



JRC TECHNICAL REPORT

# Good practice for cyanobacterial and algal bloom detection across Europe

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## Abstract

This report provides guidance on good practices for the detection of cyanobacterial and algal blooms. In recent years, bloom events are increasing worldwide, which is an environmental problem and has health implications on animals and humans.

The aim of this technical report is: to provide protocols for monitoring bloom events in waterbodies, to raise awareness of the bloom phenomena and to give an overview of monitoring/management practices implemented in the Member States (MS). This report also includes results of a JRC survey launched in 2019. In this exercise, fifteen MS provided information on parameters taken into account for the assessment of the occurrence of bloom events, warning/de-warning systems applied in national programs and measures taken to inform people and anticipate/reduce risks of these events.

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## Introduction

Cyanobacterial blooms are natural phenomena (sometimes termed “algal blooms”) but eutrophication has substantially increased their prevalence during the past decades. While in recent years, in some waterbodies, control measures are taking effect, in many others, eutrophication is continuing, blooms are increasing, and climate change can exacerbate this problem. Such blooms can occur naturally in fresh water, marine and brackish water but their observed increasing incidence is mainly reported for fresh water. Bloom events are influenced by environmental conditions, particularly eutrophication, pH and water temperature. Their occurrence can cause several problems to the environment, human health and to the economic system. Indeed, cyanobacterial blooms may contain a range of harmful cyanotoxins. Water organisms can bioaccumulate some of these toxins and transfer them through the food chain. Humans can be exposed to them through ingestion or aspiration, which can lead to illness. While cyanotoxins have resulted in the death of domestic, farm and wild animals when ingesting water contaminated with cyanobacteria, human deaths have been reported from exposure via renal dialysis. Therefore, globally, there is a strong need to develop harmonised monitoring systems which should be able to anticipate the outbreak of cyanobacteria in waterbodies.

This report is a good practice guide for cyanobacterial and algal bloom detection and provides an overview of methods applied across the European Union. Information was collected from different sources:

- The existing detection methods, currently implemented in the Member States (MS) and other countries, as outcome of the survey on monitoring and management of cyanobacterial/algal blooms, circulated by the JRC to MS in 2019, have been evaluated.
- Scientific literature published in the last 5 years has been reviewed to identify reports and articles on methods relevant to prevent and mitigate cyanobacterial blooms.
- The recently revised guidance of the World Health Organisation (WHO) has been considered, i.e. the guideline values for recreational exposure to 4 groups of cyanotoxins (WHO, 2020 a-d), the WHO guidelines on recreational water quality (WHO 2021) and the 2<sup>nd</sup> fully revised edition of the WHO guidebook “Toxic Cyanobacteria in Water” (WHO, Chorus and Welker, 2021).
- Furthermore, JRC publications on bloom events are discussed to incorporate their contribution in predicting and identifying potentially harmful blooms at their early stages.
- Additionally, reference to satellite data was evaluated to see if it could support warnings of the occurrence of cyanobacterial blooms.

The technical guidance is primarily addressed at environmental agencies of the European countries, professionals and other authorities responsible for monitoring and management of blooms in waterbodies. It also targets the public to promote awareness about cyanobacteria and harmful algae. The content is provided in two separate parts to facilitate the consultation of methods and protocols for cyanobacterial and harmful algal bloom (HAB) detection in fresh and marine water ([Part I](#)), followed by the description of conditions contributing to cyanobacterial blooms and HABs along with their effects on human health and the environment ([Part II](#)).

# Part I

Methods and protocols for the detection of cyanobacterial and harmful algal blooms in waterbodies

## 1. Protocols for monitoring cyanobacterial blooms

- Which method can be used to measure water turbidity? → See Part I, [section 1.1](#)
- Which protocols can be adopted to measure:
  - chlorophyll *a* concentration → See Part I, [section 1.2](#)
  - phytoplankton abundance and biovolume → See Part I, [section 1.3.1](#) and [section 1.3.2](#)
  - toxin concentrations → See Part I, [section 1.4.1](#)
- Which chemical methods and protocols can be used to detect cyanotoxins in water? → See Part I, [section 1.4.2](#)
- Which methods can be adopted to distinguish between cyanobacteria and algae? → See Part I, [section 2](#)
- Which warning and de-warning systems must be followed to ensure a safe use of bathing sites? → See Part I, [section 3](#)

Water turbidity ([Figure 1](#)), concentrations of chlorophyll *a*, cyanobacterial biovolume and levels of cyanotoxins are the primary means for evaluating the presence of cyanobacteria and assessing the likelihood of their development to a bloom. [Table 1](#) provides criteria for classification of waterbodies in three alert levels for possible or persisting blooms. Evidence of scum and bloom photographs can be used to document visual site inspection. Additionally, inclusion of reports on smell and notices from site users are encouraged. Based on water transparency, the presence of non-scum forming cyanobacteria can be self-assessed by wading into the waterbody up to the knees and observing the visibility of one's own feet ([Figure 1](#)) (WHO, Testai and Chorus, 2021). Vigilance and alert levels proposed by the JRC are in line with the WHO values with one exception: cyanobacterial biovolume ([Table 1](#)). For this latter, the JRC agrees with slightly higher values currently in place in Germany (see [section 3.6](#)). Novel methods and systems to predict and early detect cyanobacterial blooms are described in Part I, [section 4](#).



Figure 1. Self-assessment of water turbidity associated with the presence of cyanobacteria. The degree of turbidity can help determine vigilance or alert levels. *Source:* WHO, Testai and Chorus, 2021.

Table 1. Alert levels framework for cyanobacterial blooms. Adapted from WHO, Testai and Chorus, 2021. JRC agrees with the WHO on all the threshold values, except for the cyanobacterial biovolume (values proposed by the JRC are reported in blue).

	Vigilance level	Alert level 1	Alert level 2
Water turbidity – visual inspection	Slightly turbid, greenish discolouration Knee-deep in water: feet visible	Pronounced greenish turbidity, possible minor green film or streaks on parts of the surface Knee-deep in water: feet barely visible	Visible thick cyanobacterial scums covering most of the water surface in recreational areas
Secchi depth (m)	<1-2	<0.5-1	
Chlorophyll <i>a</i> (µg/L)	>3 to 12	>12 to 24	
Cyanobacterial biovolume (mm <sup>3</sup> /L)	<1-4 > 1	>4 to 8 > 3 to 15	> 15
Cyanotoxins (µg/L)	-	≤6 CYN or ≤24 MC or ≤30 STX or ≤60 ATX	>6 CYN or >24 MC or >30 STX or >60 ATX
Monitoring actions	<ul style="list-style-type: none"> <li>Assess conditions supporting bloom formation</li> <li>Assess for toxin-producing cyanobacteria</li> </ul>	<ul style="list-style-type: none"> <li>Assess for cyanobacterial scums</li> <li>Further assess the levels of toxins</li> </ul>	<ul style="list-style-type: none"> <li>Continuous monitoring</li> </ul>

CYN: cylindrospermopsins; MC: microcystins; STX: saxitoxins; ATX: variants of anatoxin-a.

## 1.1. Secchi depth

*Is there an increased turbidity observed in a waterbody?*

*Is there a bloom suspected?*

*How to measure the level of turbidity?*

The determination of Secchi depth is a quick and inexpensive measurement of water turbidity which is typically limited by phytoplankton growth in waterbodies such as lakes (although suspended matter also interferes with light penetration).

Secchi depth is determined using a Secchi disk, a 20 cm diameter disk with alternating black and white portions (Figure 2), and corresponds to the point at which the disk disappears when lowered by hand into the water. It is also a proxy of the euphotic zone, defined as the depth of the water in a waterbody where sunlight penetrates, enabling photosynthesis to occur. In detail, the euphotic depth is determined as 2.5 times the Secchi disk depth or the region where photosynthetically active radiation (PAR) was larger than 1% of the radiation determined immediately below the water surface.



Figure 2. Measurement of water transparency using a Secchi disk. The Secchi disk is lowered by the operator into the water and the depth at which the disk is barely no longer visible (termed “Secchi depth”) is recorded.

## SCOPE

Measurement of water turbidity using a Secchi disk (Figure 3).



Figure 3. Picture of a Secchi disk during the measurement of water turbidity.<sup>1</sup>

## MATERIALS

Ethanol (70%)

## EQUIPMENT

Boat  
Secchi disk  
Gloves  
Laboratory notebook

## METHOD

1. Reach the sampling point by boat (for a representative value for the whole lake, the sampling point may be the deepest region of the waterbody; for recreational site monitoring it may be more useful to measure in areas where most activities involving immersion of head and mouth are expected), and turn the motor off;
2. For sampling, minimise any direct sunlight reflections from the water surface by measuring in the shade of the boat; also avoid effects of the motor on water turbulence or mixing;
3. Lower the Secchi disk to the point at which it is barely no longer visible (make sure that the rope to which the disk is attached, is perpendicular to the water surface) (Figure 3);
4. Record the measurement;
5. Clean and dry all the equipment used during the campaign, before storage.

Note: Once the sampling site has been chosen, always use the same one during all sampling campaigns or select additional sampling sites based on the purpose of sampling (e.g. to characterise a waterbody over time or for public health surveillance). Where operators carrying out the measurement alternate, comparisons should be carried out to ensure using the same criteria. Sunglasses need to be taken off as they will distort the reading. It is recommended to take the measurement two times as a minimum and calculate the arithmetic average of all readings.

<sup>1</sup> From <https://serc.carleton.edu/>

## 1.2. Chlorophyll *a*

*How can it be confirmed that water turbidity is caused by a bloom?*  
*Is turbidity accompanied by a green colour or scum ("carpet")? What should be done in this case?*  
*In case of smell, which parameter can be measured first?*

In case of water smell or water turbidity, chlorophyll *a* measurement can be used to confirm that the green water colour or scum is caused by a bloom. Chlorophyll *a* is a photosynthetic pigment contained in plants, algae and cyanobacteria. The chlorophyll *a* concentration is used as an indicator of the phytoplankton biomass, which includes cyanobacteria but may also include a range of planktonic algae. Therefore, when chlorophyll *a* is selected as parameter to quantify cyanobacterial biomass, a brief qualitative analysis by microscopy is advised to assess whether the phytoplankton in water samples consists mainly of cyanobacteria. This pigment can be extracted using different chemicals, including methanol, and can be quantified spectrophotometrically at selected wavelengths by measuring the Optical Density (OD). The measurements are then applied to an equation and the resulting chlorophyll *a* quantification is usually expressed in µg/L. Two other methods can be used to quantify the concentration of chlorophyll *a*: fluorimetry and high-performance liquid chromatography (HPLC). The following protocol describes a spectrophotometric method (Figure 4). See ISO 10260 and WHO, Padiśák et al. (2021), for more detailed information.

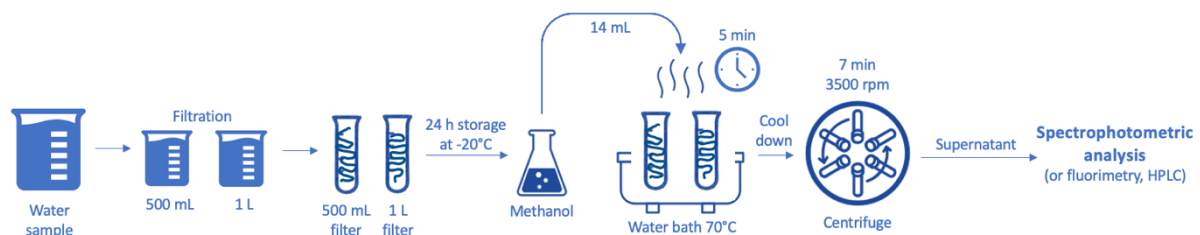


Figure 4. Schematic workflow for determination of chlorophyll *a* in water samples.

### SCOPE

Measurement of chlorophyll *a* concentration as indicator of phytoplankton biomass

### MATERIALS

- Methanol (100%)

### EQUIPMENT

- GF/C glass microfiber membranes (47 mm diameter)
- Filtration station
- Gloves
- Graduated cylinders
- Paper towels
- Plastic pipettes
- Pipet-Aid
- Micro-pipettes with disposable plastic tips
- Falcon tubes (15 ml)
- Aluminium foils
- Tweezers

- Timer
- Deep-freeze refrigerator (-20°C)
- Water bath reaching 70°C
- Centrifuge reaching 3500 rpm
- Spectrophotometer (wavelengths 665 nm and 750 nm)
- Cuvettes for spectrophotometric measurements (40 mm path)
- Laboratory notebook

## METHOD

1. Immediately upon arrival to the laboratory, filter water samples by GF/C glass microfiber membranes, stopping filtration immediately when filters fall dry to avoid rupture of cells and loss of dissolved content;
2. For each sample, prepare two filters: A) filtration of 500 mL raw water; B) filtration of 1 L raw water;
3. Fold filters and place them in 15 mL falcon-type tubes using tweezers, wrap tubes in aluminium foil and store at -20°C (note that freezing serves to rupture cells and enhance pigment extraction);
4. Freeze for at least 12 hours (storage at -18°C is possible for several days; for longer storage use -78°C); then prepare samples for spectrophotometric analysis (always minimising exposure to light):
  - set water bath at 70°C;
  - add 14 mL 100% methanol to each tube and mix well;
  - incubate samples in a water bath up to 5 minutes (to boil);
  - remove tubes from the bath and let them cool down to room temperature;
  - centrifuge the tubes for 7 minutes at 3500 rpm;
  - carefully pour the supernatant to a clean cuvette (40 mm path) and measure the OD;
  - record values for 665 nm and 750 nm wavelengths.

Chlorophyll *a* values in µg/L are determined by the following equation (HMSO, 1983):

$$\text{Chl } a \text{ (}\mu\text{g/L)} = [13.9 * (\text{OD } 665\text{nm} - \text{OD } 750\text{nm}) * v * d] / P * V$$

Where Chl*a*: chlorophyll *a*, *v*: added volume of methanol (14 mL), *d*: dilution factor (when applicable), *P*: cuvette path in cm (4 cm), *V*: filtered water sample in litres (0.5 or 1 L)

Note: Keep samples at approximately ambient lake temperature by storage in thermic containers for the transport to laboratory and filter them immediately upon arrival. OD value at 750 nm should be lower than 0.02.

For visibly thick samples, it is advisable to start filtering 50 mL of raw water and add volume until filtration slows down, taking care to note down the filtered volume.

### 1.3 Phytoplankton abundance and cyanobacterial biovolume

*What should be done in case of water turbidity, bad smell, green discoloration and/or the presence of scum?*

*How to assess whether a bloom event is taking place?*

### 1.3.1 Analysis of phytoplankton abundance

Qualitative and quantitative analysis of the phytoplankton includes identification, enumeration and calculation of biovolumes of phytoplankton, with fresh samples useful for identification, whereas microscopy for determining abundance and biovolumes is performed with preserved water samples. These analyses can be performed to assess if water turbidity, bad smell, green discolouration and/or the presence of scum are caused by a bloom, alternatively or in addition to the chlorophyll *a* measurement.

Different types of optical microscopes can be used for phytoplankton community identification. The following protocol describes the Utermöhl method (Utermöhl, 1958; for more information, see WHO, Padisák et al., 2021, EN ISO 5667-1; EN ISO 5667-3, IT, 2006; EC, 2000; EN 14996; EN 15204). This method is based on sedimentation by gravity of an aliquot of a water sample on the bottom of a chamber. The phytoplankton cells can then be identified and enumerated using an inverted microscope (see [Figure 5](#)).



Figure 5. Inverted microscope.

#### SCOPE

Analysis of phytoplankton abundance and composition using inverted microscopy

#### MATERIALS

- Lugol's solution (see recipe in WHO, Padisák et al., 2021)
- Ethanol (90%), acetone or isopropanol
- Distilled water
- Gloves

#### EQUIPMENT

- Inverted microscope
- Plastic containers or glass containers
- Plastic pipettes
- Pipet-Aid
- Micro-pipettes with disposable plastic tips
- Sedimentation chambers
- Cover glasses

- Marker
- Aluminium foil
- Timer
- Calculator
- Laboratory notebook

## METHOD

1. Allow samples and sedimentation chambers to acclimate to room temperature for at least 12 hours;
2. Clean the sedimentation chambers to avoid contamination from previous samples. The cleaning procedure is best done by using ethanol (90%), acetone or isopropanol, followed by rinsing with distilled water;
3. Before pouring the sample in the sedimentation chamber, manually homogenise it for about 2 minutes or 100 times, using gentle movements to avoid formation of bubbles and cell damage;
4. Choose a sedimentation chamber with an appropriate size and, if necessary, dilute the samples;
5. Place the sedimentation chamber on a horizontal surface and carefully fill its total volume, avoiding the formation of bubbles in the sample;
6. Cap the sedimentation column with a cover glass avoiding the formation of air bubbles;
7. Label the sedimentation chamber to ensure that it can be traced back to relevant information (e.g. sample volume, date, sampling site);
8. Keep the sedimentation chamber on the horizontal surface for 24 hours, at room temperature and protected from light. The recommended settling time is 3 hours per centimetre height of the sedimentation chamber;
9. After sedimentation, the upper cylinder of the sedimentation chamber is gently slid off from the bottom plate in order to remove the supernatant and then replaced by a cover glass, again taking care to avoid the formation of air bubbles;
10. Place the chamber on the microscope's stage;
11. Check at the microscope if the number of cells allows a correct identification and counting. If the cell density is low (less than 10<sup>5</sup> cells per litre), or the distribution is not random, the sample is discarded and a new one should be prepared. If the cell density is extremely high, the sample can be diluted with distilled water;
12. Counting of phytoplankton can be done as follows:
  - Counting part of the chamber floor by enumerating the cells contained in selected transects ([Figure 6b](#)) (taxa with intermediate dimension; e.g. magnification 250X);
  - Counting of randomly selected fields in the eyepiece grid ([Figure 6](#)) (for small taxa; magnification 400X).

A minimum value of 200 cells should be counted for each sample.

The following equation is used to convert the microscopic counts into cell abundance per unit volume of the sample:

$$C = N * \frac{(A * d)}{(a * v)}$$

Where C: concentration of phytoplankton cells or specific taxa per mL of the chamber; N: number of cells, or specific taxa, counted; A: total area of the chamber (mm<sup>2</sup>); a: total area of fields or transects counted (mm<sup>2</sup>); v: volume of the sample settled in the chamber (mL); d: dilution or concentration factor, when applicable (1X diluted d = 2; 1X concentrated d = 0.5).

In case of colonies, e.g. *Microcystis*, estimating cell number can be done by breaking up colonies using chemical or physical procedures. For the chemical procedure; a pellet of sodium hydroxide should be added to 100 mL of water sample and then heated at 90 °C for 30 min. For the physical procedure, 1 min of

ultrasound treatment ( $\sim 12 \mu\text{m}$ , 20 kHz) should be performed; the duration of sonication may vary depending on the sample (Bellinger and Sigeo, 2010).

Note: Samples collected must be preserved in a container (plastic container for short storage and glass container for long term storage periods) and quickly fixed with acidified Lugol's solution (WHO, Padišák et al., 2021) at rate of 0.2 - 0.5 mL to every 100 mL and immediately stored in the dark, at room temperature, until analysis is performed (preferably within three weeks of harvesting). Containers containing samples must be labelled with clear information including: date and place of sampling, type of analysis (e.g. cyanobacterial abundance) and description of the sample (e.g. sample collected in the metalimnion or epilimnion etc.).

The chamber (Figure 7) can be a single piece or composed by two parts: an upper cylinder containing a sample volume of 2, 5, 10, 25 or 50 mL, and a bottom plate with a thin cover glass. The thickness of the glass directly affects the resolution quality of the images obtained with a microscope, therefore it should be less than 0.2 mm. Chambers should be calibrated with water before use. The inverted microscope must be phase-contrast and/or differential interference-contrast and equipped with:

- eyepieces with 10X or 12.5X magnification;
- one eyepiece with a grid and/or the one with two parallel threads with a transverse thread (Figure 8);
- a minimum condenser numerical aperture of 0.5;
- objectives of 4-6X, 10X, 20X, 40-60X and a 100X oil immersion objective.

Taxonomic identification of phytoplankton can be performed to lower taxonomic levels (phylum and class) or to genus/species level, according to the aim of the study. Specific staff training is advised, particularly if the species and genus level is targeted to ensure proper identification and because the nomenclature of phytoplankton is constantly under revision. Text by Belcher and Swale (1976), Bellinger and Sigeo (2010) and key publications including Horner (2002), Tomas (1997), Throndsen et al. (2003, 2007) and Komárek et al. (2014) are a starting point for phytoplankton identification but they should be integrated with online sources. Taking photographs, making drawings and descriptions of algae and cyanobacteria is useful for later reference and for communication with experts.

Enumeration of the phytoplankton cells is often performed at different magnifications depending on the size of cells present in the sample. The quantification of the cell number should start at a low magnification in order to identify large species. Then, the quantitative analysis can proceed at successively higher magnifications for the identification of smaller species. Table 2 summarises magnifications recommended for different phytoplankton size classes. Specific taxa should always be counted at the same magnification in order to enable a comparison between samples. Considering that a same taxon can be found in different counting units (e.g. single cells or colonies), different magnifications can be used for their enumeration.

For filamentous taxa, measuring the length of the part of the filament within the grid rather than counting cells is often much more accurate.

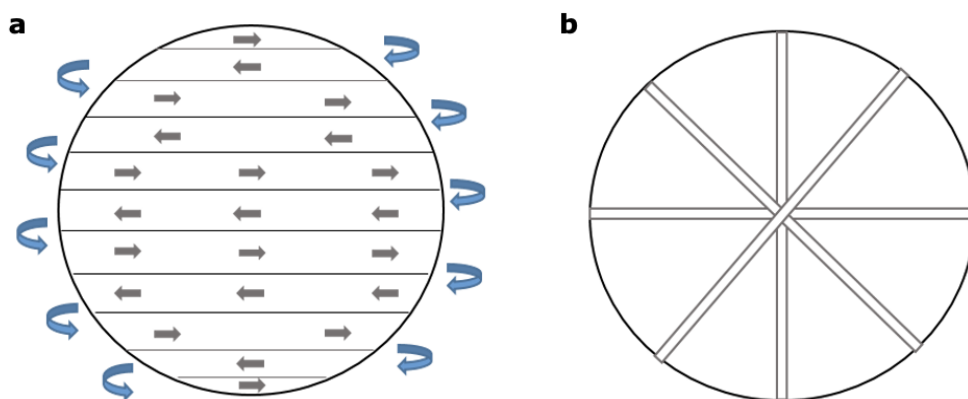


Figure 6. Enumeration of phytoplankton cells in a counting chamber. Counting of the whole floor of the chamber (a); counting of selected transects in the chamber (b).

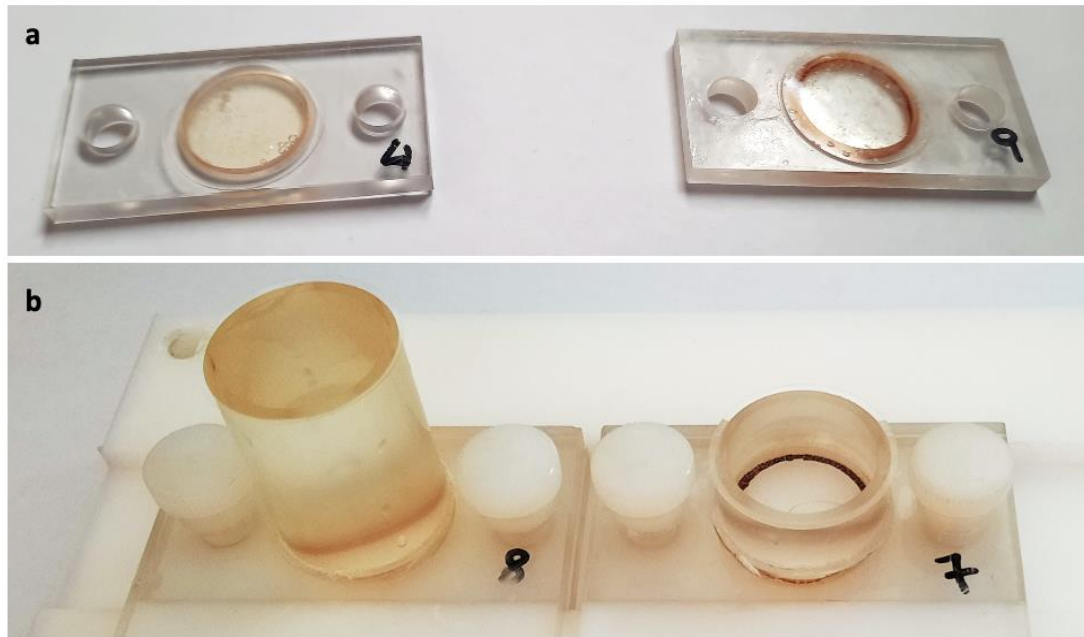


Figure 7. Sedimentation chambers. Details of the bottom plate (a) and the upper cylinder of various volumes (b).

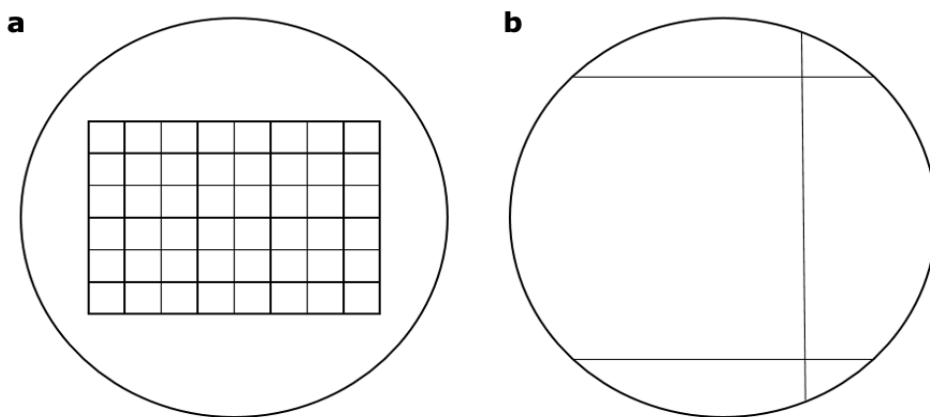


Figure 8. Schematic representation of eyepieces. Eyepiece with a grid (a) and with two parallel threads and a transverse thread (b).

Table 2. Recommended magnifications for the enumeration of different phytoplankton size classes.

