these are highly selective methods (Table 3) and can be employed for monitoring the type and levels of MCs in finished drinking water.

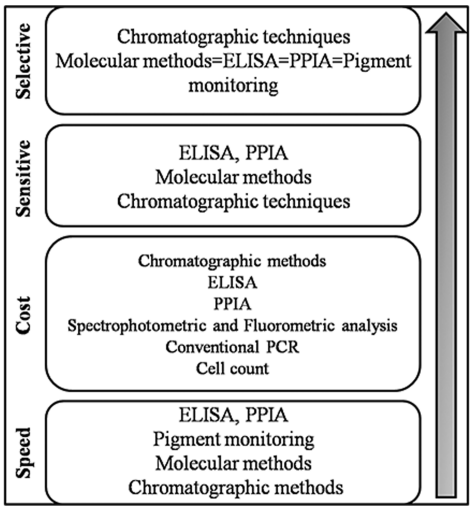

Figure 3. Monitoring approaches for cyanobacterial blooms at various parameters in ascending order.

Others. Along with the several analytical techniques described, various portable biosensors have also been developed for rapid MCs detection in environmental samples. Enzymes, antibodies or nucleic acids based biosensors are highly specific and sensitive. However, most of the biosensors have been specifically developed for the MC-LR variant. Sensors based on nanotechnology and sequence specific probes of DNA or RNA have been developed which enhanced the sensitivity of biosensors. Various biosensors related to MCs detection have been comprehensively reviewed by us.[188]

The advantages and disadvantages of the biochemical and physicochemical methods previously discussed are summarized as follows. Determination of total N and P along with high water temperature, light intensity may favor the development of cyanobacterial bloom. Such data varies with depth and distance of sampling, size of the water body, season and source of input of the nutrients. These observations cannot predict the potentially toxic strains and toxins in water bodies. ELISA and PPIA are rapid, sensitive, easy to operate and may make moderate demands on equipment (Table 3). These techniques are useful for quantitation of MCs in drinking water before and after water treatment. However, ELISA is also prone to interferences and PPIA may respond to other protein phosphatase inhibitors. HPLC is the most commonly used analytical method for the detection and identification of MCs, but this method also lacks specificity for MCs. Improved columns are needed which can offer superior cleanup and selective extraction of MCs in environmental samples. More advanced methodology such as LC-MS is suitable when further confirmation and identification of MCs is required. It enables the simultaneous separation and identification of MCs in a mixture. Monitoring approaches must involve the identification

of unknown toxins in environmental samples which can be performed by MS/MS. MALDI-TOF is useful for identification of MCs with very small sample volumes and provides the molecular mass of all of the peptides and MC variants present. GC-MS is highly sensitive and suitable for screening and accurate quantification of MCs in complex sample matrixes but it is time-consuming and costly (Table 3). NMR requires relatively large amounts of pure sample and expert interpretation therefore it is not suitable for routine monitoring.

The factors regulating the cyanobacterial growth and toxin production are still not properly understood. The genetic regulation of cyanotoxin production is an important area for further study and exploration. Various analytical methods are limited in terms of their application in routine monitoring. Determination of cyanobacterial toxicity using gene probes needs further validation involving a wider range of samples from different regions. Nucleic acid based biosensors can be further developed for obtaining the sequence-specific information more easily and rapidly. Future research must focus on the development of approaches/techniques and guidelines which can be applied in both temperate and tropical climates because differences may arise depending upon temperature and thermal stratification.

CONCLUSIONS

Predicting bloom events is an important goal of monitoring programs and fundamental in water management. A strategy for monitoring cyanobacterial blooms in water bodies will depend on various local aspects such as the intended use (drinking or recreation) and the types (ponds, lakes, rivers or oceans) of water bodies. Its success relies on how well the specified objectives of the monitoring approach are addressed. Important objectives include the identification of problematic areas, identification and quantification of cyanobacterial populations and toxins, causes and regulation of blooms, recognition of associated health risks (both human and environmental) and providing input for the development of guidelines for drinking water and use of recreational sites.

Microscopic identification, cell counting and pigment analysis have traditionally been employed in monitoring programs of water bodies having *Microcystis* blooms. The data obtained from these methods can be employed as an early warning system by comparing with the threshold limits set by WHO. Remote sensing is suitable and cost-effective for the monitoring of cyanobacterial scums and larger water bodies and/or many recreational sites. The development of a bloom is regulated by various nutrients, therefore, the source of such nutrients should be taken into account for early warning. The threshold limit of the N and P combined with light intensity and water temperature may indicate the future development of blooms in water bodies. In contrast to the approaches involved in monitoring bacterial pathogens or toxic chemicals, the unique biological and chemical characteristics of cyanobacterial blooms and cyanotoxins require different approaches mostly because the composition of cyanobacterial toxins and/or bloom change with space and time in a given water body. Toxic and nontoxic strains may be present together in blooms of *Microcystis* sp. At a particular time, toxins may be released after cell lysis and sometimes cells might be present without any detectable toxins. In the absence of cyanobacterial cells, the monitoring of toxins by the more sensitive and rapid ELISA or PPIA methods is suggested. Subsequently, molecular approaches are useful for providing the information about the abundance and dominance of key toxic species and thus furnish the best monitoring/forecasting tool available for source water control strategy. The presence of other metabolites in addition to MCs in drinking water samples can be detected by chromatographic techniques and the dissolved toxins and/or other metabolites can then be processed with water treatment processes such as, use of activated carbon (physical process), chlorination (chemical process) and sand filtration or GAC supporting a healthy biofilm (biological process).

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Notes

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