Size class	Magnification
2 – 20 $\mu\text{m}$ (nanoplankton)	100 – 400X
> 20 $\mu\text{m}$ (microplankton)	100 – 200X




Source: Edler, 1976

### 1.3.2 Calculation of biovolume

*How can the contribution of different taxa to a bloom be determined?*

Biovolume is based on phytoplankton abundance, but adds information on the contribution of each taxon to the biomass of a sample. It is important because phytoplankton cell diameters can range from 1  $\mu\text{m}$  to 10  $\mu\text{m}$ , which implies that the enumeration of phytoplankton cells in a sample can refer to a wide range of values for cell volumes (up to a 1000-fold difference between cell volumes of different taxa). The biovolume of a sample is determined by measuring the dimensions of a certain number of cells (usually 20 cells, depending on the maximum number of cells counted) for each taxon and calculating an average volume using the geometric formula that best fits the respective taxon. An example of geometric formulas used for biovolume calculation of cyanobacterial cells is reported in [Table 3](#). See also WHO, Padisák et al. (2021) for further information.

Table 3. Geometric formulas for biovolume calculation of selected cyanobacterial cells.

Shape		Taxon	Formula	Exemplary dimensions in $\mu\text{m}$	Biovolume in $\mu\text{m}^3$
Sphere		<i>Aphanocapsa</i>	$V = \pi/6 \times d^3$	$d = 0.8$	0.27
		<i>Chroococcus</i>		$d = 2$	4.2
		<i>Synechococcus</i>		$d = 3$	14
		<i>Microcystis</i>		$d = 4$	34
		<i>Microcystis</i>		$d = 5$	65
		<i>Microcystis</i>		$d = 6$	113
Prolate Spheroid (rotational ellipsoid)		<i>Aphanothece (cell)</i>	$V = \pi/6 \times d^2 \times h$	$d = 1.2, h = 2.5$	1.9
		<i>Radiocystis (cell)</i>		$d = 3, h = 4$	19
		<i>Dolichospermum (cell)</i>		$d = 4, h = 6$	50
		<i>Dolichospermum (cell)</i>		$d = 5, h = 7$	92
		<i>Dolichospermum (filament)</i>	$V = \pi/6 \times d^2 \times h \times n$	$d = 3, h = 4, n = 80$	942
		<i>Dolichospermum (filament)</i>		$d = 4, h = 6, n = 50$	3770
Cylinder		<i>Limnothrix (cell)</i>	$V = \pi/4 \times d^2 \times h$	$d = 2.5, h = 10$	49
		<i>Planktothrix (cell)</i>		$d = 5, h = 5$	98
		<i>Planktothrix (cell)</i>		$d = 8, h = 5$	251
		<i>Moorea (cell)</i>		$d = 20, h = 3$	942
		<i>Planktothrix (filament)</i>	$V = \pi/4 \times d^2 \times l$	$d = 5, l = 300$	5890
		<i>Planktothrix (filament)</i>		$d = 8, l = 450$	22 619
		<i>Moorea (filament)</i>		$d = 20, l = 1500$	471 238

Source: WHO, Testai and Chorus, 2021.

## SCOPE

Calculation of biovolume using inverted microscopy

## MATERIALS

- Lugol's solution (WHO, Padisák et al., 2021)
- Ethanol (90%), acetone or isopropanol
- Distilled water
- Gloves

## EQUIPMENT

- Inverted microscope
- Plastic containers or glass containers
- Plastic pipettes
- Pipet-Aid
- Micro-pipettes with disposable plastic tips
- Sedimentation chambers
- Cover glasses
- Marker
- Aluminium foil
- Timer
- Calculator
- Laboratory notebook

## METHOD

Follow the method described in [section 1.3.1](#) up to point 11 and then proceed to point 12 here below:

12. The biovolume of a taxon is derived by multiplying the average cell volume by the density of cells according to the following equation:

$$\text{Biovolume}_{\text{taxon}} (\text{mm}^3/\text{L}) = N * V * 10^{-9}$$

The total biovolume of a sample corresponds to the sum of biovolumes determined for different taxa:

$$\text{Total biovolume} = \sum_{i=1}^n (N_i * V_i * 10^{-9})$$

Where  $N_i$ : number of cells of each taxon/L;  $V_i$ : average cell volume of each taxon ( $\mu\text{m}^3$ ).

The final value should consider eventual concentration step of the sample.

Note: See notes in [section 1.3.1](#).

## 1.4 Cyanotoxins

*When a check for cyanotoxins should be performed?*

*How cyanotoxins can be detected?*

### 1.4.1 Enzyme-Linked Immunosorbent Assay

In case of pronounced water greenish turbidity or discolouration (see Alert level 1 in [Table 1](#)), a check of cyanotoxins should be performed to ensure they do not reach a hazardous level in water. The detection of intracellular and extracellular toxins produced by cyanobacteria during bloom events can be obtained using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits. ELISA can be performed on extracts or raw water samples, freshly collected or preserved at  $-20^{\circ}\text{C}$ , following the manufacturer's instructions of the chosen kit. It is a rapid, simple and sensitive methodology, which requires basic laboratory skills and gives quantitative results in 3-4 hours. Many samples can be analysed at one time. An example of the ELISA assay is the competitive direct ELISA ([Figure 9](#)). It is based on the concept that when a specific toxin is present in the analysed sample, a colorimetric reaction is generated and compared against a standard curve provided with ELISA kits to get the toxin concentration ([Figure 10](#)). The resulting colorimetric reaction is measured at defined wavelengths using a spectrophotometer ([Figure 11](#)). The ELISA assay does not identify specific cyanotoxin variants but the total toxin concentration. Concentrations of defined variants can be obtained using specific ELISA kits. [Table 4](#) shows literature studies where the ELISA method was adopted for the detection of microcystins, nodularin, saxitoxin, anatoxin, cylindrospermopsin and  $\beta$ -N-methylamino-L-alanine (BMAA). Further information can be found in WHO, Lawton et al. (2021).

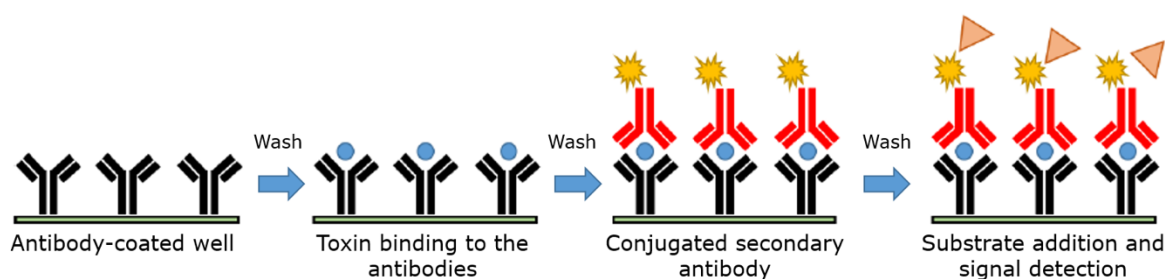


Figure 9. Schematic representation of the direct Enzyme-Linked Immunosorbent Assay (ELISA). In the ELISA assay, toxin-specific antibodies are immobilised on a 96-well plate and can bind the toxin present in the environmental samples. An enzyme-conjugated secondary antibody which can recognise the toxin is added to the plate. The interaction between the substrate and the enzyme-conjugated secondary antibody generates a colorimetric reaction which is proportional to the amount of toxin in the analysed sample. *Source:* Sanseverino et al., 2017.

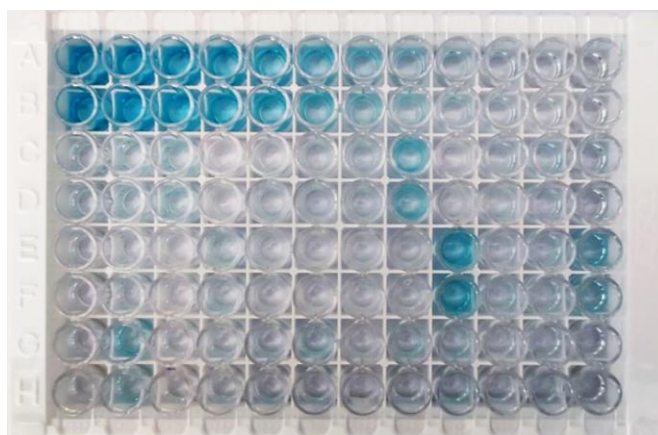


Figure 10. Colorimetric reaction on a 96-well plate. The concentration of toxin is directly proportional to colour intensity.

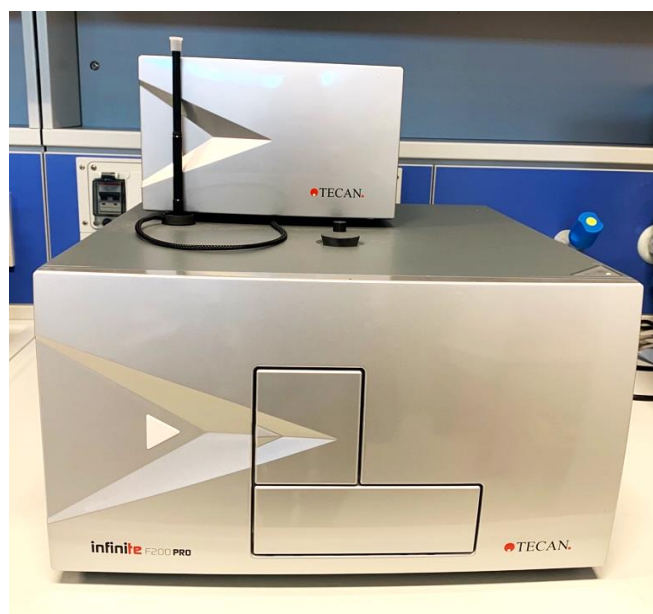


Figure 11. Tecan Infinite F200 Pro – example of a spectrophotometer for detection of toxins. The colorimetric reaction on a 96-well plate should be measured at a defined wavelength according to instruction in the ELISA kit.

Table 4. Enzyme-Linked Immunosorbent Assay (ELISA) methods for the analysis of cyanotoxins. Modified from Sanseverino et al., 2017.

Cyanotoxin	Detection limit ( $\mu\text{g/L}$ )	Extraction/sample preparation	Location	References
Microcystins Nodularin	1	n.a.	Drinking water	(Fischer et al., 2001)
Microcystins Nodularin	Not given	Filtration and sonication	Fresh water and brackish water (Finland)	(Spoof et al., 2003)
Microcystin LR	Not given	Sonication; SPE	Lake water, China	(Shen et al., 2003)
Microcystins	Not given	Lyophilisation	Lake water, New Zealand	(Mountfort et al., 2005)
Saxitoxin	n.a.	Solid phase adsorption toxin tracking (SPATT)	Coastal waters (California, USA)	(Lane et al., 2010)
Saxitoxin	0.02	n.a.	Arctic fresh water ecosystems	(Kleinteich et al., 2013)
Microcystins	0.12	n.a.	Tap and drinking water (China)	(Sheng et al., 2006)
Microcystins	Not given	Filtration	Constance Lake, Canada	(Tillmanns et al., 2007)
Microcystins Nodularin	0.1	n.a.	Surface and drinking waters (Greece)	(Triantis et al., 2010)

Microcystins in water, sediment, fish, and bloom	Not given	Water: SPE (C18) Fish: Lyophilisation Sediment: Lyophilisation	Lake Eğirdir (Turkey)	(Gurbuz et al., 2016)
Microcystin LR in clams	0.1	SPE	Coastal ponds (Sardinia; Italy)	(Sedda et al., 2016)
Microcystins	0.05	n.a.	River Ganga, India	(Dixit et al., 2017)
Anatoxin	0.1	Three freezing and thawing cycles to lyse the cells	Surface water samples (Australia)	(John et al., 2019)
$\beta$ -Methylamino-L-alanine (BMAA)	4	Filtration and freezing/thawing cycles	Surface water (Italy)	(Clausi et al., 2016)
Cylindrospermopsin	0.05	Filtration and concentration	Algal cells collected on filters (Lake Catemaco, Mexico)	(Berry and Lind, 2010)

## SCOPE

Measurement of toxins' concentration using the ELISA assay

## MATERIALS

- Deionised or distilled water

## EQUIPMENT

- ELISA kit specific for the selected toxin
- Glass container (clear or amber) or plastic containers
- Micro-pipettes with disposable plastic tips
- Multi-channel pipette, stepper pipette, or electronic repeating pipette with disposable plastic tips
- Paper towels or equivalent absorbent material
- Tape or parafilm
- Graduated cylinder
- Microtiter plate reader (wavelength 450 nm)
- Timer
- Microtiter plate washer (optional)
- Laboratory agitator

## METHOD

Follow the specific manual's instructions.

Note: The collected samples have to be stored in thermic containers for the transport to laboratory facilities. It is advised to run each sample in triplicate. It is generally recommended to avoid plastic containers for water storage other than polyethylene terephthalate glycol (PETG) as toxins will adsorb to the container's surface. However, for BMAA, the use of amber glass containers should be avoided as this toxin will adsorb to amber glass producing inaccurate results. Water samples can be stored at 4°C for up to 5 days, or frozen at -20°C. Ship overnight on ice.

## 1.4.2 Liquid chromatography and ultrahigh performance liquid chromatography tandem mass spectrometry

In liquid chromatography (LC), cyanotoxins are separated by a liquid under high pressure in columns packed with silica particles in the range of 2–50 µm in size. Ultrahigh performance LC (UHPLC) employs even smaller particles (<2 µm) and smaller columns leading to faster analysis with higher separation efficiency and minimised matrix interference. However, most protocols are developed to detect a limited number of microcystin congeners. Three methods are described below.

### SCOPE

SPE-LC-MS/MS method for simultaneous determination of multi-class cyanobacterial and algal toxins

### MATERIALS

- Analytical standards
- Methanol
- Dichloromethane (DCM)
- Acetonitrile
- Sodium hydroxide (NaOH) for sample pH adjustment
- Formic acid
- Oasis HLB (200 mg, 6 cc, 25–35 µm) Waters Corporation, USA
- HyperSep Hypercarb PGC (porous graphitic carbon, 200 mg, 3 cc, 30–40 µm)
- 12-port SPE vacuum manifold with large volume samplers (PTFE tubes)
- Diaphragm vacuum pump (KNF)
- HPLC column: Atlantis T3, 2.1 mm × 100 mm, 3 µm
- Mobile phase A: acetonitrile
- Mobile phase B: water, 0.5% formic acid

### EQUIPMENT

- HPLC: Finnigan Surveyor HPLC, equipped with a Finnigan Surveyor AS autosampler
- MS: Finnigan TSQ Quantum Discovery Max triple-stage quadrupole mass spectrometer

### METHOD

1. 4 mL of methanol are added to 400 mL of the water sample;
2. Initial sample pH is adjusted to 11 with addition of NaOH 2 M (dropwise);
3. Oasis HLB and HyperSep PGC cartridges are connected in series (top Oasis HLB, bottom PGC) and conditioned sequentially with 6 mL DCM, 6 mL methanol and 6 mL water (pH11);
4. Samples are passed through the solid phase extraction (SPE) assembly;
5. The cartridges are dried for 15 min (air under vacuum);
6. The sequence of the two cartridges in the SPE assembly is then reversed (top PGC, bottom Oasis HLB) and the analytes are eluted with a mixture of 10 mL DCM:MeOH (40:60, v/v), containing 0.5% formic acid;
7. The extract is dried under a gentle stream of nitrogen;
8. The residue is re-dissolved with 400 µL MeOH:H<sub>2</sub>O (5:95, v/v) and sonicated in water bath for 5 min;
9. The final extract is then transferred to an auto-sampler glass vial and analysed by LC-ESI-MS/MS;
10. HPLC gradient program: start at 5% A (held for 3 min), increasing to 20% A in 1 min (held for 2 min), to 35% A in 1 min (held for 7 min), to 70% A in 14 min and to 90% in 1 min (held for 3 min). An equilibration time of 10 min is needed after each sample run. Flow rate is set at 0.2 mL/min, the column is thermostated at 30°C and injection volume is 20 µL;
11. Run time: 40 min.

Note: SPE has to be carried out using an assembly of two cartridges, Oasis HLB, and HyperSep Hypercarb PGC, and a 12-port SPE vacuum manifold with large volume samplers, and a diaphragm vacuum pump (see Figure 12).

Ionisation of compounds is performed with ESI probe in positive mode. Detection is carried out in multiple reaction monitoring mode (MRM) using the three most intense and characteristic precursor/product ion transitions obtained from the MS/MS.

Examples of LC-MS/MS chromatograms of cyanotoxins, the conditions for the LC-MS-MS and the LOD values are shown in the publication of Zervou et al. (2017).

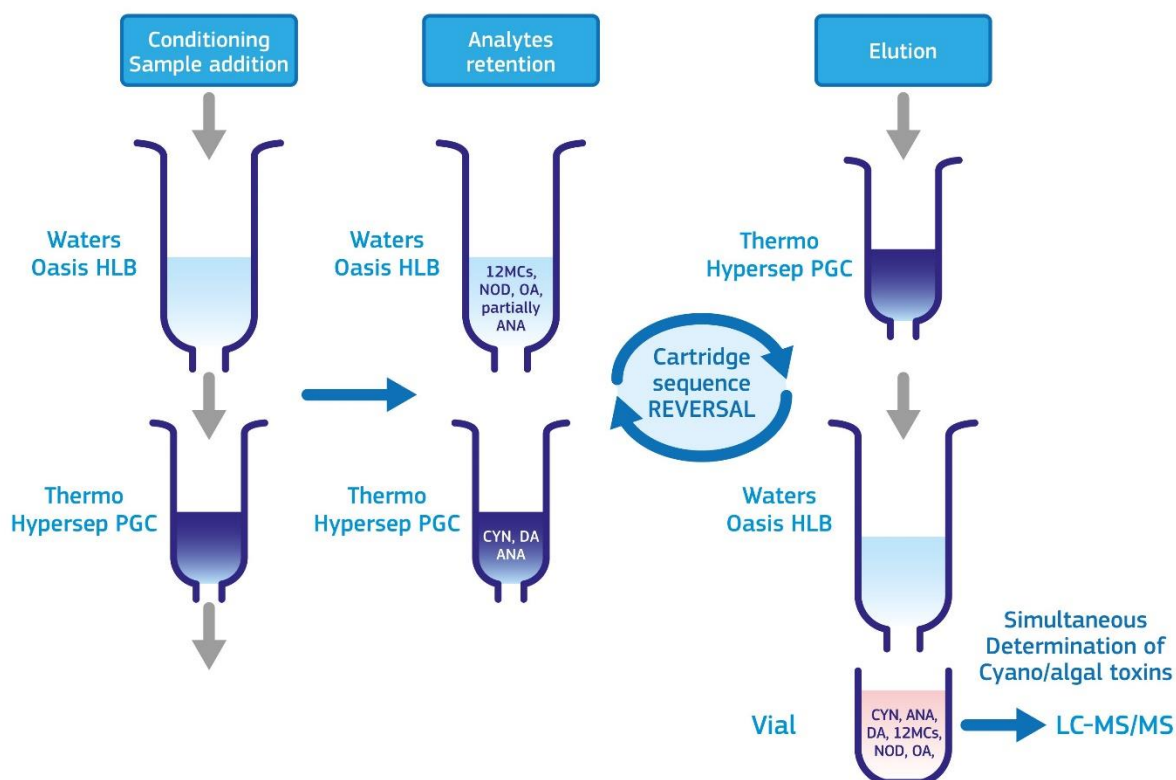


Figure 12. Dual solid phase extraction (SPE) cartridge assembly and extraction process.

## SCOPE

Analysis of microcystin-LR in surface water by solid-phase extraction ultra-performance liquid chromatography triple-quadrupole mass spectrometry (SPE-UPLC-MS/MS)

## MATERIALS

- Microcystin-LR analytical standard
- 400 mL surface water at neutral pH
- Oasis HLB 200 mg SPE cartridges (Waters)
- Methanol for SPE (conditioning and elution)
- Acquity UPLC BEH C18 column, particles 1.7  $\mu\text{m}$ ; 50 x 2.1 mm (Waters)
- HPLC eluents: water and acetonitrile (both with 0.1% acetic acid)

## EQUIPMENT

- Automated AutoTrace® solid-phase extraction (SPE) system (see [Figure 13a](#))
- Waters Acquity UPLC coupled to an AB Sciex 5500 Qtrap MS/MS (see [Figure 13b](#))

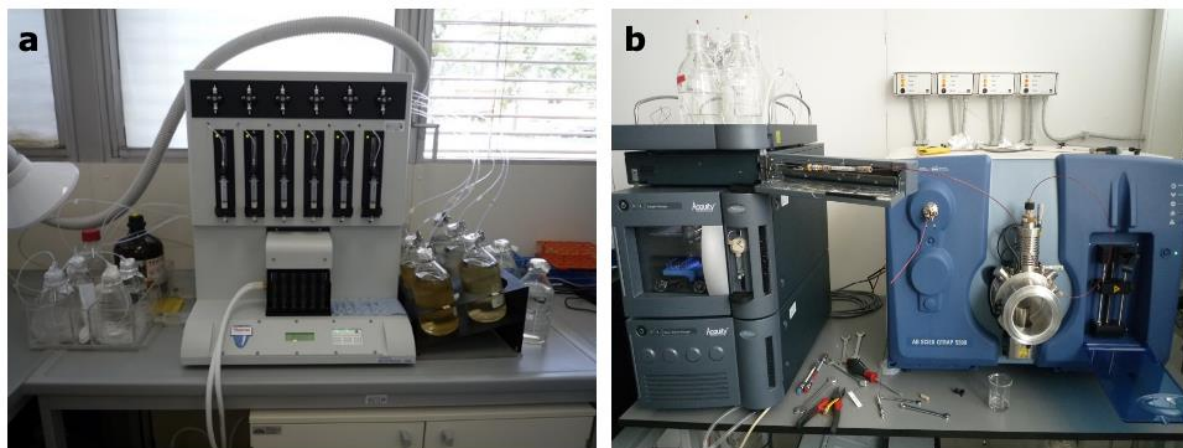


Figure 13. AutoTrace® SPE system (a) and Waters Acquity UPLC coupled to an AB Sciex 5500 Qtrap MS/MS (b).

## METHOD

1. Condition the SPE cartridges with 8 mL methanol, followed by 8 mL water; flow 5 mL/min;
2. Extraction of 400 mL water at neutral pH using Oasis HLB cartridges with automated SPE; water flow 5 mL/min;
3. Drying of cartridges with nitrogen for 30 min;
4. Elution of the cartridges with 6 mL methanol and evaporation of the extracts to 500  $\mu$ L. Recovery about 95%;
5. HPLC eluents: water and acetonitrile (both 0.1% acetic acid), gradient start with 90% water to 90% acetonitrile; injection volume 5  $\mu$ L; run time 10 min; retention time 3.2 min;
6. Microcystins can be detected in the positive or negative ionisation modes. The specific MS/MS transitions for microcystin-LR were  $m/z$   $[M+H]^+$  995.5 > 213, 135, and 553 in positive, or  $[M-H]^-$  993.5 > 265, 283, and 128 in negative ionisation mode, respectively (see [Figure 14](#) and [Figure 15](#)).

Note: LOD: 1 ng/L.

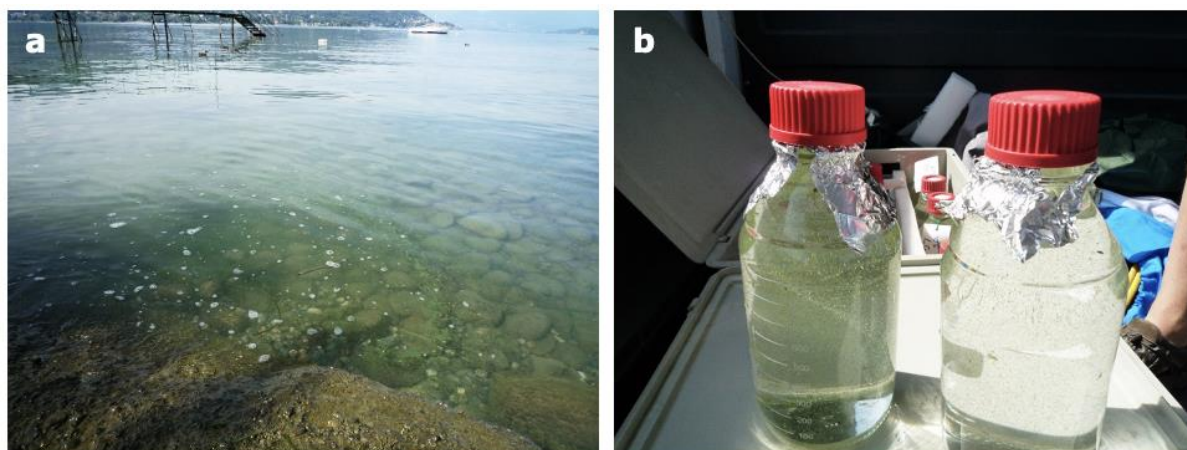


Figure 14. Cyanobacterial bloom at Lake Maggiore (Italy) (a); Water samples collected in summer 2010 (b).

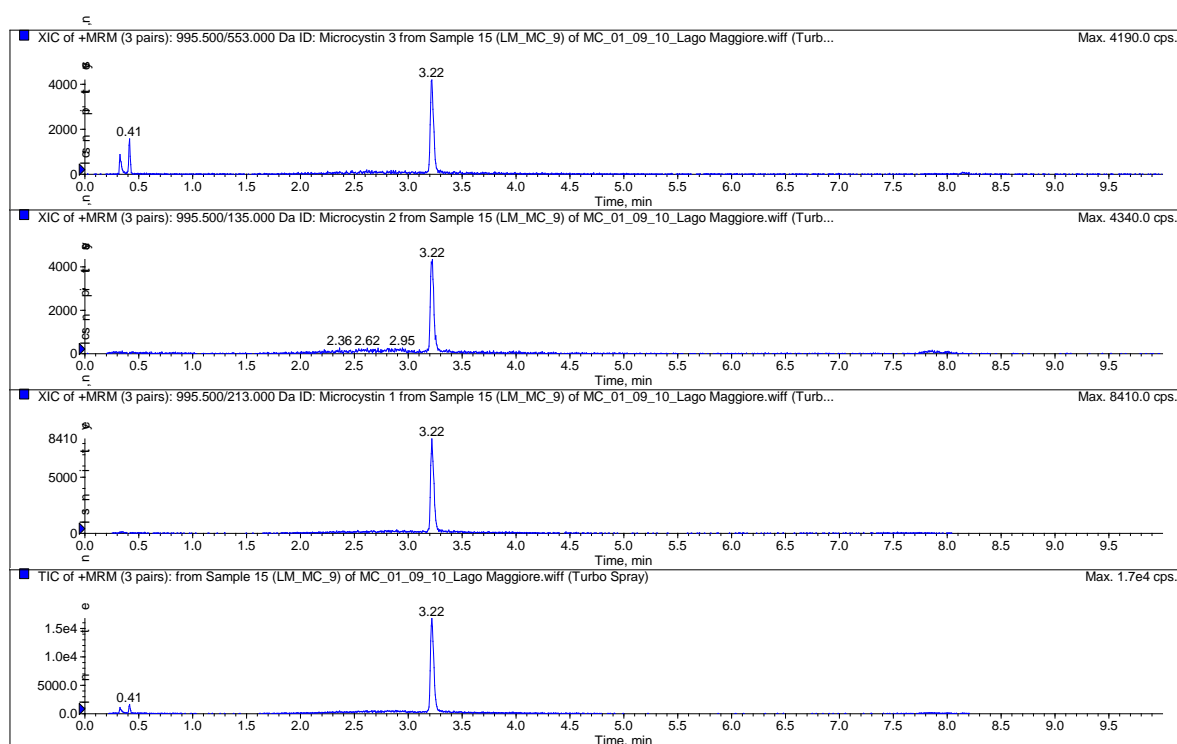


Figure 15. LC-MS/MS chromatogram of microcystin-LR in a real water sample from Lake Maggiore (Italy), 30 ng/L.

## SCOPE

Analysis of cyanotoxins, including microcystins, in drinking and surface waters by liquid chromatography-tandem quadrupole mass spectrometry (LC-MS/MS) (direct injection without the need of sample preparation)

## MATERIALS

- Cylindrospermopsin
- Anatoxin-a
- Nodularin
- Microcystin-LR, DeRR, RR, YR, LY, LW, LF

- UPLC-column: Acquity UPLC BEH C18, 1.7  $\mu\text{m}$ , 2.1 x 100 mm (Waters)
- Mobile phase A: water, 0.1% formic acid
- Mobile phase B: acetonitrile

## EQUIPMENT

- UPLC system: Acquity UPLC (Waters) (see [Figure 16](#))
- MS system: Xevo TQ-S



Figure 16. Acquity UPLC - Xevo TQ-S MS.  
*Source: Degryse et al., 2017*

## METHOD

1. Drinking and surface water samples can be injected without any further preparation;
2. UPLC flow rate: 0.4 mL/min;
3. Column temperature: 50°C;
4. Injection volume: 50  $\mu\text{L}$ ;
5. Run time: 7.5 min;
6. UPLC gradient start with 100% A, hold for 0.75 min, to 80% B in 5 min, to 100% B in 1 min, and back to 100% A in 1.5 min.

Note: For MS/MS conditions see [Table 5](#). An example of LC-MS/MS chromatograms of cyanotoxins is shown in [Figure 17](#).

Table 5. LC-MS/MS conditions. Retention time, MRM transitions, cone, and collision energy.

Compound	Retention time (min)	MRM	Cone (V)	Collision energy (eV)
CYL	1.06	416.2>194.2	60	38
		416.2>336.2	60	22
ANA	1.65	166.1>131.1	35	14
		166.1>149.1	35	12
MC-DeRR	3.41	513.2>71.0	50	50
		513.2>135.1	50	25
MC-RR	3.44	520.2>70.0	60	50
		520.2>135.1	60	28
NOD	3.65	825.4>135.1	80	60
		825.4>227.2	80	50
MC-YR	3.75	1045.7>127.1	62	85
		1045.7>135.1	60	70
MC-LR	3.81	995.6>107.1	60	80
		995.6>135.1	60	70
MC-LY	4.66	1002.7>107.1	60	76
		1002.7>135.1	60	72
MC-LW	5.03	1025.7>127.1	60	80
		1025.7>135.1	60	70
MC-LF	5.17	986.6>135.1	60	60
		986.6>249.2	60	50

Source: Degryse et al., 2017

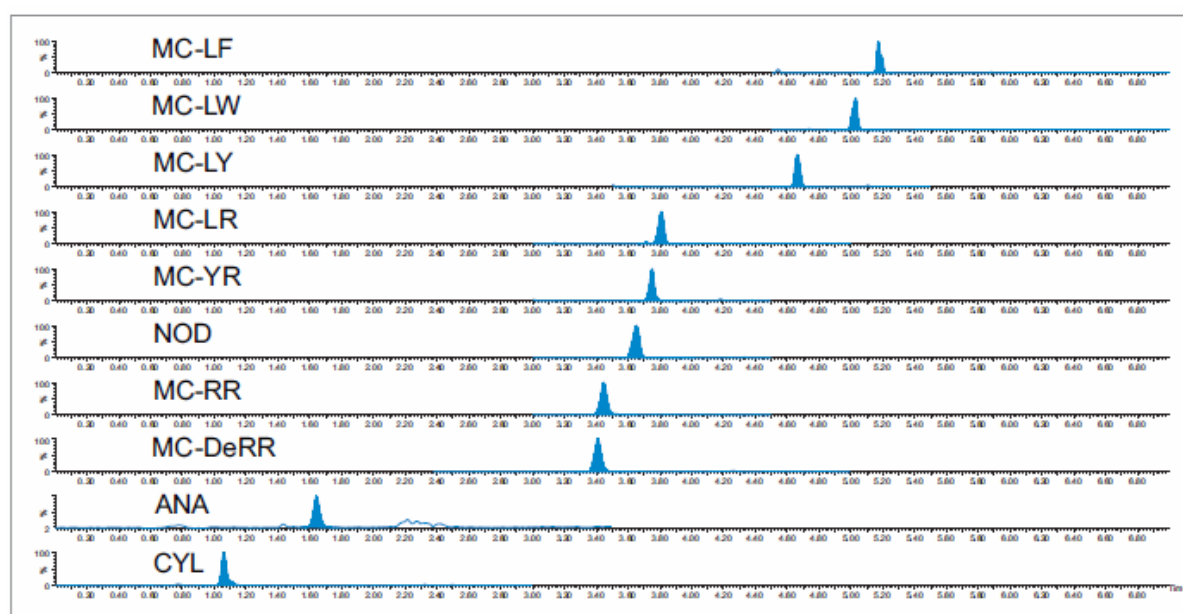


Figure 17. LC-MS/MS chromatograms of cyanotoxins of a standard in drinking water at 25 µg/L. CYL: cylindrospermopsin, ANA: anatoxin-a, NOD: nodularin and MC: microcystin. Source: Degryse et al., 2017

## 2. Distinguishing between cyanobacteria and algae

Current methods for discrimination between cyanobacteria and algae mainly rely on the identification of genera through microscopy. With this approach, differentiation at species level is not always possible and highly skilled personnel is required. Similar, identification of genotypes, which are linked to the production of toxins, cannot be achieved based on morphological characteristics (WHO, Padisák et al., 2021). To overcome these issues, some existing techniques can be adopted to understand the nature of a possible bloom and to adopt corrective actions for its mitigation.

Analysis by fluorescent microscopy performed in a laboratory allows determining the level of phycocyanin, a pigment synthesised by cyanobacteria absent in algal cells, however confounding factors must be taken into account when interpreting the results. Underestimation may occur when phycocyanin concentrations within cyanobacterial cells decrease due to lack of nitrogen in the water, when the concentration of humic substances is high and in concomitance with other algae producing different pigments which may mask phycocyanin or require a different excitation spectrum, as is the case for phycoerythrin produced by *Planktothrix rubescens*.

As a confirmatory step, this technique can be combined with light microscopy at high resolution (1000X) to verify the presence of plastids in eukaryotes (algae) or gas vesicles and non-membrane bound organelles in prokaryotes (cyanobacteria). Estimation of starch levels may point at the absence of cyanobacteria which, in contrast to eukaryotic cells, accumulate other types of polysaccharides, but this method may also generate false results when algae occur which do not rely on starch storage (e.g. *Xanthophyceae*) (Suzuki et al., 2013). Staining with Lugol's solution can facilitate the identification of starch or pyrenoids where the polysaccharide is stored in algal cells (Sánchez-Parra et al., 2020).

Molecular methods may be employed to detect and quantify invasive species even at low levels difficult to determine with morphological approaches and to monitor their concentrations before they reach bloom density. Genetic markers (*rbclX*, *rpoB*, *rpoC1* and *cpcBA*) have been validated for frequently detected cyanobacteria such as *Cuspidothrix*, *Sphaerospermopsis*, *Cylindrospermopsis*, *Chrysochloris* or *Raphidiopsis* (Kim et al., 2021). A general screening can be performed amplifying 16S rRNA and other targets, for example the internal transcribed spacer targeting the 16S-23S boundary of bacterial rRNA genes or the phycocyanin intergenic spacer (USGS, 2017).

Recently developed algorithms based on remote sensing reflectance take into account natural variation in population size, pigment concentrations (chlorophyll *a*, phycocyanin) and bloom structure, which can be adapted to distinguish among phytoplankton groups (Matthews et al., 2020).

## 3. De-warning system for cyanobacterial blooms

*How to ensure safe bathing when vigilance/risk level changes?*

De-warning of the population when parameters informing about cyanobacterial blooms are below levels of high risk to human health should be put in place to ensure a safe use of bathing sites. De-warning is particularly important after warnings or when site closures have been in place. Such a system can follow the warning system in backward direction with the advantage of there being no need to develop an additional scheme; furthermore this is readily adapted to existing national warning systems (Figure 18).

Following assessment of risk from toxic cyanobacteria based on national provisions, alerts to local authorities and the public should be communicated. A de-warning pathway implies the release of information to bathing site users when a risk level decreases, which may result in an alert for risk level 1 or recommendations when risk is substantially reduced but attention is still advised (Figure 18). Information to the public should also be released when measured parameters indicate no threat of toxic cyanobacteria in a given waterbody. Among the Member States (MS), only Germany and Slovakia have currently implemented three-step de-warning systems applied bottom-up to the warning system actions; these are described in detail in Part II [section 3.6](#) and [section 3.13](#), respectively.

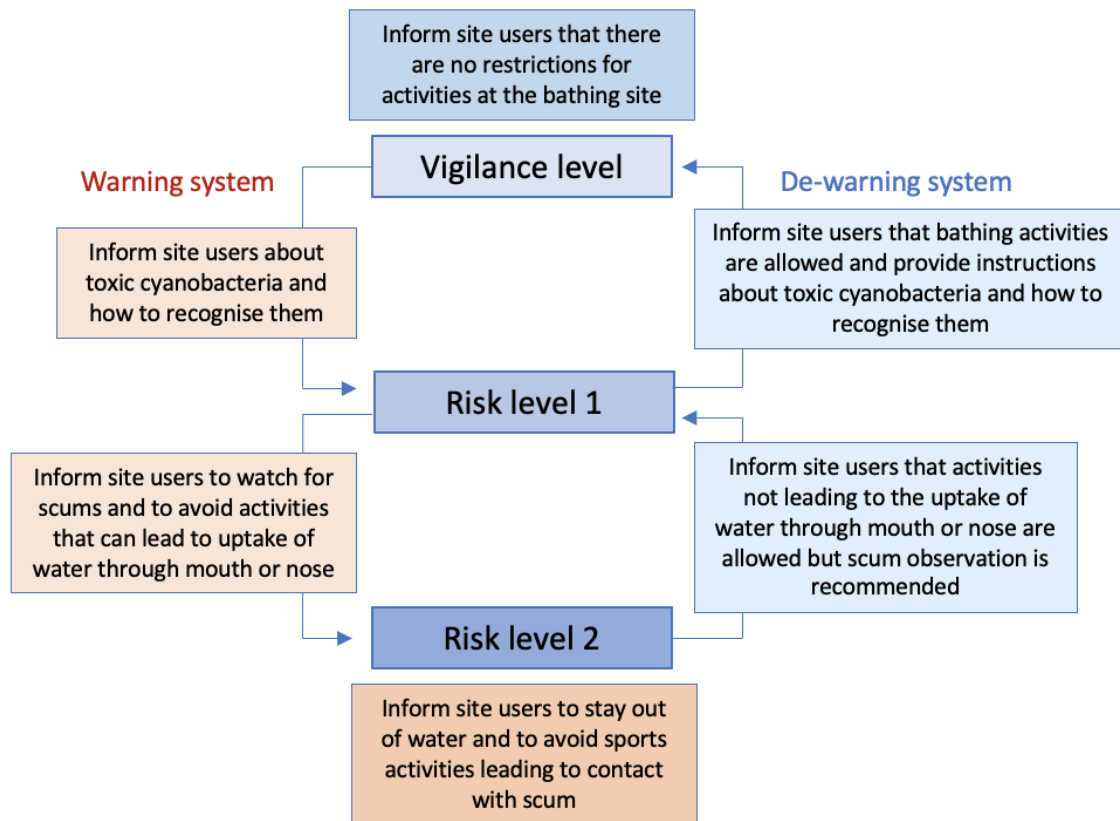


Figure 18. De-warning system as a backward application of a cyanobacterial bloom warning system. Vigilance and risk levels established based on national provisions are adaptable to the information on allowed activities in affected bathing sites.

## 4. Other methods for detecting cyanobacteria and their toxins

- *How can satellite systems and metagenomic methods help in monitoring cyanobacterial blooms and HABs in waterbodies? → See Part I, [section 4.1](#) and [section 4.2](#)*
- *Which are the newly developed methods for the monitoring and management of HABs? → See Part I, [section 4.3](#)*
- *Which method can be used to detect specific toxin variants?*

The global incidence of harmful algal blooms (HABs) along with their social and economic impacts has been widely described in the JRC reports (Sanseverino et al., 2016 and 2018). The reports highlighted the importance of effective monitoring and management systems in order to anticipate the risks of HABs events and to thus minimise economic losses. This can be achieved primarily by using sensitive methods able to predict the occurrence and extent of HABs, and consequently by adopting timely warning and de-warning systems. As experience is still being gathered with approaching this, national systems among the Member States (MS) vary (more details in Part I, [section 3](#)).

Several traditional methods (e.g., high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), Enzyme-Linked Immunosorbent Assay (ELISA), real-time quantitative PCR (see [section 1.4.1](#) and [section 1.4.2](#))) are currently being employed for the detection of cyanotoxins (Sanseverino et al., 2017; Schreidah et al., 2020). Microcystins (MC) are among the main analytical targets due to their frequent occurrence in those species that form the heaviest scums. Especially, the MC-leucine arginine (MC-LR) variant is the most prevalent and toxic MC in fresh water and marine water worldwide (Rastogi et al., 2014), although new MC are still being identified, although their concentrations during blooms are usually very low (LeBlanc et al., 2020). However, the limitations of these methods, primarily related to low sensitivity and specificity, long time to results, high cost, and/or complexity of protocols for sample preparation requiring high-skilled operators, favoured the development of novel methods in recent years, many of which proved to be a valid solution for satisfying the above-mentioned needs (Kordasht et al., 2020).

Among those methods, two approaches, obtaining different types of information and both useful for predicting the occurrence and severity of bloom events, are discussed below. Satellite systems provide geospatial data based on remote sensing which not only show scums and blooms but can also indirectly inform about factors contributing to bloom formation, such as air temperature, usually for large areas (Zibordi et al., 2020). Metagenomic methods can provide accurate information on species and strain composition, including on the occurrence of toxin-containing strains. While both methods cannot accurately quantify cyanobacterial biomass or toxin concentrations, they have specific merits for early warning. The two approaches and contexts of their application are described in sections below.

### 4.1 Satellite systems

Remote sensing-based systems are a useful tool for routine monitoring of cyanobacterial and harmful algal blooms in either fresh or coastal waters, especially in a longer-term perspective ([Figure 19](#)). They provide a low-cost support for intensified monitoring in space and time, especially in areas requiring a frequent sampling with a large number of sample collection points (Who, Welker et al., 2021). Satellite data with matched field data enable detection of bloom events, prioritisation of exposed waterbodies as well as a better spatiotemporal predictive modelling in relation to climatic parameters and environmental drivers (Zhao and Ghedira, 2014; Anderson et al., 2016; Clark et al., 2017; Shi et al., 2017; Urquhart et al., 2017; Dörnhöfer et al., 2018; Kahru et al., 2018; Mu et al., 2019; Myer et al., 2020; Mishra et al., 2021; Soto et al., 2018). For example, a study in the eutrophic Lake Varese, Italy, indicated the surface air average temperature as the best parameter of lake stratification and cyanobacterial/algae bloom predictable 2 weeks before the outbreak, providing a basis for validation in other waterbodies (Chirico et al., 2020).

In other research projects, data collected by Sentinels of the [EU's Earth Observation Programme Copernicus](#) allowed to develop methods for monitoring waters with persistent and wide-spreading cyanobacterial or algal blooms. For example, Copernicus Marine forecasts implemented with information from other sources such as the European Marine Observation and Data Network ([EMODnet](#)) were used to localise cyanobacteria and phycocyanin within 3 days in order to predict the risk of bloom occurrence near bathing sites<sup>2</sup>. Likewise, Copernicus Earth observation data enabled the development of a near real-time service improving temporal and spatial monitoring to quickly detect potentially harmful blooms in fresh water and coastal areas<sup>3</sup>.

Satellite-based methods coupled to dedicated algorithms and to machine learning can provide semi-quantitative data for cyanobacterial biomass, spatial extension of blooms and distribution of cyanobacteria and, when normalised to specific areas, enable comparisons between separated waterbodies (Shi et al., 2015; Gorham et al., 2017; Mishra et al., 2019; Nguyen et al., 2020; Soria-Perpinya et al., 2020). Satellite data combined with an integrated analysis of biological, genetic, meteorological and/or oceanographic information allowed to identify the origin of harmful algae and the long-distance pathway they followed with the water flow (Wynne et al., 2011; Gillibrand et al., 2016; Kruk et al., 2021). As well, geospatial risk modelling based on data from 771 waterbodies and using iterative Akaike information criterion (AIC) proved helpful to predict the effect of bloom mitigation measures and to identify the strongest predictors of cyanobacterial cell densities (i.e., percent forest/agriculture, minimum winter temperature, waterbody area) (Weber et al., 2020). Aerial systems (RGB band cameras and spectral enhancement techniques) were considered sufficient to provide information on surface-floating HAB distribution over the surface of 1 km<sup>2</sup> (Qu et al., 2019).

However, satellite remote sensing needs to be supplemented by ground-based methods in order to deliver information on the risk of exposure to toxic blooms and on dominant species. Such methods vary in accuracy based on assessed parameters and species. Satellite data products may require calibration with *in situ* sampling data, as the intensity and colour of light captured by satellite sensors are affected by a variety of in-water optically-significant components as well as the atmospheric composition (WHO, Welker et al., 2021). Calibration can be based on cell counts or chlorophyll *a* concentration in water samples collected during monitoring of spatiotemporal bloom dynamics (Lehmann et al., 2021). For example, improved results have been obtained with an algorithm trained using historical data on HAB events with risk classification based on water-leaving radiances of water colour discriminants to identify harmful blooms, including information on dominant species, rather than those based on chlorophyll *a* (Kurekin et al., 2014). In addition to chlorophyll *a*, concentrations of other pigments have an influence on the water optical properties that may be interpreted, in appropriate circumstances, by satellite observations to distinguish the main groups of the phytoplankton: fucoxanthin for diatoms, peridinin for dinoflagellates, 19'hexanoylfucoxanthin for haptophyte, zeaxanthin for cyanobacteria or divinyl chlorophyll *a* for *Prochlorococcus* (Puissant et al., 2021; Kramer et al., 2019 and 2022; Wang and Moisan, 2020). Satellite optical measurements are relevant only for the upper layer of waterbodies and can be limited by weather conditions (e.g. cloud cover), interference from materials other than phytoplankton or macrophyte cover in the waterbody (WHO, Welker et al., 2021; Liu et al., 2022; Shin et al., 2019). While ocean screening systems provide large amounts of data, fresh water screening is being intensified with new satellite programmes<sup>4,5</sup>.

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<sup>2</sup> [HAB-RISK service](#).

<sup>3</sup> [CyanoAlert® project](#).

<sup>4</sup> NASA: [Surface Water and Ocean Topography](#).

<sup>5</sup> [Cyanobacterial Assessment Network \(CyAN\)](#).

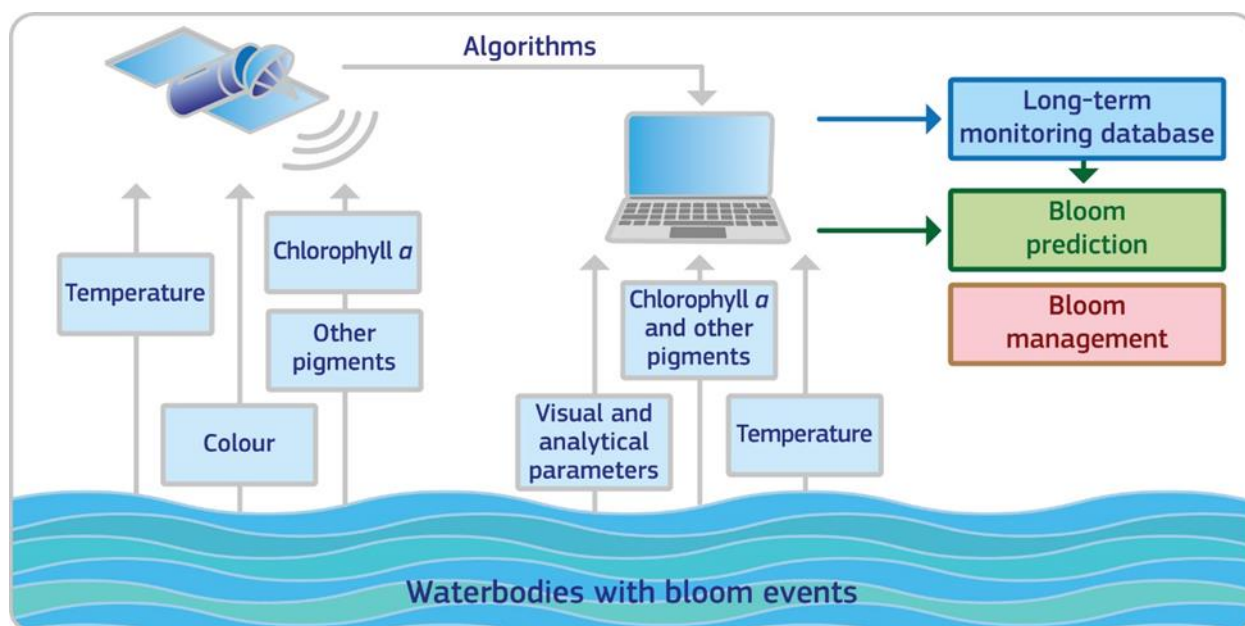


Figure 19. Satellite-based monitoring of cyanobacterial and harmful algal bloom (HAB) events. Remotely collected data primarily reflect the change of water colour due to the content of pigments in relation to the abundance of cyanobacteria and algae, besides the effects of other optically significant constituents. Other data, such as temperature can be collected by either satellite systems or from field sampling which includes a wider range of visual and analytical parameters. *In situ* sampling data can be used to calibrate the satellite data allowing the latter to give spatially integrated information and possibly also a higher frequency of data than e.g. monthly sampling can provide. Both types of data can be combined through algorithms yielding an integrated and dynamic picture of current and long-term observations which allows prediction and management of bloom events at early stages.

## 4.2 Quantitative polymerase chain reaction

Molecular methods, such as quantitative polymerase chain reaction (qPCR), may provide information on the presence of specific strains and production of toxins within a given bloom. Such information, along with the data on environmental conditions, can help early prediction of a bloom event during monitoring of waterbodies and to understand key factors responsible for its occurrence (Lu et al., 2020; Panksep et al., 2020; Pham et al., 2021). Furthermore, identifying toxin producers and non-toxin producing strains improves water management strategies to mitigate the impact of blooms on water quality and human health.

In contrast to the ELISA method which allows for measuring the sum of all congeners of e.g. microcystins, qPCR could estimate the amount of specific toxin variants through targeted detection of genes involved in toxin production (e.g. genes belonging to the *mcy* cluster for microcystins). Thus far, qPCR protocols have been developed mainly for the detection of microcystins and to a lesser extent for other classes of toxins (Pacheco et al., 2016). The presence of the particular targets, such as *mcyA*, *mcyB*, *mcyD*, and *mcyE* genes, in water samples indicates the genetic potential to produce microcystins. Of these, *mcyE* proved in many studies to be particularly suitable molecular marker for early detection of microcystin producers in lakes even when cyanobacterial biomass was low (Tanabe et al., 2004; Rinta-Kanto et al., 2005; Mankiewicz-Boczek et al., 2006; Rantala et al., 2006; Fortin et al., 2010; Sevilla et al., 2010; Panksep et al., 2020; Zupančič et al., 2021; Duan et al., 2022).

DNA-based qPCR quantifies the gene encoding a given toxin even in the absence of its active transcription or from dead/lysed cells, thus leading to a possible overestimation of toxinogenicity. Monitoring of active toxin producers would, therefore, be more reliable (Sipari et al., 2010). RNA-based qPCR uses mRNA as matrix facilitating the detection of toxin producing cyanobacteria that are alive and actively transcribe the toxin gene, thus circumventing the interference from cyanobacterial genotypes with inactive toxin synthesis relatively common among some strains (Tillet et al., 2001; Mikalsen et al., 2003; Kurmayer et al., 2004). However, RNA being less stable than DNA, adequate temperature conditions should be ensured during sample collection and

analysis in order to maintain predictive power (Li X et al., 2019a and 2019b; Lu et al., 2020). Also, interpretation of results should be performed with caution based on the number of copies of the selected gene within the cyanobacterial genetic code and its correlation with the overall abundance of cyanobacterial cells (Pacheco et al., 2016).

### 4.3 Metagenomic methods

Next-generation metagenomic sequencing technologies have been described as highly promising method for the development of site-specific management strategies for cyanobacterial blooms in waste water treatment plants (WWTPs) (Romanis et al., 2021) and waterbodies affected by multi-toxin blooms (Steffen et al., 2015; Casero et al., 2019) (Figure 20). Their application, combined with quantitative chemo-analytical approaches and microscopy (Teta et al., 2015; Zamyadi et al., 2019), to long-term observations, is supposed to facilitate the identification of indicator toxicogenic species and the development of site-specific HAB dynamics models of cyanobacterial blooms. Monitoring expression levels of key genes in physiological pathways of adaptation to environmental conditions, such as those associated with nitrogen fixation and phosphorus scavenging, with subsequent expression of genes for toxin production (e.g. microcystin/nodularin (*mcyE*), saxitoxin (*sxtA*), cylindrospermopsin (*cyrA*), phycocyanin (*cpcA*)), was employed in the investigation of cyanobacterial communities in lake, river and estuary waters during emergency events (Davis et al., 2015; Otten et al., 2017; Kramer et al., 2018; Lu et al., 2019). Besides identifying species-specific genes, metagenomics allows to detect shifts in populations, and to determine early and late bloom stages (Sanseverino et al., 2022); for example, *xisH* and *xisJ* genes encoding excision proteins are associated with lysogeny in cyanobacteria (Steffen et al., 2015; Meyer et al., 2017).

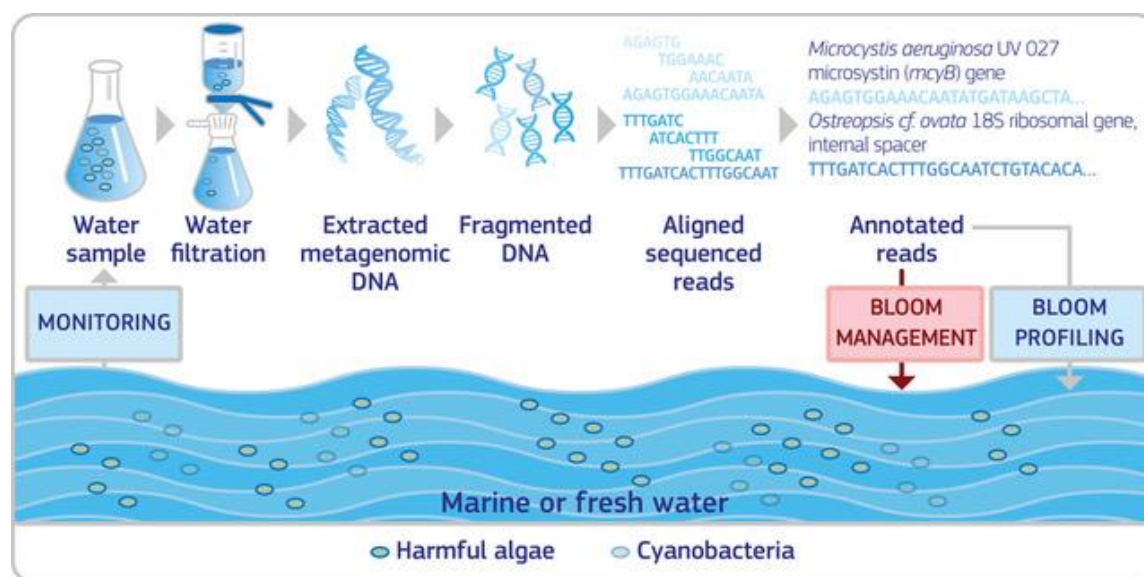


Figure 20. Metagenomic monitoring of cyanobacterial and harmful algal blooms (HABs). After collecting marine or fresh water, samples are filtered for extraction of the total DNA content. The extracted genomes are then fragmented and subjected to sequencing. The sequenced reads are aligned and annotated to identify target genes, such as those encoding toxins or allowing determination of the abundance of distinct species. Based on the results, clustering of waterbodies according to bloom profiles can be performed which facilitates decisions regarding bloom prevention and management.

### 4.4 Other methods

As adequate selection of methods is crucial for an efficient monitoring and management of HABs, the introduction of new approaches should be carefully evaluated.

Adaptation of already employed methods may increase their efficiency, as has been shown for the analysis of *Lyngbya wollei*-specific toxins (LWT1-6) with optimised sample extraction protocol and LC-MS instrument

parameters (Smith et al., 2019). Optimised extraction of cells as well as animal tissues or crop biomass may allow a simultaneous analysis of several toxins in one sample, which in combination with a sensitive technique such as isotope dilution LC-MS using zwitterionic hydrophilic interaction liquid chromatography column could be employed for a more complex, non-targeted analysis and preliminary screening (Haddad et al., 2019). Furthermore, two-site sandwich format lateral-flow immunoassay was developed as a rapid method for detection of microcystins/nodularin-R at concentrations  $\leq 4 \mu\text{g/L}$  (Akter et al., 2019).

An established method used by the Environmental Protection Agency of Hamburg, Germany, relies on spectral fluorometers. It allows to distinguish algae class by simultaneously determining cyanobacteria, green and brown algae, and cryptophytes through the measurement of chlorophyll *a* content in the range of 0-200  $\mu\text{g/L}$  and other pigments generating typical fingerprints (Harris and Graham, 2015). The measurement provides results in a few minutes and does not require concentration or extraction.

Clustering of phytoplankton could be a valid solution to determine community dynamics to control and anticipate cyanobacterial blooms. Imaging flow cytometry has been suggested as a method of choice for the classification of phytoplankton species according to taxonomic and morphologically-based functional groups, with the advantage to investigate single-cell traits and to archive microscopic images (Dunker, 2020). Fluorescence signature of organic matter derived from harmful cyanobacteria was proposed as indicator of upcoming blooms, which contribute to the overall pool of chromophoric dissolved organic matter (Hipsler et al., 2020).

In long-term perspective, methods originally developed for analysing harmful bacteria of the human gut (e.g., killer and inhibitor bacteria, phages and phagemids, bacterial transplant) have been proposed as candidate approaches for the monitoring also of HABs in lakes (Hellweger et al., 2019).

Biosensors, in particular those employing antibodies (immunosensors), combine high selectivity of specific antibodies and high sensitivity of electrochemical methods with a high limit of detection (LOD). While they can provide accurate biovolume quantification and determination of bloom-forming species upon adequate calibration, limitations of this method are discussed in Bertone et al. (Bertone et al., 2019). A surface-enhanced Raman scattering (SERS) spectroscopic immunosensor was proposed for an early detection and continuous monitoring of MC-LR levels at a LOD of 0.014  $\mu\text{g/L}$  and a linear detection up to 100  $\mu\text{g/L}$  (Li M et al., 2019), which is an improvement compared to ELISA. The expanding biosensor-based methods for detection of MC and other cyanobacterial toxins have been reviewed by Kordasht et al., 2020 and Vogiazzi et al., 2019 (Vogiazzi et al., 2019; Kordasht et al., 2020).

Molecularly imprinted polymer (MIP) transducing elements used in conjunction with sensitive analytical techniques are a useful tool for microcystin detection and quantification (Fernando et al., 2021). This approach couples the selectivity provided by MIPs to various analytical methods including those adaptable for in-field analysis.

Probability modelling and artificial intelligence methods can provide a complementary approach to analytical methods, most of which are unable to efficiently predict HAB events. Some models include variables related to weather forecast (direction and speed of the wind), cyanobacterial biomass and other parameters of the bloom dynamics which allow to predict the location and time of scum formation many days in advance (Ibelings et al., 2003; WHO, Ibelings et al., 2021a). Based on 141 phytoplankton datasets from 90 sites, it was demonstrated that monitoring chemico-physical parameters in water may provide robust information and thresholds for a rapid cyanobacterial proliferation have been proposed on the basis of large datasets: total phosphorus (TP) 0.13-0.22 mg/L, water temperature range 19.5-32.5°C, pH 7.0-9.38, ammonium nitrogen level 0.38-0.63 mg/L, chemical oxygen demand 10.5-17.5 mg/L and dissolved oxygen levels 4.97-8.28 mg/L (Zhao et al., 2019). Carvalho et al. (WHO, Ibelings et al., 2021; Carvalho et al., 2013) showed that a concentration of TP between 0.02-0.1 mg/L strongly supports an increase of phytoplankton biomass and high levels of cyanobacterial biomass usually occur at TP concentrations above 0.02-0.05  $\mu\text{g/L}$ , depending on the mixing conditions of the waterbody (WHO, Burch et al., 2021). In many freshwater environments, TP is the nutrients which most often limits the phytoplankton growth but N limitation is also common in water environments. Reynolds et al. (Reynolds et al., 2006; WHO, Ibelings et al., 2021) reported that N limitation is unlikely if concentrations of dissolved inorganic N are above 0.03-0.1 mg/L. Therefore, predictive models for phytoplankton biomass should always take into account nutrients limitations.

A bloom forecast system based on the stability of thermal stratification, validated against 191 previous bloom events and on a data-driven artificial neural network (ANN) model which includes predictions of sea surface temperature (SST), vertical temperature and salinity, has been applied to marine aquaculture without relying on chlorophyll *a* measurements (Guo et al., 2020).

## Part II

Conditions contributing to cyanobacterial and harmful algal blooms and effects on human health and aquatic ecosystems

## 1. Cyanobacterial blooms and cyanotoxins

- Which cyanotoxins are produced by cyanobacteria and what kind of effects can cyanobacterial toxins elicit in humans and animals? → See Part II, [section 1.1](#)
- What is the contribution of nutrients and environmental conditions to cyanobacterial bloom events occurring in freshwater? → See Part II, [section 1.2](#) and [section 1.3](#)

Cyanobacterial blooms are a worldwide environmental problem affecting aquatic ecosystems, including fresh water and brackish water, with increasing reports of their occurrence mainly for fresh water (Whitton and Potts, 2000). Cyanobacteria are photosynthetic bacteria, some of which may have a key role in the nitrogen cycle as they are specialised to converting inert atmospheric nitrogen into reduced forms, such as ammonia and amino acids, which are needed for plant growth. In recent years, climate change and – in many waterbodies – increasing anthropogenic pressures (O’Neil et al., 2012), have contributed to the intensification of bloom phenomena, with consequences on the quality of drinking and recreational water and, where water treatment and restrictions of recreational site use are not in place, also on human health. Also, some cyanobacterial metabolites are toxic particularly to birds and mammals, and while the impact of these metabolites on aquatic organisms is yet not very clear, the negative impacts of high amounts of cyanobacterial biomass on aquatic ecosystems are well established. Indeed, when blooms occur, this biomass can lead to water discolouration (lending a distinct blue-green or olive or – with some taxa – also reddish-brown colour to the water), some species emit unpleasant odour, and generally the often prolonged persistence of blooms as well as their decomposition can cause problems inducing hypoxic conditions in deeper water layers, resulting in the death of animals. Typically, prolonged blooms also kill submerged plants by lack of light due to the pronounced turbidity that they cause. Additionally, during blooms, some cyanobacteria contain toxins which can cause an array of human and animal illnesses (see overview in WHO, Chorus and Welker, 2021). Cyanotoxins are classified by their toxicological target in hepatotoxins, cytotoxins, neurotoxins and dermatotoxins (Sanseverino et al., 2016). However, not all cyanobacterial blooms are toxic, and toxic and non-toxic cyanobacterial species often coexist during a bloom, while environmental conditions can influence the growth and toxicity of specific strains (van der Westhuizen and Eloff, 1985; Geada et al., 2017). Within a morphologically defined species, toxic and non-toxic cyanobacterial strains are morphologically undistinguishable and toxin analyses or molecular techniques are necessary to assess their toxicity.

### 1.1 Effects of cyanobacterial toxins on humans and animals

*What are the most common cyanobacterial genera producing cyanotoxins?*

*Through which pathways does exposure to cyanotoxins occur?*

*Which organs are mainly affected by cyanotoxins?*

Humans and animals can be directly or indirectly affected by cyanotoxins by oral uptake (ingestion or aspiration of water containing cells and/or dissolved toxins) and by inhalation route (inhalation of aerosolised toxins) (Sanseverino et al., 2017; Plaas and Paerl 2022). While severe dermal lesions can be caused by lyngbyatoxins in some marine cyanobacteria that grow on submerged surfaces in tropical and subtropical waterbodies, the agents causing skin irritation which is sometimes reported from fresh waters upon contact with blooms are yet unknown and may coincidentally co-occur with cyanobacteria or be constituents of the mucilage of some cyanobacterial colonies (chapter 5 in WHO, Chorus and Welker, 2021; WHO, 2022).

Humans can be affected by harmful toxins by the intake of contaminated food (WHO, Ibelings et al., 2021b; Sanseverino et al., 2017). For example, the dietary exposure pathway to *Mycrocystis* spp. blooms evaluated through an integrated approach in threadfin shad (*Dorosoma petenense*) sampled from the affected San Francisco Estuary (USA) showed the accumulation of MC in the gut and liver tissues of the fish (Acuña et al., 2020).

Respiratory exposure to spray and aerosol containing cyanobacteria and their toxins from fresh water systems is poorly characterised as compared to spray aerosols from marine waters affected by HABs and has chiefly been studied for microcystins (Plaas and Paerl, 2021). These authors conclude that inhalation of cyanotoxins can potentially affect several pathways causing effects at different levels of biological complexity.

Symptoms people occasionally report after exposure to cyanobacteria are usually mild and self-limiting, including gastroenteritis, skin irritation, headache and fever and, as for the dermal symptoms noted above, the agents causing these are yet unclear (see Table 6). In contrast to animal deaths, human death is only known from patients undergoing haemodialysis with water containing cyanotoxins (Jochimsen et al., 1998). Clarifying the cause of human symptoms reported upon cyanobacterial exposure is typically hampered by a delay between exposure and symptom manifestation and lack of awareness of the cyanobacteria as possible cause. Moreover, a range of microorganisms in waterbodies may cause similar health conditions. In most of the studies, there is a lack of sufficient data to support cyanobacteria as the causative effect of the reported symptoms and scientifically reliable epidemiological studies on this topic are very few. Two epidemiological studies confirmed that only mild symptoms are reported in swimmers who came in contact with cyanobacteria in bathing areas (Stewart et al., 2006; Lévesque et al., 2014).

A different situation is instead reported for animals. Cases of livestock, pets and wild mammals lethally poisoned by consumption of cyanotoxins-contaminated water have been documented in a number of studies (e.g. Stewart et al., 2008; Backer et al., 2013; Trevino-Garrison et al., 2015). Animals can be severely affected by cyanotoxins as they are more likely than humans to swim in waterbodies contaminated by toxic cyanobacteria, and particularly where they lack an alternative drinking-water source, they are prone to oral uptake of far larger volumes of water than swimmers do. However, these observations clearly suggest that accidental intake of water containing cyanobacteria by humans during recreational and professional activities should be avoided. Therefore it is important to limit restriction of recreational site use to situations in which cyanotoxin concentrations are in a range in which risks need to be avoided.

For this aim, the World Health Organisation (WHO, 2020a-d) has derived guideline values (GVs) for recreational exposure to cyanotoxins (microcystins, cylindrospermopsin, anatoxin-a and saxitoxins) below which exposure through recreational activities is not likely to be hazardous (Table 6). These are calculated based on the scenario of a 15 kg toddler swallowing 250 mL of water; in contrast, a 60 kg adult would reach a dose corresponding to the GV by ingesting 1 L of water with the toxin concentration of the respective GV (WHO, 2020).

As recreational site monitoring may more readily include an estimate of cyanobacterial biomass than cyanotoxin analyses, WHO also proposes values characterising the level of cyanobacterial biomass at which the cyanotoxin GV is unlikely to be exceeded. These parameters can either be the concentration of chlorophyll *a* (provided qualitative microscopy shows chlorophyll *a* to be chiefly due to cyanobacteria), or biovolume assessed by microscopy (see Part I, [section 1.2](#) and [section 1.3.2](#)). An advantage of using such biomass parameters is that they may offer protection also against the above-mentioned self-limiting unspecific symptoms reported after bloom contact. However, with respect to the cyanotoxin hazard it is important to realise that these values for chlorophyll *a* and biovolume are based on worst-case scenarios for toxin/biomass ratios. While for, e.g., microcystins up to 3 µg per mm<sup>3</sup> biovolume and 1 µg per µg of chlorophyll *a* are occasionally observed in field samples, usually ratios are several-fold lower (WHO, Chorus and Welker, 2021, chapter 5). In consequence, to avoid undue restrictions and particularly for de-warning, the analysis of cyanotoxins may be warranted. This can be especially relevant for decisions on calling off or allowing major sports events. The WHO Alert Levels Framework designates cyanobacteria biovolume levels between 1-4 mm<sup>3</sup>/L or a chlorophyll *a* concentration up to 3-12 µg/L as “vigilance level” corresponding to a low probability that water may contain hazardous toxin concentrations.

To compare with the effects of marine toxins on humans and animals, see Part II, [section 2.1](#).

Table 6. Toxins produced by cyanobacteria: their effects and primary targets. From Sanseverino et al., 2016 and WHO, 2020a-d.

Toxin classification (Section in WHO, Chorus and Welker, 2021)	Toxins and WHO GV* for recreation (WHO, 2020a-d)	Most common cyanobacteria genera producing toxins	Main organ affected	Effects	Main targets
Hepatotoxins 2.1	Microcystins 24 µg/L	<i>Microcystis, Anabaena, Anabaenopsis, Aphanizomenon, Planktothrix, Oscillatoria, Phormidium</i>	Liver	Liver inflammation, liver hemorrhage, dermatitis diarrhea, vomiting, weakness	Serine/ threonine protein phosphatases
	Nodularin	<i>Nodularia, Nostoc</i>	Liver	Diarrhea, vomiting, weakness, liver inflammation, liver hemorrhage	Serine/ threonine protein phosphatases
Cytotoxins 2.2	Cylindrospermopsin 6 µg/L	<i>Cylindrospermopsis, Anabaena, Aphanizomenon, Raphidiopsis, Oscillatoria, Lyngbya, Umezakia</i>	Liver	Gastroenteritis, liver inflammation, liver hemorrhage, pneumonia,	Protein synthesis
Neurotoxins 2.3-2.5; 2.7	Anatoxins 60 µg/L	<i>Anabaena, Aphanizomenon, Planktothrix, Cylindrospermopsis, Oscillatoria</i>	Nervous system	Muscle twitching, burning, numbness, drowsiness, salivation, respiratory paralysis leading to death	Nicotinic receptors or acetylcholinesterase
	Saxitoxins 30 µg/L	<i>Anabaena, Aphanizomenon, Cylindrospermopsis Lyngbya, Planktothrix, Raphidiopsis</i>	Nervous system	Muscle twitching, burning, numbness, drowsiness, headache, vertigo, respiratory paralysis leading to death	Sodium channels
	BMAA* (currently under debate as possible cyanotoxin)	<i>Nostoc, Microcystis, Anabaena, Aphanizomenon, Nodularia</i>	Nervous system	No specific clinical symptoms, ALS/PDC under debate as possible outcome of long-term consistent exposure	NMDA* excitotoxicity, ROS production
Marine dermatoxins 2.6	Lyngbyatoxins	<i>Lyngbya</i>	Skin	Massive skin lesions and irritation, eye irritation, respiratory problems	Protein kinase C
	Aplysiatoxin	<i>Lyngbya, Schizothrix, Oscillatoria</i>	Skin	Massive skin lesions and irritation, asthma	Protein kinase C

(\*) GV: guideline value; BMAA: β-Methylamino-L-Alanine; NMDA: N-Methyl-D-Aspartate. Source: Sanseverino et al., 2016 and WHO, 2020a-d

## 1.2 Promotion of cyanobacterial blooms by phosphorus and nitrogen

*How phosphorus and nitrogen may contribute to cyanobacterial blooms?*

*What are additional factors enhancing the role of nutrients in bloom events?*

The basis for massive amounts of cyanobacteria is the release of plant nutrients, primarily phosphorus (P) and nitrogen (N), into the aquatic environment, causing eutrophication of waterbodies. Excessive loads of N and P reach waterbodies both via runoff from land treated with mineral fertilisers or manure (for P with the erosion of soil) and from sewage, particularly where treatment does not sufficiently reduce the concentrations of N and P. A further, usually less significant source is the combustion of fossil fuels (via atmospheric deposition). In temperate climates, cyanobacteria tend to achieve their peak biomass, forming blooms and scums, during summer and early autumn. Many species can survive over winter on the sediment surface, and where periods of ice cover are short or absent, some species may persist perennially in the plankton. Several studies using databases from many lakes have investigated concentrations of total phosphorus (TP) above which to expect the proliferation of cyanobacteria, provided concentrations of N are not limiting. While TP thresholds for blooms also depend somewhat on hydrophysical conditions ([Table 7](#)), findings agree that blooms should be expected above TP-concentrations of 20–50 µg/L (WHO, 2018 and 2020; Chorus et al., 2021). These findings demonstrate the TP concentrations to achieve or undercut to effectively control blooms, and although throughout the EU concentrations of TP have been declining as the Water Framework Directive and waste water legislation are taking effect, for many waterbodies to date sufficiently low concentrations have not yet been attained. This may require further measures. For example, despite an ongoing process of restoration, at TP concentrations still exceeding 0.2 µg/L (along with insufficient zooplankton to reduce cyanobacteria via grazing), the bloom in Swarzędzkie Lake persists (Rosińska et al., 2019).

While blooms are primarily found in eutrophic waterbodies, they can be transported downriver to sites with low levels of nutrients (Kruk et al., 2021). While cyanobacteria are occasionally found to form scums in oligotrophic waterbodies, these are typically minor, locally confined (to downwind bays) and short-lived. Indeed, cyanobacteria possess specific phosphate uptake systems favouring their persistence under nutrient-limited conditions (Aubriot and Bonilla, 2018), and they can store sufficient P for up to 4 cell divisions, thus enabling a more than 10-fold increase of their biomass even if the concentration of dissolved inorganic phosphorus (DIP) is below the limit of detection. For this reason, where monitoring detects DIP at concentrations > 3 µg/L this shows that DIP is “left over” by the phytoplankton and thus is currently not limiting uptake rates, growth or biomass. While cyanobacteria also need nitrogen to build biomass, their storage mechanisms for N are not as pronounced. As rule of thumb, nitrogen is currently not limiting if concentrations of DIN (dissolved inorganic N, i.e. nitrate and ammonia) exceed 100-130 µg/L. Some cyanobacteria can compensate N-limitation by fixation of atmospheric N<sub>2</sub> and thus sustain a bloom (Lu et al., 2019). An overview of the traits of cyanobacteria that promote their dominance over other phytoplankton is given by Ibelings et al. (WHO, Ibelings et al., 2021a).

Taken together, to take actions against cyanobacterial blooms, information on cyanobacterial traits relative to waterbody conditions can be used to understand causes for blooms, plan management interventions and optimise remediation processes. According to WHO guidance (WHO, Burch et al., 2021), once basic information on the conditions in waterbodies has been collected, including nutrient loads and hydro-physical parameters (e.g. water exchange and vertical mixing of the water), it may suffice to check key parameters only periodically, for example, once a year (WHO, 2020). A summary of TP concentrations with other conditions affecting the likelihood of cyanobacterial bloom events is provided in [Table 7](#). The contribution of nutrients to blooms in marine water can be found in Part II, [section 2.2](#).

### 1.3 Effect of temperature, water turbidity and water mixing on cyanobacterial blooms

*How do hydrophysical parameters including air and water temperature, wind, light, currents and water mixing impact cyanobacterial blooms?*

*Which are the parametric values indicating a risk of a bloom event?*

In the context of climate warming, the significance of water temperature as condition triggering cyanobacterial blooms has been subject of many investigations, showing that the mechanisms by which elevated temperatures change growth conditions for phytoplankton are complex. Indeed, in eutrophic environments with high concentrations of nutrients, high temperatures can favour cyanobacterial growth and their dominance over other forms of phytoplankton. A recent study performed at the JRC showed that air temperature can be considered predictive of surface water temperature and together with information on total phosphorus, could be used as an early warning tool to predict the occurrence of cyanobacteria blooms in Lake Varese (Italy) two weeks in advance (Chirico et al., 2020). Elevated temperatures also change patterns of thermal stratification, extending its seasonal duration and stability. While this can favour cyanobacteria, if nutrient concentrations are moderate, during the course of summer it can lead to their sufficient decline in the near-surface layer (via sedimentation of plankton) to reduce bloom intensity. Furthermore, in conjunction with water turbidity, the depths to which surface-near water layers are mixed is critically important for the amount of light to which the average plankton cell is exposed, and this also shapes the outcome of competition between cyanobacteria and other phytoplankton. Low water exchange rates also promote cyanobacterial blooms: while they are not found in rivers unless flow rates are very low, creating impoundments can lead to blooms if the water residence time in the reservoir is longer than approximately 1 month (Figure 21). For a summary of the effects of environmental factors on cyanobacterial blooms, see Figure 21; to compare with marine conditions promoting marine HABs, see and Part II, section 2.2.

While cyanobacterial blooms are a freshwater concern, they can occur in brackish waters, and they can reach beaches from heavy blooms in slow-flowing rivers. An example of a long-distance transportation of harmful algae belonging to *Microcystis aeruginosa* complex (MAC) has been reported in Río de la Plata (RdLP) basin, Uruguay (Kosten et al., 2012). Intensive precipitation enhanced the river's flow, shifting its bloom downstream to the estuary and Atlantic beach. Favourable climatic conditions such as high temperature and weak winds in the source-to-estuary direction contributed to transport of the bloom from fresh water to marine water. On the contrary, wind at a speed of 0.5 m/s was able to reduce the HAB's surface coverage by half within 1 h. Moreover, analysis of the effects of water mixing on *Microcystis aeruginosa* during subsequent calm conditions showed over 90% of the cyanobacteria to be floating within 1 h as aggregates, ribbons and patches (Qu et al., 2019).

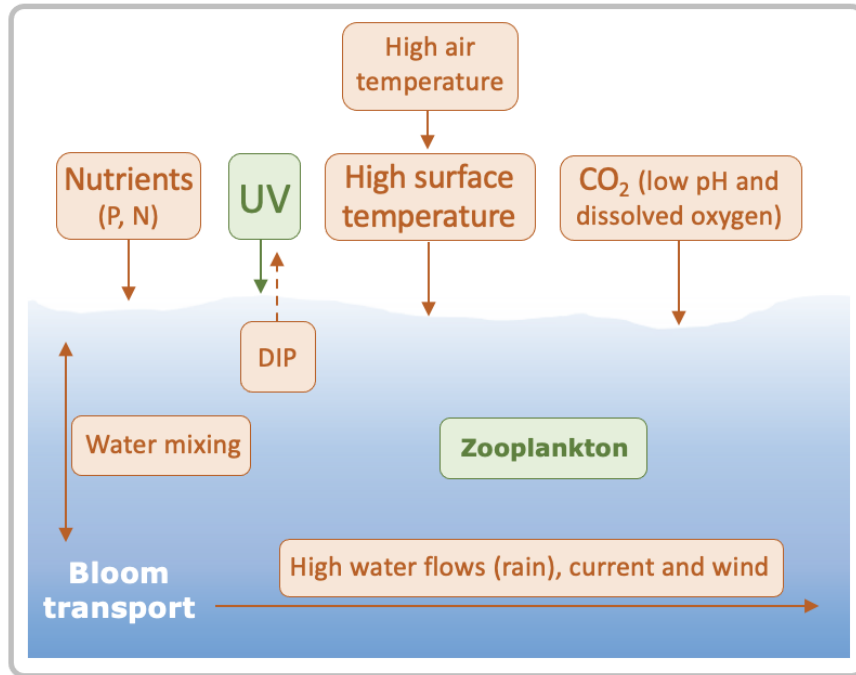


Figure 21. Environmental factors contributing to cyanobacterial bloom events. Zooplankton and UV light (green boxes) are helpful in reducing the abundance of cyanobacteria, while factors in orange boxes contribute to their increase. Interaction between those factors may further modulate the concentration of cyanobacterial cells, for example dissolved inorganic phosphorus (DIP) may contrast the detrimental effect of UV light.

Table 7 describes conditions which may indicate a high likelihood of a cyanobacterial bloom to occur, and Burch et al. (WHO, Burch et al., 2021) give a more detailed introduction to assessing this likelihood.

Table 7. Conditions affecting or indicating the likelihood of high cyanobacterial biomass. Adapted from WHO, Burch et al, 2021. Note that these conditions serve for rough first assessments and exceptions may occur.

Total P (µg/L)	Water transparency	Mixing conditions		pH
>50	Low <ul style="list-style-type: none"> <li>• Secchi depth often &lt;1 m</li> </ul>	Stagnant, depth >5–10 m, with stable thermal gradients: favours scum-forming taxa (e.g. <i>Microcystis</i> , <i>Dolichospermum</i> , <i>Aphanizomenon</i> )	Stagnant, shallow and well mixed: favours non-scum-forming taxa (e.g. <i>Planktothrix agardhii</i> ) and other fine filamentous forms (e.g. <i>Limnothrix</i> )	>7 (often >8 or possibly >9 due to high rates of photosynthesis caused by high biomass)
>20 to <50	Moderate <ul style="list-style-type: none"> <li>• Secchi depth ~1–3 m</li> </ul>	Stagnant, mixing deeper than 10 m, stratified: potential for mass development of <i>Planktothrix rubescens</i> , which accumulates at the metalimnion		≥7
>10 to <20	High <ul style="list-style-type: none"> <li>• Secchi-depth ~3–7 m</li> </ul>	Fast flowing river	Lake or reservoir with water residence time <1 month	6–7
<10	Very high – clear water <ul style="list-style-type: none"> <li>• Secchi depth often &gt;7 m</li> </ul>	Mountain stream or brook		<6
Exception: cyanobacteria attached to surfaces				

## 2. Marine algal blooms and toxins

- Which toxins are produced by harmful algae in marine water and what kind of effects can algal blooms have on human and animal health? → See Part II, [section 2.1](#).
- What is the contribution of nutrients and environmental parameters on bloom events? → See Part II, [section 2.2](#).

While cyanobacteria are principally responsible of blooms in fresh water, eukaryotic algae and in particular dinoflagellates and diatoms are the main cause of blooms in marine water (Figure 22). When a marine bloom event causing a water discolouration occurs, these phenomena are referred to as “red tides” due to the characteristic red water colour, which however could vary from red to brown, or yellow. Not all algal blooms cause water discolouration.

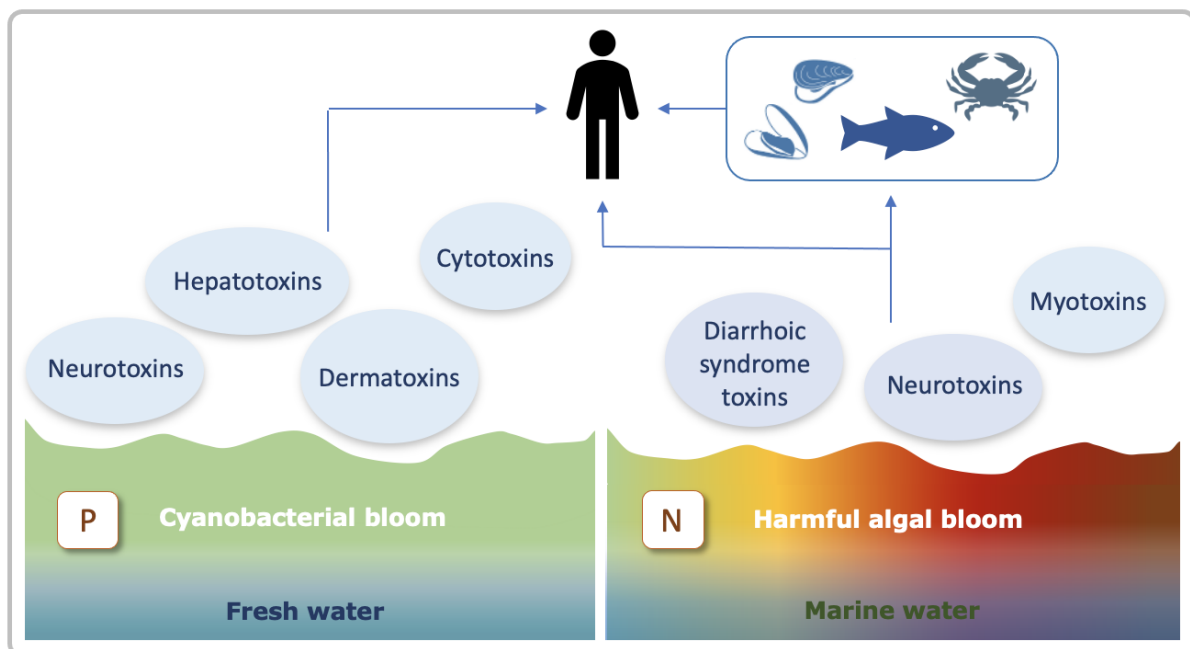


Figure 22. Main differences between cyanobacterial and harmful algal blooms (HABs). While water discolouration caused by cyanobacteria is usually blue-green, harmful algae cause a wide range of colouring, from green and yellow to red and brown. In fresh water, the contribution of phosphorus (P) seems to have major impact on bloom occurrence, whereas in marine water nitrogen (N) has been indicated as a nutrient with major contribution. Categories of toxins produced by cyanobacteria and harmful algae are shown, however not in all cases of blooms toxins are produced. Cyanobacterial toxins are more likely to directly affect human health upon exposure to contaminated water. This route of intoxication by toxins also occurs in case of HABs, although secondary poisoning due to ingestion of contaminated fish, shellfish and crabs is frequent.

## 2.1. Effects of marine toxins on humans and animals

*Which species are the most common producers of marine toxins?*

*What are the main ways of exposure to marine toxins?*

*What are the main targets of marine toxins?*

*Which organisms are the primary vectors of marine toxins?*

Like cyanobacteria, eukaryotic algae can release harmful toxins in waterbodies. Marine toxins are classified based on effects they cause to organisms in: Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Amnesic Shellfish Poisoning (ASP), Neurotoxic Shellfish Poisoning (NSP) and Azaspiracid Shellfish Poisoning (AZP) (Table 8).

Worldwide records of algal blooms are stored in the Harmful Algae Event Database (HAEDAT)<sup>6</sup> (Sanseverino et al., 2016). In the period between 1980 and 2015, more than 3000 bloom events were reported globally by single countries to the HAEDAT, most of which were associated to seafood toxins (Sanseverino et al., 2016).

Currently, the Bathing Water Directive (BWD) has not provided any specific alert level approach or guidance levels for controlling and managing marine bloom events. However, as outlined in the BWD, in case a potential for proliferation of HAB organisms is detected, adequate management actions, including information to the public, are required from Member States when health risks are identified (WHO, 2018).

No threshold values concerning marine blooms are present in the World Health Organisation (WHO) guidelines for recreational water quality (WHO, 2020), but management actions together with parameters and methods for monitoring are indicated. An example of a plan to manage *Moorea* blooms in Australia is also described.

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<sup>6</sup> <http://haedat.iode.org>

Table 8. Toxins produced by harmful algae: their effects and primary targets.

Poisoning	Main toxins	Most common organisms producing toxin	Effects	Main targets	Primary vector
PSP*	Saxitoxins	<i>Alexandrium</i> spp., <i>Gymnodinium</i> spp., <i>Pyrodinium</i> spp.	Muscle twitching, burning, numbness, drowsiness, headache, vertigo, respiratory paralysis leading to death	Sodium channels	Shellfish
NSP*	Brevetoxins	<i>Kerenia brevis</i> , <i>Chattonella marina</i> , <i>C. antiqua</i> , <i>Fibrocapsa japonica</i> , <i>Heterosigma akashiwo</i>	Tingling, numbness, nausea, muscular pain, neurologic symptoms	Sodium channels	Shellfish
CFP*	Ciguatoxins	<i>Gambierdiscus toxicus</i>	Tingling, itching, hypotension, bradycardia, vomiting, diarrhoea, nausea	Sodium channels	Coral reef fish
AZP*	Azaspiracids	<i>Protoperidinium crassipes</i> , <i>Azadinium spinosum</i>	Diarrhoea, nausea, vomiting, stomach cramps	Calcium channel	Shellfish
DSP*	Okadaic acid, Dinophysins toxins	<i>Dinophysis</i> spp., <i>Prorocentrum</i> spp.	Diarrhoea, nausea, vomiting, abdominal cramps	Serine/threonine protein phosphatases	Shellfish
Palytoxin poisoning	Palytoxins	<i>Ostreopsis siamensis</i>	Weakness, nausea, vomiting, myalgia, fever	Na <sup>+</sup> -K <sup>+</sup> pumps	Shellfish
Yessotoxin poisonings	Yessotoxins	<i>Protoceratium reticulatum</i> , <i>Lingulodinium polyedrum</i> , <i>Gonyaulax spinifera</i>	Restlessness, dyspnea, shivering, jumping, cramps	Calcium/sodium channel?	Shellfish
Pectenotoxin Poisoning	Pectenotoxins	<i>Patinopecten yessoensis</i>	Hepatotoxic effects	Na <sup>+</sup> -K <sup>+</sup> ATPase	Shellfish
ASP*	Domoic acid	<i>Pseudo-nitzschia</i>	Amnesia, hallucinations, confusion, vomiting, cramping	Glutamate receptor	Shellfish, anchovies, crabs

(\*) PSP: Paralytic Shellfish Poisoning; NSP: Neurotoxic Shellfish Poisoning; CFP: Ciguatera Fish Poisoning; AZP: Azaspiracid Shellfish Poisoning; DSP: Diarrhetic Shellfish Poisoning; ASP: Amnesic Shellfish Poisoning. *Source*: Sanseverino et al., 2016.

As reported in the Harmful Algae Event Database (HAEDAT), most of the marine bloom events reported by countries worldwide are associated to foodborne poisonings, and in particular to the syndromes diarrhetic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP), which are caused by harmful toxins released by algae during blooms (see [Table 8](#)) (Sanseverino et al., 2016). Humans can be exposed to marine toxins via inhalation, when these molecules are incorporated into aerosols, skin contact or consumption of toxin-contaminated seafood ([Figure 23](#)). Due to their temperature stability, marine toxins are not destroyed by cooking or freezing. In addition, they do not modify the taste of food. The variety of illnesses caused by marine toxins are reported in [Table 8](#) and in general, they can cause intoxication, gastrointestinal problems and even death in humans and animals. See Part II, [section 1.1](#) to compare with effects of toxins on human health in fresh waters.

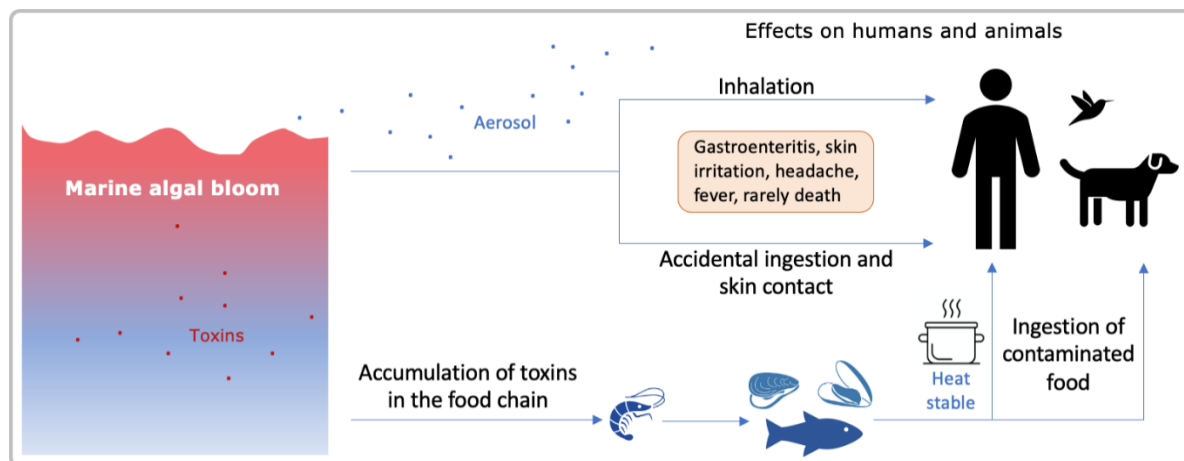


Figure 23. Possible effects of algal toxins and ways of exposure.

In the Mediterranean Sea, species producing PSP toxins such as *Gymnodinium catenatum* have been identified. No deaths have been associated to PSP cases in Europe, but severe outbreaks and human fatalities have been reported in Philippines from 1983 to 2002 (Ching et al., 2015), Nicaragua, Southeast Asia and Latin America (Visciano et al., 2016). Domoic acid (DA), the marine toxin which causes the amnesic shellfish poisoning (ASP), has been described to cause human intoxication and severe prognosis in North America, but no cases have been registered in Europe. Sea lions, marine birds and mammals are particularly sensitive to the toxic effects of DA (Ramsdell and Zabka, 2008; Visciano et al., 2016). In particular, documented cases of neurologic diseases and reproductive failure associated with exposure of California sea lions to DA occurred for the first time in 1998 and have followed since 2006. Outbreaks due to DSP and ciguatera fish poisoning (CFP) toxins have been observed in Europe and affected people after consumption of okadaic acid (OA) and azaspiracid (AZA)-contaminated mussels (Visciano et al., 2016), while cases in Northern Germany were attributed to ciguatoxins (CTX) (Mattei et al., 2014). In addition, episodes of palytoxin poisoning have been registered in France and Italy after exposure of people to aerosolised palytoxins (PITX) (Biré et al., 2013). Neurotoxin shellfish poisoning episodes have not been registered in Europe, however species belonging to the genus *Karenia* have been detected in Ireland (Vilariño et al., 2018). Differently from other marine toxins (e.g. OA, AZA, DA and saxitoxins - SX) for which legal limits in seafood have been set for ensuring the safety of consumers, brevetoxins (BTX), CTX and PITX are still not regulated in the European Union legislation (EC No. 853/2004; 854/2004; 15/2011, 786/2013).

Recently, toxins like PITX and CTX have been reported in new geographical areas where they had not been previously described. *Ostreopsis* spp. associated with the production of PITX and mainly reported in Japan and Hawaii, have been recently observed in Europe (Estevez et al., 2019). In addition, species belonging to the genus *Gambierdiscus* and *Fukuyoa* and producing CTX, have been detected in Spain (Canary Islands) and in the Mediterranean Sea, despite their presence was usually associated to tropical regions (Estevez et al., 2019). The increasing incidence of these bloom events in Europe represents a reason of concern since PITX and CTX can contaminate seafood and therefore expose humans and animals, which feed on them, to their toxic effects. Further research at European level is therefore necessary in order to evaluate the risk linked to the presence of these marine toxins in seafood and set regulatory limits to protect consumers.

## 2.2. Effect of nutrients and environmental parameters on marine blooms

How parameters such as air and water temperature, salinity, nutrients, wind, currents and water mixing impact marine blooms?

Which parameters contribute to the transportation of marine blooms over large areas and across water ecosystems?

It is envisaged that harmful algae in marine environments form blooms only under certain stable conditions combining temperature, salinity and dynamics in the water column, and with sufficient supply of nutrients and light (Figure 24). Those conditions underlie the expansion of harmful algal blooms (HABs) from fresh water to marine interconnected environments and determine whether motile species aggregate to prevail over non-motile species, particularly utilising bottom nutrients in calm waters, or favour rapid growth of non-motile species (Hu et al., 2016; Paerl et al., 2018). To compare effects of environmental parameters on fresh water blooms, see Part II, [section 1.3](#).

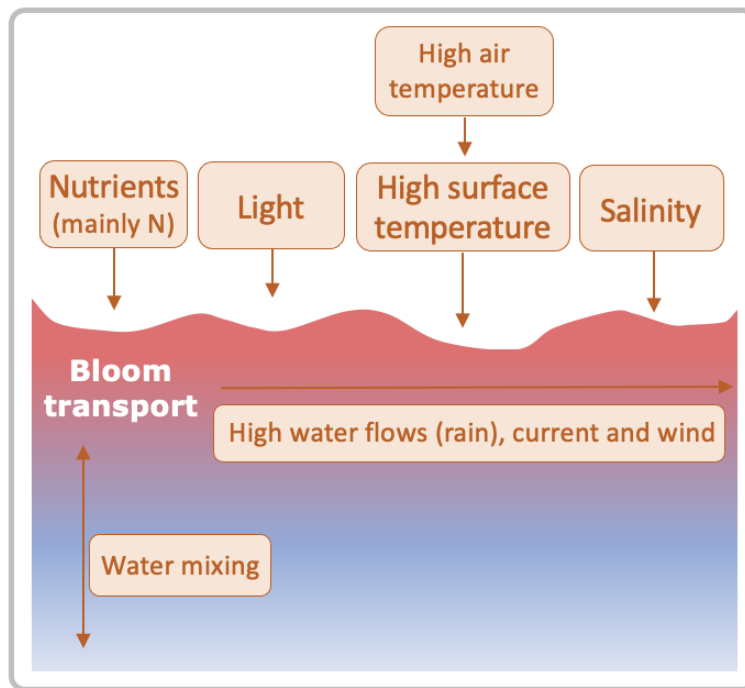


Figure 24. Environmental factors contributing to harmful algal bloom events in marine waters. Most factors are common to those determining cyanobacterial blooms in fresh waters, except salinity which is unique to marine environments. Among nutrients, nitrogen (N) seems to be determinant for algal growth, while analogous role in fresh waters is attributed to phosphorus.

Numerous studies reported the association of increasing sea surface temperatures with higher growth rates of harmful algae, competitive advantage of some toxinogenic species and extended bloom season including areas where HABs have become newly established (Brandenburg et al., 2017 and 2019; Ekstrom et al., 2020; Gobler et al., 2017; Griffith et al., 2019; Huang et al., 2018; Lee et al., 2019; Lotliker et al., 2018; Zhu et al., 2017). For example, it has been experimentally assessed that the optimal growth of *Dinophysis* spp. associated with red tides takes place at 18-24°C and salinity 22, at 25°C and salinity 20-40 for *Paragymnodinium shiwhaense*, while *Mesodinium rubrum* and *Teleaulax amphioxeia* grow optimally at 24°C and salinity 30-34 (Fiorendino et al., 2020; Jeong et al., 2018). Contrarily for other species (*Alexandrium catenella*), anomalously low temperatures in the range 10-15°C combined with light wind, high rainfall and land run-off promoted temperature and salinity stratification in coastal waters favouring in turn the formation

of blooms (Condie et al., 2019). Temperature and salinity were also associated with toxin content. During heat waves, peaks in toxicity of benthic dinoflagellate *Coolia malayensis* appeared at 26°C even though the maximum growth was registered at 28°C (Li X et al., 2020). Positive correlation of these environmental conditions has been found with yessotoxin (YTX) production by *Lingulodinium polyedra*, however it did not affect the overall toxin profile produced by the strains present in the bloom (Peter et al., 2018).

Currents are classified as driving factors which determine transportation of harmful algae even under unfavourable wind conditions, red tide assembly and dispersal. In turn, red tides govern the redistribution of nutrients and regulate dominant species in the phytoplankton community (Zhang W et al., 2020; Zhang Y et al., 2020). Floating algal ecotypes associated with green tide may be transported over long distance along the coast during blooming time, often becoming exotic species in invaded areas (Zhao J et al., 2018).

While in fresh waters the concentrations of phosphorus are considered the main nutrient determining HABs (see Part II, [section 1.2](#)), in marine waters this role is assigned to nitrogen (Paerl et al., 2018). In areas with high anthropogenic pressure where HABs are likely events, the input of nutrients from land may account for over 70% of overall contribution to blooms (Lin et al., 2020a and 2020b). Transportation of nutrients in aerosol with subsequent deposition during favourable weather conditions was also linked to HABs (Tian et al., 2018). Fluctuations in nutrient availability, especially phosphorus and silicate, and ability of their assimilation is at the basis of shifts in diatom-to-dinoflagellate phytoplankton community composition (Zhang Y et al., 2019). Similarly, the affinity of *Ostreopsis cf. ovata* to ammonium (NH<sub>4</sub><sup>+</sup>) was attributed to the global success of this species in bloom formation under low nitrogen and phosphorus supply (Jauzein et al., 2017; Pezolesi et al., 2016). Conditions enriched in nutrients were positively associated with high temperatures and bloom occurrence indicating the ratio between initial nitrate and chlorophyll *a* concentration as a critical factor (Lee et al., 2019). Along with increased nutrient content, pH variations and elevated concentrations of carbon dioxide (CO<sub>2</sub>) dissolved in water contribute to the HAB formation and this phenomenon is intensified due to climate change (Raven et al., 2020). As gas exchange occurs between atmosphere and sea surface water, higher availability of CO<sub>2</sub> promotes its assimilation by several HAB-related species accelerating their growth rate and toxicity (Brandenburg et al., 2019; Tatters et al., 2012). Combined effects of co-stressors related to climate change such as thermal extremes, low pH and low dissolved oxygen, yield different outcomes on aquatic organisms than the same stressors assessed individually (Griffith and Gobler, 2020).

### 3. Cyanobacterial monitoring in bathing waters

- *Which parameters for monitoring and detecting cyanobacterial blooms have been implemented so far in the European Member States? → See Part II, from [section 3.1](#) to [section 3.15](#).*
- *Which parametric thresholds have been developed by some countries to determine alert levels and relative actions during bloom events? → See Part II, from [section 3.1](#) to [section 3.15](#).*

In 2019, the JRC circulated a survey to all Member States (MS) to collect information on methods used at national level for monitoring cyanobacterial/algal blooms and on parameters to confirm the occurrence of such events in bathing waters in the context of recreational exposure. Additionally, the survey included a question on measures taken to inform people, anticipate and reduce risks of bloom events. The MS were also asked to provide information on threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal blooms. Finally, the survey addressed the question of de-warning systems implemented in national management programs.

Results of the survey have been received from fourteen MS (AT, BE, CY, DE, ES, FI, FR, HU, LT, NL, PL, PT, RO, SK, UK<sup>7</sup>) and are reported in the following sections and in [Table 11](#).

#### 3.1. Austria

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

As communicated by the Federal Ministry of Labour, Social Affairs, Health and Consumer Protection, methods used at national level for monitoring cyanobacterial/algal bloom events may differ between the federal provinces of Austria and include:

- regular site inspection of the bathing area;
- measurement of water transparency;
- microscopic identification (= qualitative analysis);
- cell counts of cyanobacteria and assessment of biovolume (= quantitative microscopic analysis);
- assessment of chlorophyll *a*;
- assessment of the total phosphorus;
- use of *in situ* monitoring fluorometers for chlorophyll *a* determination;
- toxin analysis: ELISA and/or HPLC-MS.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

The parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events include microscopic analysis and/or HPLC-MS analysis of toxins.

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<sup>7</sup> At the time of the JRC survey, the United Kingdom was a Member State of the EU

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

There exists no uniform warning system for cyanobacteria. Different alert and threshold values are in use across the federal provinces of Austria:

#### *Carinthia:*

Above an alert value of a total biovolume of cyanobacteria equal to 1 mm<sup>3</sup>/L (recommended by the German Environmental Agency)<sup>8</sup> and a water transparency until 2 m of distance there is an increased attention on a possible bloom event. In the next step, a cyanotoxin measurement via HPLC-MS is carried out and, in the absence of a binding limit value, bathing prohibition (including information to the public) is issued when microcystin concentrations exceed 100 µg/L, according to the recommendations of the Austrian Agency for Health and Food Safety (AGES)<sup>9</sup>.

#### *Vorarlberg:*

When the routine site inspection of a bathing area indicates a reasonable suspect of cyanobacterial proliferation, an indicative cyanotoxin measurement via ELISA is performed. Below microcystin concentration of 10 µg/L (alert value according to the recommendations of DWA-M 624<sup>10</sup>), the situation is classified as non-hazardous to health. If the measured cyanotoxin concentration is >100 µg/L, bathing is prohibited.

#### *Vienna:*

If the water transparency does not exceed 1 m and cyanobacteria are the dominant component of the plankton community, the cyanotoxin content is measured via HPLC-MS. The German Environmental Agency<sup>11</sup> recommends a limit value of 30 µg/L for microcystin (including toxicological findings and taking into account the lower body weight of most children at risk), which is applied for a definite bathing prohibition.

#### *Rest of Austria:*

In case the measured concentrations of cyanotoxins exceed 100 µg/L, bathing is prohibited.

There is an urgent need to establish a threshold value for cyanotoxin concentrations as a standardised criterion for the introduction of bathing ban. Furthermore, a warning system including surveillance/alert/action values should be established.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

Austria has no uniform de-warning system to manage cyanobacterial bloom events. Nevertheless, the following conditions should be fulfilled before a bathing ban can be lifted:

- water transparency >1 m;
- no scums or cloudiness visible during the site inspection;
- cyanotoxin concentration below a defined threshold value.

Information for the public is provided via information boards at the bathing site and via the internet<sup>12</sup>.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

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<sup>8</sup> Empfehlung zum Schutz von Badenden vor Cyanobakterien-Toxinen. Bundesgesundheitsblatt – Gesundheitsforschung 58:908-920. Deutsches Umweltbundesamt (2003).

<sup>9</sup> Cyanobakterien in Schwimmteichen und Badeseen – AGES Info-Folder (2017).

<sup>10</sup> Merkblatt DWA-M 624: Risiken an Badestellen und Freizeitgewässern aus gewässerhygienischer Sicht (2016).

<sup>11</sup> Empfehlung zum Schutz von Badenden vor Cyanobakterien-Toxinen. Bundesgesundheitsblatt – Gesundheitsforschung 58:908-920. Deutsches Umweltbundesamt (2015).

<sup>12</sup> <https://www.ages.at/en/environment/water/bathing-water-monitoring>

Targeted measures to reduce the risk of cyanobacterial/algal blooms are based on reduction of the nutrient input into bathing waters. These are two exemplary projects with a focus on improving the water quality:

- Austrian Agri-Environmental Program ÖPUL (2015-2020): Reduction of diffuse pollution sources<sup>13</sup>;
- LIFE Old Danube Project<sup>14</sup>.

Is there in place any model-based system to inform and anticipate the risk?

There is no general model-based system in use to predict the risk of cyanobacterial blooms in Austrian bathing waters, but in 2013 a model was created to estimate/forecast the cyanobacterial abundance in Austrian lakes depending on climate effects<sup>15</sup>. This model could possibly be adjusted to the current requirements to help in the risk assessment of cyanobacterial proliferation.

### 3.2. Belgium

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

The monitoring pathway provided by the Flanders Environment Agency (VMM) combines visual assessment with analytical determination of chlorophyll *a* and microcystin (Figure 25). As communicated by the Institute of Social, Economic and Political Sciences, the framework developed in Wallonia is based on monitoring chlorophyll *a* concentrations along with the identification of harmful species using optical microscopy when bloom events have not occurred.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

Measurement of chlorophyll *a* and microcystin are the parameters of choice.

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

Although the main parameters are common to different regions in Belgium, the safety threshold levels vary. In Flanders, the thresholds of concentration have been established at 75 µg/L for chlorophyll *a* and at 20 µg/L for microcystin. In Wallonia, the levels of cyanotoxins are monitored when chlorophyll *a* exceeds 50 µg/L or when cyanobacterial bloom is already present. Figure 26 shows the monitoring workflow and warning values adopted in Wallonia.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events? Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

In bathing and recreational waters, the presence of cyanobacteria determined visually results in a ban of swimming and recreation, otherwise decision is taken based on detected chlorophyll *a* and microcystin levels.

Is there in place any model-based system to inform and anticipate the risk?

No information has been provided on any model-based system used in Belgium to inform and anticipate the risk of bloom events.

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<sup>13</sup> <https://www.bmlrt.gv.at/english/agriculture/rural-development/oepul2015until2020.html>

<sup>14</sup> <https://www.wien.gv.at/english/environment/waterbodies/old-danube/life-project/project-description/index.html>

<sup>15</sup> RADICAL - Risk analysis of direct and indirect climate effects on deep Austrian lake ecosystems. Wanzenböck, J et al. (2013). <https://www.uibk.ac.at/limno/research/projects/radical/index.html>

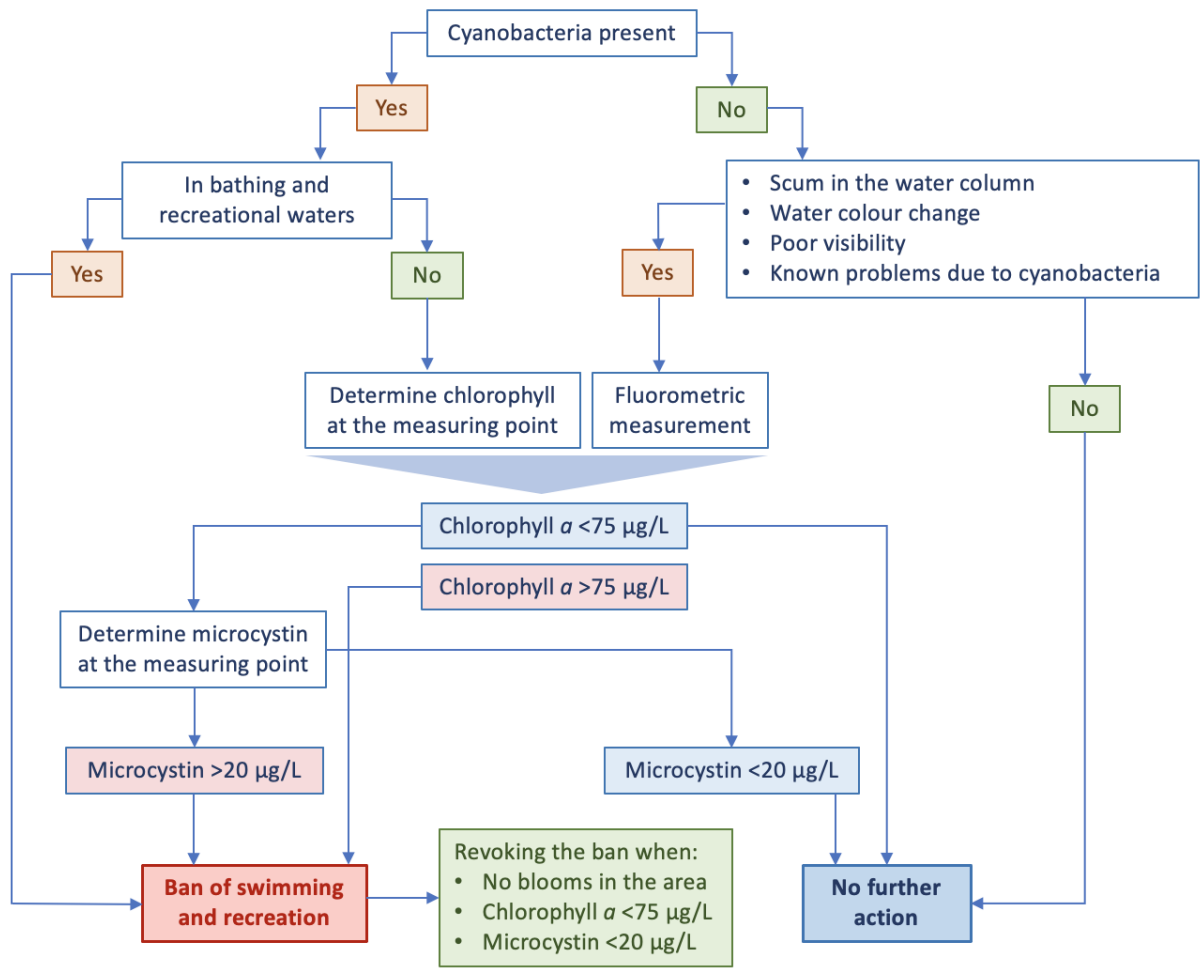


Figure 25. Pathway for monitoring blooms of cyanobacteria and measures implemented in Flanders, Belgium.

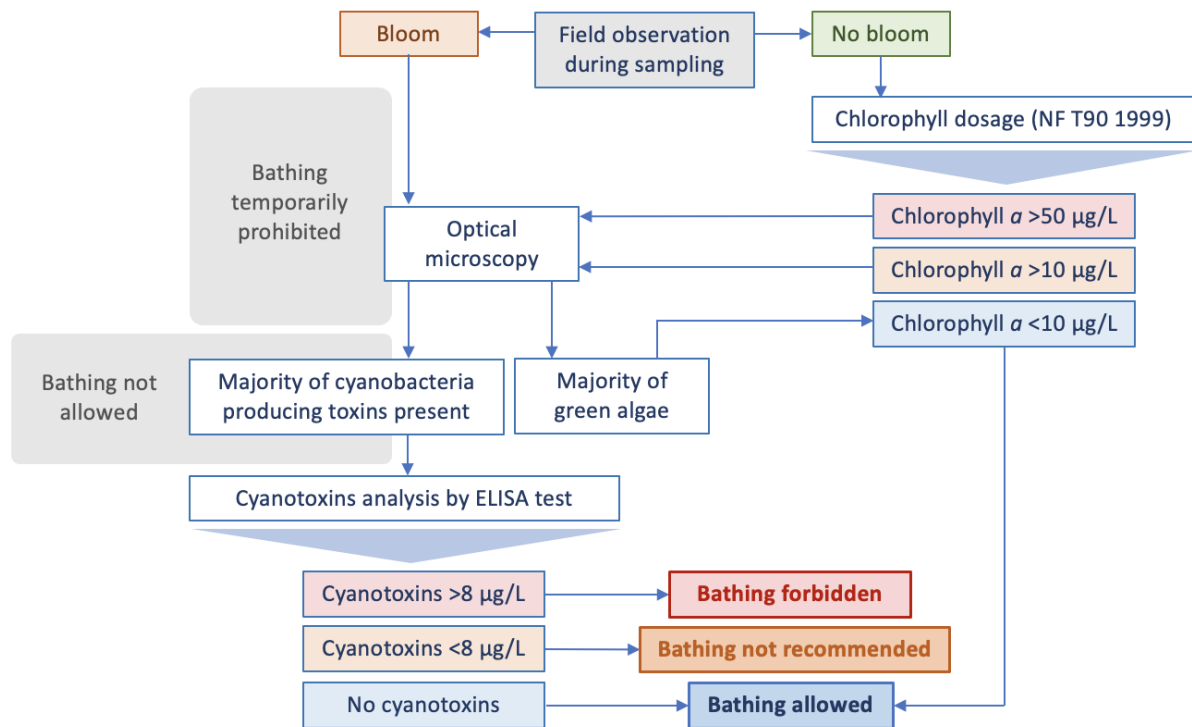


Figure 26. Monitoring framework of waterbodies in Wallonia, Belgium, for the presence of cyanobacterial blooms.

### 3.3. Cyprus

The Ministry of Agriculture, Rural Development and Environment of Cyprus referred that assessment of cyanobacterial proliferation in the bathing waters of Cyprus indicates almost no risk of having systematically persistent blooms in the bathing waters. This is due to the island's climatic conditions and the increase salinity of the sea in the area. Overall, the bathing waters of Cyprus are oligotrophic and chlorophyll *a* levels are usually low which presents an unfavourable environment for these microorganisms.

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events? Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

At present, there are no measures or parameters used at national level for monitoring and confirming the occurrence of cyanobacterial/algal bloom events, therefore related systems have not been put in place.

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events? Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

According to the risk assessment, there are chances of having blooms forming only at specific locations where nutrient concentrations are above average (i.e. harbours, where ships are present and especially when poor management practices of ballast water are applied), and in stagnant waters with increased nutrients' concentrations where cyanobacterial species can make it into the marine environment. These areas are not bathing areas.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

Thus far, contamination of bathing waters from inland waterbodies has been detected in only one waterbody in Cyprus, that is Polemidia Dam, which has no effluents to the sea. In case of cyanobacterial proliferation, the Department of Fisheries and Marine Research will perform investigation and propose action measures to involve parties. The risk of cyanobacteria appearance in Cypriot waters is included in the revised bathing water profiles.

Furthermore, the Department of Environment will prepare an action plan to take measures in case of detection of cyanobacteria. The measures to combat cyanobacterial blooms will be included.

Is there in place any model-based system to inform and anticipate the risk?

No model-based systems to inform and anticipate the risk are in place.

### 3.4. Finland

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

In Finland, according to the Ministry of Social Affairs and Health and the National Institute for Health and Welfare, monitoring of cyanobacteria is based on visual inspection of cyanobacterial cells in water which is an easy, rapid and reliable method for detecting the proliferation of cyanobacteria in bathing waters. The monitoring of harmful cyanobacterial blooms is a challenge due to the rapid occurrence and disappearance of blooms (calm weather or suitable wind direction towards or off the beach). Visual inspection allows detection of cyanobacterial cells even if the wind had mixed the cells into the water column.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

For a rapid warning to the public of a possible health risk, the basic monitoring of blooms is based on the visual inspection of bathing water (Figure 27). If blooms or dense algae occurrence are detected, it is advised that water samples are taken for microscopic analyses to confirm the presence of cyanobacteria. Microscopic methods and toxin analyses are not, however, used in routine monitoring of cyanobacteria in bathing waters due to the relatively long-time delay that the analyses take. The toxin concentrations in bathing water may vary and a toxin result may already be outdated when received from the laboratory. In addition, it is not possible to analyse routinely all known toxins produced by cyanobacteria (e.g. different microcystin variants, anatoxin-a, anatoxin-a(S), different saxitoxin variants and cylindrospermopsin). Studies conducted in Finland have shown that all these toxins may be present in cyanobacterial blooms. In addition, adverse health effects may originate from toxic or non-toxic blooms and due to different exposure routes. Other bacteria present in blooms may also cause or increase symptoms (LPS-toxins).

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

According to the bathing water legislation in Finland, municipal health protection authority has to take management actions to protect bathers' health. In practice, it has been assessed that the occurrence of cyanobacteria in bathing water may pose threat for bathers' health and additional public information is needed. The visual inspection of cyanobacterial cell aggregates in bathing water is adequate for management actions. Further analyses are not required. Information on cyanobacteria including advice against or ban of using the bathing site, if necessary, has to be available at bathing sites (the use of symbols is recommended). General information on cyanobacteria is also included in the bathing water profile if cyanobacteria have been detected in bathing water during the previous bathing seasons.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

According to the information on cyanobacteria in Finnish bathing waters, it is not possible to predict and manage the cyanobacterial blooms in short term. Management requires reduction of nutrient loads to the waterbody, which is taken into consideration in the River Basin Management Plans (RBMP) compiled in accordance to Water Framework Directive (WFD) (EC, 2000).

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

To reduce the risk of cyanobacterial/algal bloom, rapid and targeted on-site information at the bathing site is needed. Health problems could be eliminated and/or reduced by guidance and public education. In Finland, a webpage system where public can report visual inspections on cyanobacteria in the inland waters (not only bathing waters), has been developed and is available along with a mobile application. There are also pre-determined monitoring sites throughout the country, most of them at bathing water sites, where the occurrence of cyanobacteria is monitored weekly during the summer time. The information on cyanobacteria is updated weekly, and can be found on a dedicated webpage<sup>16</sup>.

Is there in place any model-based system to inform and anticipate the risk?

Currently, there is no any model-based system to inform and anticipate the risk. Due to the reasons mentioned above, the occurrence of cyanobacteria and their toxins in bathing water may vary largely and is therefore difficult to predict.

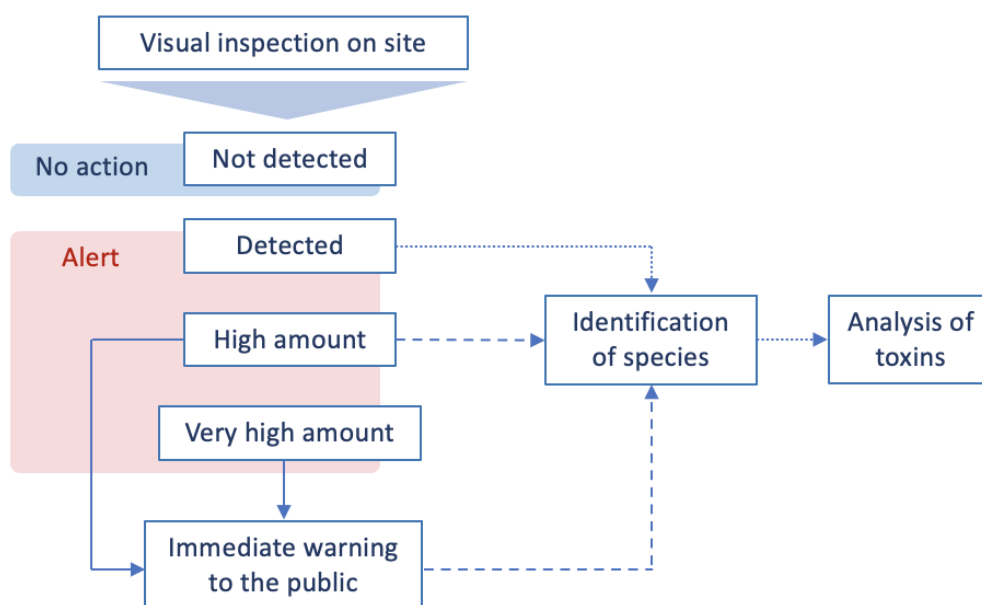


Figure 27. Decision chart for detection and actions for cyanobacteria in the bathing water sites in Finland. Continuous line: compulsory actions. Dashed line: preferred actions. Dotted line: actions in special situations, e.g. persistent bloom, popular beach, adverse health effects or animal poisoning reported.

### 3.5. France

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

As reported by the Ministry of Health, the monitoring of cyanobacterial/algal bloom events in France is established based on the enumeration of cyanobacteria with identification of gender, including toxinogenic

<sup>16</sup> [http://www.jarviwiki.fi/wiki/Algal\\_situation](http://www.jarviwiki.fi/wiki/Algal_situation)

species, according to standard NF EN 15204. The search for microcystin must be done according to ISO 20179, which determines the intracellular (solid fraction) and extracellular (liquid fraction) toxins.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

To confirm the occurrence of cyanobacterial/algal bloom events at national level, the bathing manager must set up a regular monitoring of the bathing water to detect the changes of characteristics of the medium corresponding to early signs of a possible phenomenon of proliferation of cyanobacteria. Monitoring is based on enumeration of cyanobacteria with identification of gender, including toxigenic species.

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

For the assessment of warning systems and corresponding level of risk associated to cyanobacterial/algal blooms, graduated management measures are implemented within the monitoring system for water quality which foresees sampling at least once a month for determination of cell and toxin levels, with the following threshold values:

- presence of cyanobacteria ( $1 \times 10^5$  cells/mL);
- microcystin  $\geq 13$   $\mu\text{g/L}$  (microcystin-LR equivalent);
- toxoid A at the concentration equal or higher than 40  $\mu\text{g/L}$ .

This risk management must then be established on the basis of the knowledge available on the configuration of the site, its vulnerability to algal contamination, the periodicity of the pollution, the nature of leisure activities and attendance.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

De-warning system and measures to inform and anticipate the risk have not been put in place.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

The long-term prevention of cyanobacterial blooms, and thus the risk of production of cyanotoxins, is based on the reduction of nutrients, and in particular phosphorus resources. Some regional policies take this issue into account in their programs.

Is there in place any model-based system to inform and anticipate the risk?

There are no model-based systems to inform and anticipate the risk in place.

### 3.6. Germany

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

The German Environment Agency (UBA) first issued recommendations on the monitoring of cyanobacteria and on the interpretation of the results in 1997 (updated in 2003 and 2015). It consists of a three-step system: increased monitoring/warning/temporary bathing ban ([Figure 28](#)). The regional authorities use these recommendations as basis for their management systems.

Additional monitoring may be necessary where toxic benthic cyanobacteria occur. Intoxication of dogs with these cyanobacteria (e.g. *Microcoleus*, *Tychonema*) have appeared in some waterbodies (lakes, rivers) in

Germany with low nutrient content and resulting high transparency. In this case, additional monitoring of anatoxin-a is performed.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algae bloom events?

The parameters for visual analysis comprise water turbidity/visibility and the presence of scums or visible layers of cyanobacteria, as well as the levels of chlorophyll *a* and/or – via microscopy – cyanobacterial biovolume as accessory or alternative parameter reflecting biomass and thus indicating maximum likely toxin concentrations (Figure 8).

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algae bloom events?

The warning system in place includes three levels of risk as shown in [Figure 28](#). The threshold calling for increased attention is established at 1 mm<sup>3</sup>/L for cyanobacterial biovolume and at 5 µg/L for chlorophyll *a*. The alert level which may result in a temporary bathing ban is recommended at respective values of 15 mm<sup>3</sup>/L and 75 µg/L.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algae bloom events?

If the situation concerning cyanobacteria is improving, the three-step system is used bottom-up. For example, the bathing ban is released as soon as the results of the measurements or the on-site observation indicate that this hazard level is no longer valid. Usually, this results in a replacing the ban with warning and information. It may also result in a complete release of the restrictions if the improvement leads to sufficiently reduced turbidity and occurrence of cyanobacteria to levels not requiring elevated attention.

Are there any targeted measures to reduce the risk of cyanobacterial/algae bloom?

To reduce the risk of cyanobacterial/algae bloom events, most of the measures undertaken by the regional authorities target reducing nutrient inputs (especially those of phosphorus) of both urban and rural origin, aiming at levels sufficiently low to prevent massive cyanobacterial blooming or at rendering other conditions less favourable for cyanobacteria. These include:

- improvement of waste water treatment;
- reduction of waste water discharge;
- reduction of storm water overflow events;
- phosphate precipitation in the waterbody;
- aeration or artificial mixing;
- sediment removal;
- measures against water fowl and fish;
- extended riparian buffer strips;
- timing of fertilisation and fine-tuning of amounts according to crop demand.

Is there in place any model-based system to inform and anticipate the risk?

None of the regions in Germany is using modelling to predict cyanobacterial blooms in order to warn bathers.

Step	Assessment by visual examination (on-site and microscopy)	Action	Alternative and/or additional assessment through analysis durch Analysen
<b>1. Elevated attention</b>	Turbidity with visibility <2 m and occurrence of cyanobacteria	Where appropriate, increase monitoring frequency during the bathing season; general education and information about this bathing water	>1 mm <sup>3</sup> /L cyanobacteria BV or >5 µg/L cyanobacterial chlorophyll <i>a</i> *
<b>2. Warning level</b>	Streaks from cyanobacteria, but not a closed layer of cyanobacteria or no streaks, but greenish turbidity due to cyanobacteria with a depth of visibility <1 m	Publish warning notices (on effects of cyanobacteria) with behavioural advice for bathers (especially small children) and those engaging in water sports	>3 mm <sup>3</sup> /L cyanobacteria BV or >15 µg/L cyanobacterial chlorophyll <i>a</i> *
<b>3. Alert level</b>	Large areas completely covered by scum or pronounced greenish turbidity caused by cyanobacteria with visibility <0.5 m	Publish warning notices (on effects of cyanobacteria) with behavioural advice for bathers (especially small children) and those engaging in water sports; if necessary, temporary bathing ban or site closure**	>15 mm <sup>3</sup> /L cyanobacteria BV or >75 µg/L cyanobacterial chlorophyll <i>a</i> *

Figure 28. Monitoring scheme for bathing and recreational waters with increased potential for development of cyanobacterial mass. BV: biovolume.<sup>17</sup>

Note: Supplementing visual inspection with analyses increases the reliability of the assessment, however decisions are already possible on the basis of the visual inspection. \*This value applies to cyanobacterial chlorophyll *a* level determined directly on site (using *in situ* fluorometry) as well as to wet-chemical analyses in the case of pronounced cyanobacterial dominance (which is determined by qualitative microscopic examination). \*\*Exception: If microcystin or nodularin <30 µg/L, the measures under the warning level are applied; the value of 30 µg/L can also be used for the other cyanobacterial toxins.

### 3.7. Hungary

The Hungarian Government Decree 78/2008<sup>18</sup> transposes provisions of the Directive 2006/7/EC and lays down additional national requirements, including provisions for the monitoring and management of cyanobacteria.

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

When the bathing water profile or previous experience indicates the risk of cyanobacterial blooms, visual inspection during monitoring should extend to the signs of cyanobacterial proliferation, such as reduced transparency, discolouration of the water or foaming.

<sup>17</sup> Based on Empfehlung zum Schutz von Badenden vor Cyanobakterien-Toxinen. Doi: [10.1007/s00103-015-2192-8](https://doi.org/10.1007/s00103-015-2192-8).

<sup>18</sup> <https://njt.hu/jogszabaly/2008-78-20-22>

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

If visual signs are detected, samples should be taken for the analysis of phytoplankton, cyanobacterial cell count and chlorophyll *a* upon the responsibility of the local public health authority. Samples should be sent to an accredited laboratory to be analysed within 36 h. Whenever the results indicate the risk of cyanobacterial proliferation (cyanobacterial count is over 100,000 cells/mL and chlorophyll *a* is >50 µg/L), and the analysis of phytoplankton shows the presence of potentially toxin-producing taxa, microcystin-LR should also be measured ([Table 9](#)).

Table 9. Quality categories related to cyanobacterial blooms.

Parameter	Excellent	Good	Sufficient	Analytical method
Chlorophyll <i>a</i> (in case of cyanobacterial dominance) (µg/L)	10	25	50	MSZ ISO 10260:1993
Cyanobacterial cell count (cells/mL)	20 000	50 000	100 000	MSZ EN 15204:2006
Microcystin-LR equivalent toxin	4	10	20	ISO 20179:2003

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

In case the parameters associated with cyanobacterial bloom indicate “poor” quality ([Table 9](#)), the bathing site should be closed. It can reopen if the visible signs of blooming are no longer present. The local public health authority should inform the operator of the bathing site and the public on the health risk associated with cyanobacterial bloom, the analytical results and the proposed management measures. Additionally, a special advice should be given to the vulnerable population.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

No de-warning systems are currently in place.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

No information on measures to reduce the risk of cyanobacterial/algal bloom has been reported.

Is there in place any model-based system to inform and anticipate the risk?

There are no model-based systems applied to inform and anticipate the risk.

### 3.8. Lithuania

As reported by the Centre for Health Education and Diseases Prevention, the new edition of Lithuanian Hygiene Norm HN 92:2018 "Beaches and the quality of their bathing water" was approved on 23<sup>rd</sup> of January 2018 by the order No V-76 of Minister of Health of the Republic of Lithuania. This document regulates actions in case of cyanobacterial abundance and the establishment or presumption of a health threat (Crf. 18.3 paragraph).

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

The authorities responsible for the management of beaches and bathing waters have to ensure that monitoring is carried out visually according to the established timetable for the quality monitoring of the bathing water during the bathing season.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

The monitoring is based on examination of cyanobacteria at least every two weeks until the amount of cyanobacteria falls to the recommended value ( $<2 \times 10^4$  cells/L) during the intensive blooms.

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

The public is immediately informed of the health threat at the beach, in places of social gathering, in the city or district press, on the website of responsible authorities and/or by using other tools of awareness raising. It is recommended not to use a bathing site if the level of cyanobacteria is  $>2 \times 10^4$  cells/L and to forbid bathing if the concentration of cyanobacteria is  $>1 \times 10^5$  cells/L.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

No information about de-warning systems in place has been provided.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

No information on measures to reduce the risk of cyanobacterial/algal bloom has been reported.

Is there in place any model-based system to inform and anticipate the risk?

There are no model-based systems applied to inform and anticipate the risk.

### 3.9. The Netherlands

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

In 2020, the National Institute for Public Health and the Environment (RIVM) established a protocol for the monitoring of cyanobacteria in bathing waters (RIVM, 2020). It is based on a visual inspection performed daily during the bathing season in sites where the proliferation of cyanobacteria is highly probable, as well as on the fluorescence analysis of cyanobacterial chlorophyll *a*, which is a proxy of the cyanobacterial density at a bathing site. These methods constitute mandatory steps to ensure a rapid response to a bloom event and adjustment of bathing site's risk level ([Figure 29](#)). Facultatively, microscopic and analytical analysis can be performed when scum is present.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

The basic parameters are based on determination of chlorophyll *a* concentrations and scum category ([Figure 29](#)). The scum category is determined by means of visual inspection, facultatively supplemented with microscopic examination of the cyanobacterial composition of the scum. This analysis includes determination of toxic cyanobacterial genera and biovolume when fluorescence analysis of chlorophyll *a* is doubtful. Microcystin analysis can be performed when chlorophyll *a* concentration exceeds 75  $\mu\text{g/L}$  and the abundance of microcystin-producing cyanobacteria is  $>50\%$ .

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

Risk levels are determined based on the scum category, concentration of chlorophyll *a* and, when dominance of toxin producing cyanobacteria are observed, by microcystin concentrations (Figure 29). Weekly monitoring is performed when chlorophyll *a* levels do not exceed 12 µg/L and microcystin is below 10 µg/L. Additionally, public should be informed when the respective values fall in the range of 12-75 µg/L and 10-20 µg/L. Swimming can be banned when the highest concentrations in these ranges are exceeded.

If a category I or II scum is detected, additional samples can be taken for facultative determination of cyanobacterial genera. The presence of non-toxic cyanobacteria can be used to downward adjust a risk level according to chlorophyll *a* concentrations. The presence of scum category II automatically leads to the highest risk level.

Information for the public is provided by physical signs placed on site and at the Dutch bathing water information website<sup>19</sup>. At this interactive website with a clickable map of The Netherlands, the most recent information about every official bathing site can be obtained. Additionally, it provides background information about safe swimming, health risks and other topics of relevance for the general public.

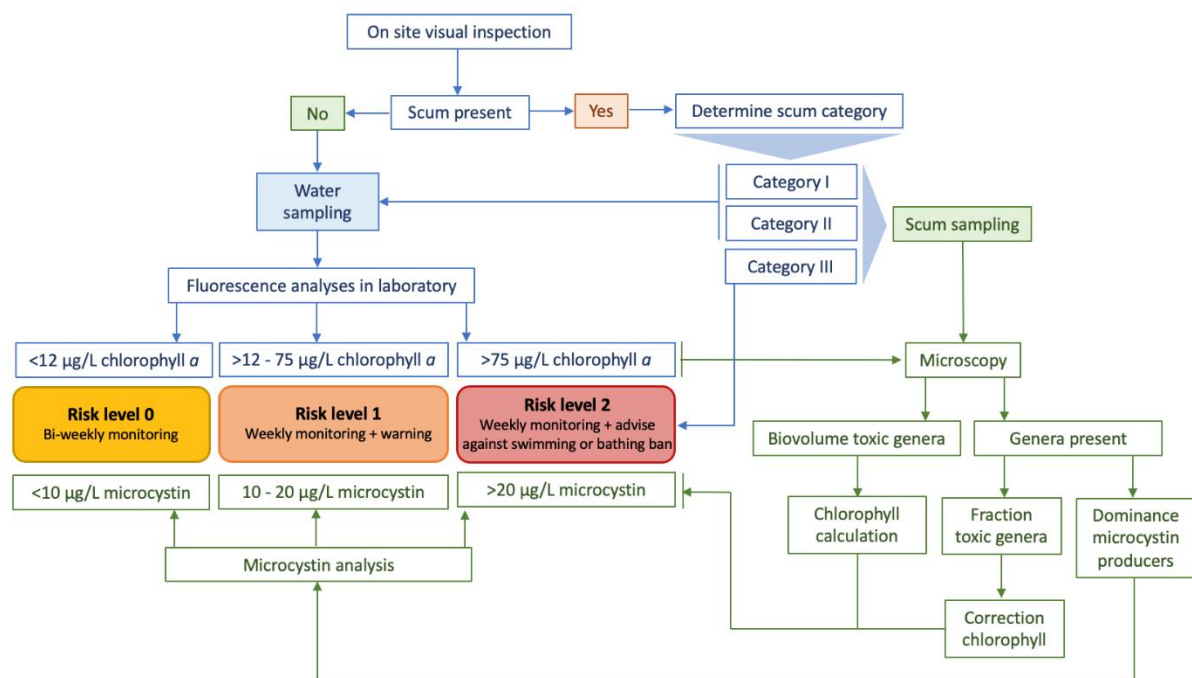


Figure 29. Schematic representation of the protocol for cyanobacterial monitoring in the Netherlands. Mandatory workflow is shown in blue, while facultative measurements are indicated in green.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

<sup>19</sup> [www.zwemwater.nl](http://www.zwemwater.nl)

No specific information about de-warning systems in place has been provided. However, if monitoring at a bathing site results in a changed risk level, the accompanying measure (warning, advice against bathing, or swimming ban) may change backward as well.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

Following the precautionary principle, the basic assumption is that all cyanobacteria are potentially toxic. All cyanobacteria are therefore taken into account by applying fluorescence analyses to determine the cyanobacteria-associated chlorophyll *a* concentrations in water samples. Occasionally, however, not all cyanobacteria present at a bathing site are toxic. For some bathing sites, it is actually known that only non-toxin producing cyanobacteria are present or dominant. In this case, the assumption that all cyanobacteria are toxic leads to an overestimation of the risk. Therefore, the microscopically determined ratio of toxic and non-toxic cyanobacteria may be used to correct the measured cyanobacteria-associated chlorophyll *a* concentration. The list of potentially toxic cyanobacteria that are currently relevant in the Netherlands can assist in this process (RIVM, 2020).

Is there in place any model-based system to inform and anticipate the risk?

Information of model-based systems to inform and anticipate bloom risk has not been provided.

### 3.10. Poland

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

According to the Department of Environmental Hygiene of the Polish Chief Sanitary Inspectorate, a visual monitoring is executed daily by the managers of the bathing sites and by the competent state sanitary inspector in case of cyanobacterial/algal bloom occurrence report.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events? Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

There are no parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events nor established threshold values for the assessment of warning systems and the corresponding levels of risk.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

In the case of cyanobacterial/algal bloom events, information about occurrence is provided on the information board in the close area of the bathing water and on the national bathing water quality website<sup>20</sup> as well as on local websites of competent state sanitary inspectors, bathing water managers and local authorities.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

Poland takes measures to reduce the cyanobacteria/algal blooms by implementing European regulations aimed at reducing environmental pressures and protecting the aquatic environment.

Is there in place any model-based system to inform and anticipate the risk?

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<sup>20</sup> <https://sk.gis.gov.pl>

Regarding model-based systems to inform and anticipate the risk, there is ongoing HAB Risk project<sup>21</sup>, which aims to set up a demonstration service on a web and mobile platform in order to support monitoring of cyanobacteria in the Baltic Sea. The development of this service is supported and funded by MERCATOR OCEAN, under the program for development and promotion of the downstream services Copernicus Marine Environment Monitoring Service (CMEMS). Partners of the project include i-Sea (prime), Planetek, N7 mobile and Hydro Cote.

An advanced tool for large scale monitoring of cyanobacterial blooms in the Baltic Sea, such as HAB Risk, is expected to benefit local population, tourism, and aquaculture. It leverages the near real time satellite data and provides the bloom information through the mobile and web applications.

### 3.11. Portugal

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

As informed by the Portuguese Environment Agency, cyanobacterial proliferation in form of bloom, mat or scum in Portuguese bathing waters has not a high occurrence. The monitoring, meaning the detection of an eventual cyanobacterial bloom, is done by visual surveillance, being this surveillance more attentive in bathing waters where, according to the bathing water profile, the development of blooms is more likely.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

To confirm the occurrence of cyanobacterial/algal bloom events, when visual signs are detected, a sample is taken to determine density of cyanobacteria and whether toxic species are present. The parameter used is cyanobacterial cell density (cells/mL).

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

The alert can be issued when toxic species are present or when the WHO guidelines of cyanobacterial cell density, relating to probability of adverse health effects, are exceeded.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

De-warning occurs when toxic species are no longer detected or when cyanobacterial cell density drops to a value lower than the guideline values.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

There are some generic measures to reduce the risk of cyanobacterial/algal bloom, also within the WFD, Urban Waste Water Directive (UWWDD) and Nitrates Directive (ND), which aim to reduce nutrient load into waterbodies.

Is there in place any model-based system to inform and anticipate the risk?

No model-based system to inform and anticipate the risk is used.

### 3.12. Romania

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

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<sup>21</sup> <https://marine.copernicus.eu/services/use-cases/monitoring-harmful-algal-bloom-baltic-sea-hab-risk-service>

The National Institute of Public Health of Romania reported that methods used at national level for monitoring cyanobacterial/algae bloom events are based on a visual detection and determination of phytoplankton/cyanobacteria performed by ABA Dobrogea-Litoral and INCDM „GrigoreAntipa”. Cyanobacterial occurrence is not characteristic for the Black Sea.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algae bloom events?

The parameters used to confirm the occurrence of cyanobacterial/algae bloom events consist in phytoplankton cell/L count and determination of the dominant species (occurrence: cryptophyte *Hillea fusiformis*, chlorophyta *Carteria* sp., *Pseudoanabaena*, *Merismopedia tenuissima* and *Protoperidinium quinquecorne*).

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algae bloom events?

In case of occurrence of algae bloom events in bathing waters, actions are taken to remove the bloom immediately. Threshold values have not been provided.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algae bloom events?

De-warning is based on a visual inspection once there is no risk from a bloom.

Are there any targeted measures to reduce the risk of cyanobacterial/algae bloom?

To reduce the risk of cyanobacterial/algae bloom, phytoplankton determinations are performed where cyanobacteria are also present but do not proliferate as a bloom, mat or scum (not characteristic of the Black Sea).

Besides measurements of nutrient concentrations in water, there are no other targeted measures to reduce the risk of cyanobacterial/algae bloom.

Is there in place any model-based system to inform and anticipate the risk?

There is no any model-based system to inform and anticipate the risk.

### 3.13. Slovakia

Which are the methods used at national level for monitoring cyanobacterial/algae bloom events?

The methods used at national level for monitoring cyanobacterial/algae bloom events in bathing waters have been defined by the Public Health Authority of the Slovak Republic in the water quality assessment scheme ([Figure 31](#)) in accordance with the European project Cyanobacteria and toxins in waters: Occurrence, health impact and measures (COSTES1/05). The methods used are summarised in [Table 10](#).

Table 10. Methods and parameters used for monitoring cyanobacterial/algal bloom events in Slovakia.

Parameter	Method
Cyanobacterial proliferation	Visual inspection in the field, by microscopic quantification
Measurement of water transparency	Secchi disk
Determination and quantification of cyanobacterial and algal taxa	STN 75 7715 (microscopic analysis)
Acute toxicity	Ecotoxicological tests: STN ISO 14380 for <i>Thamnocephalus platyurus</i> STN EN ISO 11348-1 for <i>Vibrio fischeri</i> STN EN ISO 8692 for <i>Desmodesmus subspicatus</i> STN 838303 for <i>Sinapis alba</i>
Detection and quantification of cyanotoxins	HPLC
Measurement of chlorophyll <i>a</i> concentration	STN ISO 10260

Every year before bathing season, the scheme (Figure 30) is reviewed and provided to all Regional Public Health Authorities, which are responsible for visual inspection, measurement of water transparency and sampling on local level. In general, a few Regional Public Health Authorities have designated accredited laboratories to carry out laboratory analyses of samples; and only the Public Health Authority of the Slovak Republic has designated accredited laboratories to carry out determination of acute toxicity of samples taken by all Regional Public Health Authorities.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

The parameters used at national level to confirm the occurrence of cyanobacterial bloom events are listed in Table 10 and comprise:

- in water: transparency, cyanobacteria, dominant taxa of cyanobacteria and algae, acute toxicity, cyanotoxins (microcystins - LR, YR, RR and cylindrospermopsin) and chlorophyll *a* – concentration;
- in biomass of cyanobacteria: percentage of cyanobacteria, acute toxicity and cyanotoxins (microcystins - LR, YR, RR and cylindrospermopsin).

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

There are three classes of water quality in the scheme (Figure 30) in terms of visual inspection and laboratory testing of biological indicators and ecotoxicological examination in Slovakia:

- Suitable water quality - degree I. Visual inspection (cyanobacterial colonies or flakes are not visible to the naked eye, water transparency is over 1 meter) and simultaneously  $<2 \times 10^4$  cells/mL cyanobacteria,  $<10$  µg/L chlorophyll *a*. It is possible to carry out common activities, without special restrictions.
- Acceptable water quality - degree II. Visual inspection (flakes or clusters of cyanobacterial colonies are observable with the naked eye, water transparency is at least 1 meter) and simultaneously from  $2 \times 10^4$  to  $1 \times 10^5$  cells/mL cyanobacteria, from 10 to 50 µg/L chlorophyll *a*. It is possible to carry out common activities, but in case of deterioration of water quality it is necessary to advise against bathing for children, allergic people and individuals with weakened immune system, and to disseminate this information to the public.

- Unacceptable water quality - degree III. Visual inspection (flakes or clusters of cyanobacterial colonies are dispersed in the water column or in larger amounts accumulated on the surface, the occurrence of cyanobacteria in the form of bloom or slurry flowing off the shore, water transparency is less than 1 meter) or  $>1 \times 10^5$  cells/mL cyanobacteria, or  $>50 \mu\text{g/L}$  chlorophyll *a*, or acute toxicity  $>30\%$  impact. Bathing ban is advised temporarily. It is necessary to disseminate this information to the public.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

Over the past years, there were highlighted problems with the repeated increasing growth of cyanobacteria only in some bathing waters in Slovakia. Specifically, during the 2018 bathing season, exceeded values of cyanobacteria were detected only at two bathing water sites out of 30 reported. De-warning systems in Slovakia are based on information provided to the public to avoid entering the water, which is disseminated through local water quality websites. Information on cyanobacterial occurrence and proliferation are also provided through the Public Health Authority's website at weekly interval during the bathing season.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

The Public Health Authority of the Slovak Republic does not have the competence to deal with the quality of bathing waters because it is the responsibility of the municipal districts, operators of bathing waters, fishing organisations and others. Inspectors only control the quality of bathing waters from a health point of view and cannot intervene in the management of bathing waters nor order measures to reduce the risk of the cyanobacterial bloom events in vulnerable locations.

Is there in place any model-based system to inform and anticipate the risk?

Public Health Authority of the Slovak Republic collects up-to-date information on waterbodies' characteristics, history of bathing waters, etc. from the Ministry of the Environment of the Slovak Republic, Slovak Hydrometeorological Institute, Water Research Institute, Slovak Environment Agency and Regional Public Health Authorities in accordance with Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC (Article 6, ANI EX III) in order to establish, review and update bathing water profiles. There is no any model-based system to inform and anticipate the risk of cyanobacterial/algal bloom events, but during the bathing season Public Health Authority of the Slovak Republic provides information to the public through its website at weekly interval.

Under a project of the Public Health Authority of the Slovak Republic monitoring of selected bathing waters, especially those with cyanobacterial bloom events registered in the past, is performed. This project aims to obtain an overview of the current occurrence of cyanobacteria, to detect the presence of cyanotoxins in water and in cyanobacterial biomass, to determine the species composition of cyanobacterial water blooms, with an emphasis on the presence and spreading of invasive cyanobacterial species.

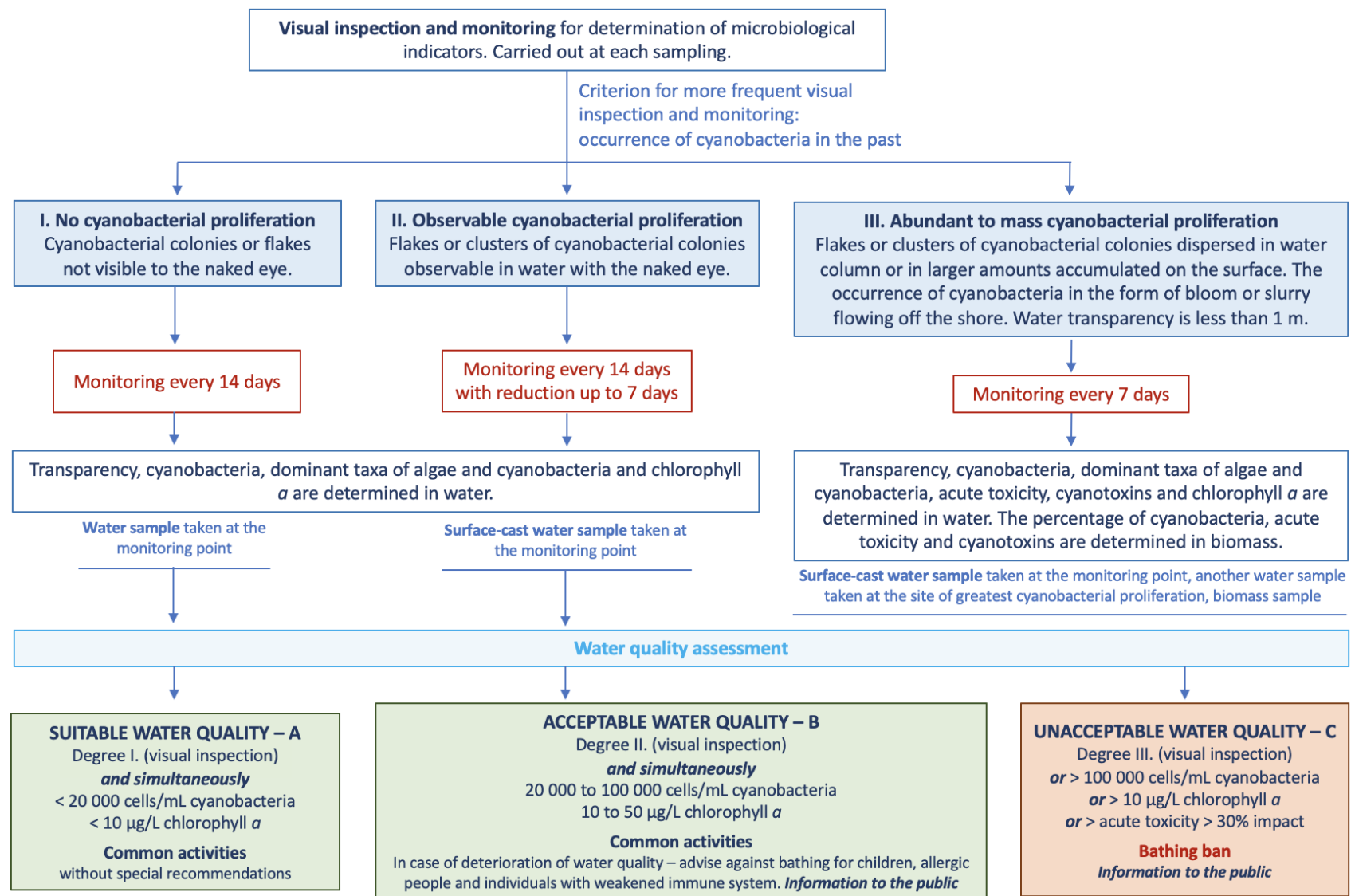


Figure 30. Procedure for visual inspection and monitoring of cyanobacterial proliferation within the water quality assessment in Slovakia.

### 3.14. Spain

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

For the monitoring of cyanobacterial/algal bloom events in Spain, in inland bathing waters, the Ministry for the Ecological Transition has elaborated two protocols:

1. Protocol for the evaluation of the proliferation propensity of cyanobacteria. The information collected according to this protocol forms part of the profile of each bathing area.

The protocol is based on a decision tree that leads to the different levels of decision depending on whether some criteria or others are exceeded. The choice of critical values at each of the levels has been based on the existing scientific literature, on the guidance or recommended values of WHO, on the regulations and legislations of other European and non-European countries (such as Australia) and on the experience provided by the UAM (Autonomous University of Madrid) after studying and researching toxins and cyanobacteria in Spain. A methodology similar to that used for other types of risk analysis has been applied, where the propensity of cyanobacteria to proliferate is considered as risk.

2. Phytoplankton sampling protocol in lakes and reservoirs.

For coastal bathing waters of the Canary Islands, methods used will depend on the type of bloom concerned: planktonic or benthic. The methods are those proposed by IOC-UNESCO. In Catalonia, the Catalan Water Agency has 21 control points within risk areas that are sampled during the bathing season (from the end of June to the middle of September). An exhaustive control of the *Ostreopsis* sp. algae is carried out for its repeated presence in some points of the Catalan coast, although without showing important blooms.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

The parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events include in inland bathing waters (Figure 31):

1. Visual examination of the waterbody;
2. Transparency (depth of Secchi Disk);
3. Parameters related to the presence of biomass of cyanobacteria:
  - total phosphorus;
  - phosphate ( $\text{PO}_4^{3-}$ );
  - DIN/ $\text{PO}_4^{3-}$  ratio (Dissolved Inorganic Nitrogen = nitrate + ammonium + nitrite/phosphate);
  - concentration of chlorophyll *a* total;
4. Presence of potentially toxic cyanobacterial genera (producers of cyanotoxins);
5. Presence of toxins, mainly microcystins, anatoxin and cylindrospermopsin.

In coastal bathing waters (Canary Islands and Catalonia), the parameters include the concentration of cells by volume, per surface or per gram of algae. Depending on the identification of the species in question, the type of toxin and its concentration is studied.

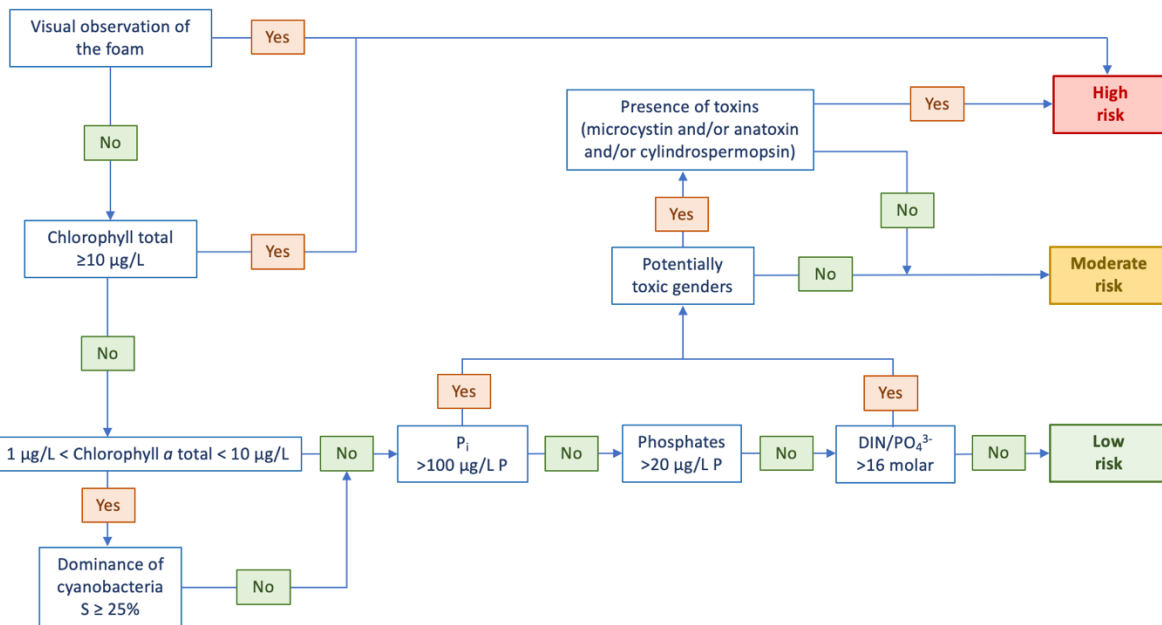


Figure 31. Pathway of monitoring cyanobacterial proliferation and actions implemented in Spain for bathing areas. DIN: nitrate, ammonium, nitrite.

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

Threshold values for the assessment of warning systems and the corresponding levels of risk associated with cyanobacterial/algal bloom events in inland bathing waters are those recommended by the WHO<sup>22</sup>, while for coastal bathing waters the levels have been developed for the central Macaronesian region based on local literature and local measurements. When the mass of the bloom approaches the coast, the warning of prohibition of swimming is activated.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

De-warning measures include the communication between the competent authorities (hydrographic and health services) up to the local authorities (town halls) for inland bathing waters. For coastal bathing waters (Canary Islands), at the moment of bloom detection, the majority of the species is identified and, based on their toxicity, the alert is activated. Bloom appearance is dependent on weather conditions: rising seawater temperature, calm sea, lack of wind generally by reduction of the trade winds, accompanied by long episodes of Saharan air intrusion or by small continuous episodes in the weather. Under these weather conditions, small blooms of *Trichodesmium erythraeum* were observed in 2004 and 2011, which affected mainly the coasts of the eastern islands and subsided in a few days. However, during the summer of 2017, as a consequence of the long duration of the meteorological conditions described, there was a bloom that lasted 90 days and affected the western islands more sharply. During this time, the health system did not detect any effect on the health of the population. However, the alert was activated over the entire episode and as a precautionary measure, although the literature does not describe important effects for health.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

Measures to reduce the risk of cyanobacterial/algal bloom in inland bathing waters include:

<sup>22</sup> WHO (2003). Algae and cyanobacteria in coastal and estuarine waters. Chapter 7. In: Guidelines for Safe Recreational Water Environments. Volume 1: Coastal and Fresh Waters. World Health Organisation, Geneva, Switzerland.

1. The river basin districts within its river basin management plans include a series of general measures such as:

- improvements on sanitation and waste water treatment systems;
- environmental recovery measures;

2. Other competent bodies: agricultural activity;

3. Other measures: innovative such as the application of eucalyptus bark as a measure against the proliferation of cyanobacteria in the Caldas de Reis reservoir (Pontevedra).

In coastal bathing waters (Canary Islands) the blooms constitute a natural phenomenon, since *Trichodesmium* is found in the depths in its natural habitat and only emerges when the meteorological conditions described remain in time, therefore it is not possible to adopt measures to reduce bloom. Although the scientific literature does not describe any relationship between the formation of these blooms and the existence of waste waters on the coast, an agreement has been established with the University of Las Palmas of the Canary Islands to study a possible relationship between both factors. To date, *Trichodesmium erythraeum* bloom in the Canary Islands has been formed in the Mar de Las Calmas on the Island of El Hierro, where there is no trace of contamination.

Recently, a small ship was acquired to collect the cyanobacterial mass in the high seas, and prevent it from reaching the coasts. It has not been possible to assess its effectiveness, because in 2018 there were small blooms that dispersed by themselves without reaching the coasts, as the weather conditions changed.

Is there in place any model-based system to inform and anticipate the risk?

There is no any model-based system to inform and anticipate the risk in place for inland and coastal bathing waters. For the latter, the weather conditions are the only indicator that allows to predict a possible formation of blooms, and the sightings by the surveillance helicopters track their displacement. The Catalan Water Agency participates in the WateXr Project<sup>23</sup> in which it is planned to develop an efficient prediction model.

### 3.15. United Kingdom

Which are the methods used at national level for monitoring cyanobacterial/algal bloom events?

The methods used in UK<sup>24</sup> for monitoring cyanobacterial/algal bloom events include visual inspection for blooms/scums as a first measure, if observed algal species are determined and number of cells per unit volume quantified to assess risk.

Which are the parameters used at national level to confirm the occurrence of cyanobacterial/algal bloom events?

The parameters to confirm the occurrence of blooms are based on microscopic analysis to establish genus level identification of cyanobacteria. Algal cells are counted using either a Sedgwick-Rafter, or Lund cell. If the analysis finds any blue-green algae taxa present, cell counts are compared with warning thresholds for cyanobacteria.

Which are the threshold values for the assessment of warning systems (surveillance/alert/action) and the corresponding levels of risk (low/medium/high) associated with cyanobacterial/algal bloom events?

If cyanobacterial species are identified and  $2 \times 10^4$  cell/mL or 10 µg chlorophyll *a*/L with dominance of cyanobacteria, the action is to:

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<sup>23</sup> <https://watexr.eu/>

<sup>24</sup> At the time of the JRC survey, the United Kingdom was a Member State of the EU

Watch for scums or conditions conducive to scums;

- Advise owners to restrict swimming and other immersion activities;
- Advise owners to post on-site risk advisory signs;
- Inform public and relevant authorities;
- Monitor until below guidance level;
- Inform public and relevant authorities.

Which are the de-warning systems (if any in your management program) to manage cyanobacterial/algal bloom events?

The de-warning measures to manage cyanobacterial/algal bloom events are based on a visual observation and, if necessary, further analysis.

Are there any targeted measures to reduce the risk of cyanobacterial/algal bloom?

There are no specific measure packages in place to protect bathing waters from cyanobacterial blooms but pollution prevention measures to reduce nutrients and promote balanced macrophyte dominated community in place of algal dominated communities will help to reduce bloom formation.

Is there in place any model-based system to inform and anticipate the risk?

There are no current models in place specifically to estimate bathing risk from cyanobacteria.

Table 11. Results of the survey on monitoring and management of cyanobacterial/algal blooms circulated by the JRC to Member States in 2019.

AT: Austria, BE: Belgium, CY: Cyprus, DE: Germany, ES: Spain, FI: Finland, FR: France, LT: Lithuania, PL: Poland, PT: Portugal, RO: Romania, SK: Slovakia, UK: United Kingdom.

Country	Methods for monitoring bloom events	Parameters to confirm the occurrence of bloom events	Threshold values for the assessment of warning systems	De-warning systems	Measures to reduce the risk of blooms	Model-based system to inform and anticipate the risk
AT	<ul style="list-style-type: none"> <li>• Site inspection of the bathing area</li> <li>• Measurement of water transparency</li> <li>• Microscopic identification of cyanobacteria</li> <li>• Cell counts of cyanobacteria and assessment of biovolume</li> <li>• Assessment of chlorophyll <i>a</i> (<i>in situ</i> fluorometer analysis)</li> <li>• Assessment of the total phosphorus</li> <li>• Toxin analysis</li> </ul> <p># Monitoring methods may differ between the federal provinces of AT</p>	<ul style="list-style-type: none"> <li>• Cyanobacteria identification, cell density and biovolume (microscopic analysis) and/or</li> <li>• Toxin analysis with ELISA and/or HPLC-MS</li> </ul>	<p>Different threshold values are in use in the different provinces of AT:</p> <p>Carinthia: in case the total biovolume of cyanobacteria is &gt;1 mm<sup>3</sup>/L and water transparency is &lt;2 m, the cyanotoxin measurement (HPLC-MS) is performed. A bathing prohibition is issued above a value of 100 µg/L microcystin.</p> <p>Vorarlberg: when microcystin concentration is &gt;100 µg/L (ELISA), bathing is prohibited. The situation is non-hazardous to health when microcystin concentration is &lt;10 µg/L.</p> <p>Vienna: in case water transparency is &lt;1m, the microcystin measurement (HPLC-MS) is performed. A bathing prohibition is issued when microcystin concentration is ≥30 µg/L.</p> <p>Rest of AT: bathing prohibition when microcystin concentration is &gt;100 µg/L</p>	<ul style="list-style-type: none"> <li>• Water transparency &gt;1m</li> <li>• No scums or cloudiness visible during the site inspection</li> <li>• Cyanotoxins' concentrations below a defined threshold value</li> <li>• Information for the public is to be provided via information boards at the bathing site and via the internet (<a href="https://www.ages.at/en/topics/environment/water/bathing-water-monitoring/">https://www.ages.at/en/topics/environment/water/bathing-water-monitoring/</a>)</li> </ul>	<p>Reduction of nutrient input:</p> <ul style="list-style-type: none"> <li>• Austrian Agri-Environmental Program ÖPUL (2015-2020): Reduction of diffuse pollution sources</li> <li>• LIFE Old Danube Project</li> </ul>	<p>No model-based system in use although a model was created in 2013 (*)</p>

BE	<ul style="list-style-type: none"> <li>• Site inspection of the bathing area</li> <li>• Assessment of water transparency</li> <li>• Assessment of water colour</li> <li>• Presence of clumps in the water column</li> <li>• Chlorophyll <i>a</i> determination</li> <li>• Determination of toxin-producing cyanobacteria</li> </ul>	<ul style="list-style-type: none"> <li>• Chlorophyll <i>a</i> determination (fluorometric measurement) and/or</li> <li>• Toxin analysis (ELISA): Microcystin in Flanders and cyanotoxins in Wallonia</li> <li>• Visual identification of bloom</li> </ul>	<p>A bathing prohibition is issued when there is a visible bloom in the swimming or recreation zone and when there is no a visible bloom in these areas, but:</p> <p>Flanders: chlorophyll <i>a</i> concentration is &gt;75 µg/L or microcystin concentration is &gt;20 µg/L; Chlorophyll <i>a</i> concentration is also measured in case a different water colour or a poor visibility is observed. If chlorophyll <i>a</i> concentration is &lt;75 µg/L, no further actions are taken.</p> <p>Wallonia: chlorophyll <i>a</i> concentration is &gt;50 µg/L or cyanotoxin concentration is &gt;8 µg/L. Cyanotoxins are monitored until their concentration decreases to &lt;5 µg/L. If chlorophyll <i>a</i> concentrations are &lt;10 µg/L, no further actions are taken.</p> <p>A swimming or recreation ban is enforced when chlorophyll <i>a</i> levels are &gt;75 µg/L (Flanders) or &gt;50 µg/L (Wallonia).</p>	<ul style="list-style-type: none"> <li>• No visible blooms</li> <li>• Chlorophyll <i>a</i> &lt;75 µg/L in Flanders and &lt;50 µg/L in Wallonia</li> <li>• Microcystin &lt;20 µg/L in Flanders and cyanotoxins &lt;8 µg/L in Wallonia</li> </ul>	Not reported	Not reported
CY	Not applicable for CY (**)	Not applicable for CY (**)	Not applicable for CY (**)	Not applicable for CY (**)	Not applicable for CY (**)	Not applicable for CY (**)

DE	<ul style="list-style-type: none"> <li>• Site inspection of the water</li> <li>• Measurement of water transparency</li> <li>• Chlorophyll <i>a</i> determination</li> <li>• Assessment of cyanobacteria biovolume</li> </ul>	<ul style="list-style-type: none"> <li>• Chlorophyll <i>a</i> determination (<i>in situ</i> fluorometry)</li> <li>• Assessment of cyanobacteria biovolume (microscopic analysis)</li> </ul>	<p>Three alert levels are in place:</p> <p>High attention: water transparency &lt;2 m, or cyanobacteria biovolume &gt;1 mm<sup>3</sup>/L, or chlorophyll <i>a</i> &gt;5 µg/L</p> <p>Warning level: water transparency &lt;1 m, or cyanobacteria biovolume &gt;3 mm<sup>3</sup>/L, or chlorophyll <i>a</i> &gt;15 µg/L</p> <p>Alert level: water transparency &lt;0.5 m, or cyanobacteria biovolume &gt;15 mm<sup>3</sup>/L, or chlorophyll <i>a</i> &gt;75 µg/L</p> <p>An additional monitoring of anatoxin-a is required in case of benthic cyanobacteria (e.g. <i>Tychonema</i>)</p>	<ul style="list-style-type: none"> <li>• No presence of visible bloom in water</li> </ul> <p>Additional assessment:</p> <ul style="list-style-type: none"> <li>• &lt;1 mm<sup>3</sup>/L cyanobacteria biovolume</li> <li>• &lt;5 µg/L chlorophyll <i>a</i></li> </ul>	<ul style="list-style-type: none"> <li>• Improvement of waste water treatment</li> <li>• Reduction of waste water discharge</li> <li>• Reduction of storm water overflow events</li> <li>• Phosphate precipitation</li> <li>• Aeration or artificial mixing</li> <li>• Sediment removal</li> <li>• Measures against water fowl and fish</li> <li>• Extended riparian buffer strips</li> <li>• Timing of fertilisation and fine-tuning of amounts according to crop demand</li> </ul>	No model-based system in use
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ES	<p>Inland bathing water:</p> <p><i>Bathing areas:</i></p> <ul style="list-style-type: none"> <li>• Site inspection of the water</li> <li>• Measurement of water transparency</li> <li>• Assessment of the total phosphorus</li> <li>• Assessment of phosphate (PO<sub>4</sub><sup>3-</sup>)</li> <li>• Assessment of DIN/PO<sub>4</sub><sup>3-</sup> ratio (dissolved inorganic nitrogen = nitrate + ammonium + nitrite/phosphate)</li> <li>• Chlorophyll <i>a</i> determination</li> <li>• Presence of potentially toxic cyanobacterial genera (producers of cyanotoxins)</li> <li>• Presence of toxins, mainly microcystins, anatoxin and cylindrospermopsin</li> </ul> <p><i>Lakes and reservoirs:</i></p> <p>Chlorophyll <i>a</i> determination, cell counts of cyanobacteria and assessment of biovolume,</p>	<p>Inland bathing water:</p> <p><i>Bathing areas:</i></p> <ul style="list-style-type: none"> <li>• Presence of visible bloom in water</li> <li>• Water transparency (Secchi Disk)</li> <li>• Cyanobacteria identification</li> <li>• Chlorophyll <i>a</i> concentration</li> <li>• Total phosphorus content</li> <li>• PO<sub>4</sub><sup>3-</sup> content</li> <li>• DIN/PO<sub>4</sub><sup>3-</sup> ratio</li> <li>• Toxin analysis (microcystins, anatoxin and cylindrospermopsin)</li> </ul> <p><i>Lakes and reservoirs:</i></p> <p>Chlorophyll <i>a</i> determination (fluorometric probe), cell counts of cyanobacteria and assessment of biovolume (microscopic analysis), cyanobacteria identification,</p>	<p>Inland bathing water:</p> <p><i>Bathing areas:</i></p> <p>Low risk of blooms: Chlorophyll <i>a</i> concentration &lt;1 µg/L, total phosphorus &lt;100 µg/L, PO<sub>4</sub><sup>3-</sup> &lt; 20 µg/L, DIN/PO<sub>4</sub><sup>3-</sup> ratio &lt; 16 M and cyanobacterial dominance &lt;25% (even if "1 µg/L &lt; chlorophyll <i>a</i> concentration &lt;10 µg/L").</p> <p>Moderate risk of blooms: Chlorophyll <i>a</i> concentration is between 1 µg/L and 10 µg/L, but cyanobacterial abundance is &lt;25, or when chlorophyll <i>a</i> concentration is &lt;1 µg/L. In both cases, other values to check are for total phosphorus (&gt;100 µg/L) or PO<sub>4</sub><sup>3-</sup> (&gt;20 µg/L) or DIN/PO<sub>4</sub><sup>3-</sup> ratio (&gt;16 M) and no harmful toxins are detected.</p> <p>High risk of blooms: same parameters as the ones for confirming the moderate risk of blooms with the difference that harmful toxins are detected in water. The high risk is also defined when there is a visible bloom in water and when chlorophyll <i>a</i> concentration is ≥10 µg/L.</p> <p>Coastal water (Canary Islands and Catalonia):</p> <p>The swimming ban is activated when the bloom approaches the coast.</p>	<p>Inland bathing water:</p> <p><i>Bathing areas:</i></p> <ul style="list-style-type: none"> <li>• No presence of visible bloom in water</li> <li>• Chlorophyll <i>a</i> and cyanotoxins concentration, total phosphorus, PO<sub>4</sub><sup>3-</sup> content and DIN/PO<sub>4</sub><sup>3-</sup> ratio below defined threshold values</li> </ul> <p>Coastal water (Canary Islands and Catalonia):</p> <ul style="list-style-type: none"> <li>• Not reported</li> </ul>	<p>Inland bathing water:</p> <p><i>Bathing areas:</i></p> <ul style="list-style-type: none"> <li>• Improvements on sanitation and waste water treatment systems</li> <li>• Other measures: e.g. application of eucalyptus bark against the proliferation of cyanobacteria in the Caldas de Reis reservoir (Pontevedra)</li> </ul> <p>Coastal water (Canary Islands and Catalonia):</p> <p>Relationship between the formation of <i>Trichodesmium erythraeum</i> bloom and the existence of waste waters on the coast is under</p>	<p>Inland bathing water:</p> <p><i>Bathing areas:</i></p> <p>No model-based system in use</p> <p>Coastal water (Canary Islands and Catalonia):</p> <p>Weather conditions are the only indicator that allows predicting the possible bloom formation. A prediction model has been planned to be developed during the WateXr Project (***)</p>
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	<p>cyanobacteria identification, water transparency  Coastal water (Canary Islands and Catalonia):</p> <ul style="list-style-type: none"> <li>• Concentration of cells by volume, per surface or per gram of algae</li> <li>• Control of the <i>Ostreopsis</i> algae (Catalonia) and <i>Trichodesmium erythraeum</i></li> <li>• Toxin analysis</li> </ul>	<p>water transparency (Secchi Disk)  Coastal water (Canary Islands and Catalonia):</p> <ul style="list-style-type: none"> <li>• Cyanobacteria identification and concentration</li> <li>• Toxin analysis</li> </ul>			<p>investigation. Collection the cyanobacterial mass in the high seas and prevention that the bloom reaches the coasts.</p>	
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FI	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> </ul>	<ul style="list-style-type: none"> <li>• Cyanobacteria identification (microscopic analysis) and/or</li> <li>• Toxin analysis</li> </ul>	<p>Cyanobacterial monitoring is based on visual inspection. Further analyses are not required unless popular beaches are affected by bloom or in case a persistent bloom or adverse health effects on humans and animals are reported. In those cases, the cyanobacteria identification and toxin analyses are performed. When visual inspection of high amount or very high amount of cyanobacteria are reported in water, an immediate warning is launched to inform the public.</p>	<ul style="list-style-type: none"> <li>• No presence of visible bloom in water</li> <li>• A webpage system and a mobile application publicly available for reporting visual inspections of blooms</li> </ul>	<ul style="list-style-type: none"> <li>• Measures for reducing nutrient loads in waterbodies recommended in the River Basin Management Plans compiled in accordance to the Water Framework Directive</li> <li>• Weekly monitoring of cyanobacterial blooms at selected sites during the summer period</li> </ul>	No model-based system in use
FR	<ul style="list-style-type: none"> <li>• Cyanobacteria identification and enumeration</li> <li>• Toxin analysis</li> </ul>	<ul style="list-style-type: none"> <li>• Identification and enumeration of cyanobacteria (NF EN 15204)</li> <li>• Toxin analysis (ISO 20179 for microcystins)</li> </ul>	<p>Recommendations:          Sampling implemented once a month, at least, and closure of bathing sites and nautical activities in case of presence of:</p> <ul style="list-style-type: none"> <li>• Cyanobacteria (<math>1 \times 10^5</math> cells/mL)</li> <li>• Toxoid A (<math>\geq 40</math> <math>\mu\text{g/L}</math>)</li> <li>• Microcystin (<math>\geq 13</math> <math>\mu\text{g/L}</math>, microcystin-LR equivalent)</li> </ul>	<p>Recommendations:</p> <ul style="list-style-type: none"> <li>• Cyanobacteria <math>&lt; 1 \times 10^5</math> cells/mL</li> <li>• Toxoid A <math>&lt; 40</math> <math>\mu\text{g/L}</math></li> <li>• Microcystin <math>&lt; 13</math> <math>\mu\text{g/L}</math> (microcystin-LR equivalent)</li> </ul>	<ul style="list-style-type: none"> <li>• Reduction of nutrient loads in the waterbody (in particular, phosphorus)</li> </ul>	No model-based system in use

HU	<ul style="list-style-type: none"> <li>• Visual inspection</li> <li>• Chlorophyll <i>a</i> determination</li> <li>• Enumeration of cyanobacterial</li> <li>• Toxin analysis</li> </ul>	<ul style="list-style-type: none"> <li>• Chlorophyll <i>a</i> determination (MSZ ISO 10260)</li> <li>• Cyanobacterial cell density (cells/mL) (MSZ EN 15204)</li> <li>• Toxin analysis (ISO 20179 for microcystins)</li> </ul>	<ul style="list-style-type: none"> <li>• Chlorophyll <i>a</i> concentration &gt;50 µg/L</li> <li>• Cyanobacteria (1x10<sup>5</sup> cells/mL)</li> <li>• Microcystin (≥20 µg/L, microcystin-LR equivalent)</li> </ul>	<ul style="list-style-type: none"> <li>• Chlorophyll <i>a</i> concentration &lt;10 µg/L</li> <li>• Cyanobacteria (2x10<sup>4</sup> cells/mL)</li> <li>• Microcystin &lt;4 µg/L, microcystin-LR equivalent)</li> </ul>	Not reported	Not reported
LT	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> </ul>	<ul style="list-style-type: none"> <li>• Cyanobacterial cell density (cells/mL)</li> </ul>	<ul style="list-style-type: none"> <li>• Recommendations not to bathe: Cyanobacterial cell density &gt;2x10<sup>4</sup> cells/L</li> <li>• Bathing ban: Cyanobacterial cell density &gt;1x10<sup>5</sup> cells/L</li> <li>• During intensive blooms, analysis of cyanobacteria implemented at least every two weeks until the amount &lt;2x10<sup>4</sup> cells/L</li> </ul>	<ul style="list-style-type: none"> <li>• Cyanobacterial cell density &lt;2x10<sup>4</sup> cells/L</li> </ul>	Not reported	Not reported
NL	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> <li>• Chlorophyll <i>a</i> determination</li> <li>• Cyanobacteria identification</li> <li>• Toxin analysis</li> </ul>	<ul style="list-style-type: none"> <li>• Scum classification</li> <li>• Chlorophyll <i>a</i> concentration (fluorescence analysis)</li> <li>• Cyanobacteria biovolume and presence of toxin producers (facultative)</li> <li>• Microcystins' analysis (facultative)</li> </ul>	<ul style="list-style-type: none"> <li>• Scum category II and III</li> <li>• Chlorophyll <i>a</i> concentration &gt;75 µg/L for swimming ban and &gt;12-75 µg/L for warning</li> <li>• Microcystin concentration &gt;20 µg/L for swimming ban and &gt;10-20 µg/L for warning</li> </ul>	<ul style="list-style-type: none"> <li>• Scum category I</li> <li>• Chlorophyll <i>a</i> concentration &lt;12 µg/L</li> <li>• Microcystin concentration &lt;10 µg/L</li> </ul>	Not reported	Not reported

PL	<ul style="list-style-type: none"> <li>• Visual inspection of the water (every day in case of bloom events)</li> </ul>	Not available	Not available	<ul style="list-style-type: none"> <li>• Information for the public is to be provided via information boards in the close area of the bathing site and on the national bathing water quality website (<a href="https://sk.gis.gov.pl/">https://sk.gis.gov.pl/</a>) as well as on local websites</li> </ul>	<ul style="list-style-type: none"> <li>• Implementation of European regulations aimed at reducing environmental pressures and protecting the aquatic environment</li> </ul>	<p><u>HAB Risk project</u>. This project leverages the near real time satellite data and provides the bloom information through the mobile and web apps.</p>
PT	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> </ul>	<ul style="list-style-type: none"> <li>• Cyanobacterial cell density (cells/mL)</li> <li>• Toxin analysis</li> </ul>	<ul style="list-style-type: none"> <li>• Threshold values defined according to the World Health Organisation (WHO) guidelines of cyanobacterial cell density</li> <li>• The alert can be issued when toxic species are present in water.</li> </ul>	<ul style="list-style-type: none"> <li>• No presence of visible bloom in water</li> <li>• Cyanobacterial cell density &lt; WHO guidelines values</li> </ul>	<ul style="list-style-type: none"> <li>• Reduction of nutrient loads to the waterbody (in accordance to the Water Framework Directive, Urban Waste Water Directive and Nitrates Directive)</li> </ul>	No model-based system in use
RO	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> <li>• Cyanobacteria identification</li> </ul>	<ul style="list-style-type: none"> <li>• Cyanobacteria identification and biovolume</li> </ul>	<ul style="list-style-type: none"> <li>• No defined threshold values</li> </ul>	<ul style="list-style-type: none"> <li>• No presence of visible bloom in water</li> </ul>	<ul style="list-style-type: none"> <li>• Reduction of nutrient loads</li> </ul>	No model-based system in use

SK	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> <li>• Measurement of water transparency</li> <li>• Cyanobacteria identification and quantification</li> <li>• Presence of toxins</li> <li>• Chlorophyll <i>a</i> concentration</li> </ul>	<ul style="list-style-type: none"> <li>• Water transparency (Secchi Disk)</li> <li>• Cyanobacterial cell density (cells/mL) (microscopic analysis)</li> <li>• Chlorophyll <i>a</i> concentration (STN ISO 10260)</li> <li>• Toxin analysis (HPLC)</li> </ul>	<p>Suitable water quality: no presence of visible bloom in water (water transparency is over 1 m), and simultaneously <math>&lt;2 \times 10^4</math> cells/mL cyanobacteria and <math>&lt;10 \mu\text{g/L}</math> chlorophyll <i>a</i>.</p> <p>Acceptable water quality: cyanobacterial colonies in water (water transparency is at least 1 m), and simultaneously cyanobacterial density is between <math>2 \times 10^4</math> and <math>1 \times 10^5</math> cells/mL, and chlorophyll <i>a</i> concentration is <math>10\text{-}50 \mu\text{g/L}</math>.</p> <p>Unacceptable water quality (bathing ban): cyanobacterial colonies in water (water transparency <math>&lt;1</math> m) or <math>&gt;1 \times 10^5</math> cells/mL cyanobacteria, or <math>&gt;50 \mu\text{g/L}</math> chlorophyll <i>a</i>, or acute toxicity <math>&gt;30\%</math> impact</p>	<ul style="list-style-type: none"> <li>• Water transparency <math>&gt;1\text{m}</math></li> <li>• Cyanobacterial cell density <math>&lt;2 \times 10^4</math> cells/mL</li> <li>• Chlorophyll <i>a</i> <math>&lt;10 \mu\text{g/L}</math></li> <li>• Information on cyanobacterial occurrence provided through the Public Health Authority's web site in weekly interval during the bathing season</li> </ul>	<ul style="list-style-type: none"> <li>• Under responsibility of municipal districts, operators of bathing waters, fishing organisations etc.</li> </ul>	<ul style="list-style-type: none"> <li>• No model-based system in use</li> </ul>
UK <sup>25</sup>	<ul style="list-style-type: none"> <li>• Visual inspection of the water</li> <li>• Cyanobacteria identification and concentration</li> </ul>	<ul style="list-style-type: none"> <li>• Cyanobacteria identification (microscopic analysis)</li> <li>• Cyanobacterial cell density (Sedgwick-Rafter, or Lund cell)</li> </ul>	<p>Restriction of swimming and other immersion activities: presence of cyanobacterial scum in water or cyanobacterial cell density <math>\geq 2 \times 10^4</math> cells/mL or chlorophyll <i>a</i> <math>\geq 10 \mu\text{g/L}</math></p>	<ul style="list-style-type: none"> <li>• No presence of visible bloom in water</li> <li>• Cyanobacterial cell density <math>&lt;20\,000</math> cells/mL</li> <li>• Chlorophyll <i>a</i> <math>&lt;10 \mu\text{g/L}</math></li> </ul>	<ul style="list-style-type: none"> <li>• Pollution prevention measures to reduce nutrients</li> </ul>	<ul style="list-style-type: none"> <li>• No model-based system in use</li> </ul>

(\*) RADICAL - Risk analysis of direct and indirect climate effects on deep Austrian lake ecosystems. Wanzenböck, J; Kurmayer, H; Grillitsch, B (2013)

<https://www.uibk.ac.at/limno/research/projects/radical/index.html.en> (only in German).

(\*\*) Chlorophyll *a* levels are usually low due to climatic conditions and increase salinity of the sea.

(\*\*\*) <https://watexr.eu/>

<sup>25</sup> At the time of the JRC survey, the United Kingdom was a Member State of the EU

## Conclusions

The intensification of bloom phenomena in recent years is one of the consequences of climate change and the increased anthropogenic pressure in waterbodies. This good practice guide for cyanobacterial and harmful algal bloom detection across Europe, responds to the request of Member States of better knowing the environmental impact of bloom events and the methods for their monitoring and management applied across the EU. The detailed description of protocols for the cyanobacterial and harmful algal bloom detection in fresh and marine waters has been included in this report for the purpose of representing a valid technical support for professionals and authorities working in the fields of water-quality monitoring and management. The conditions contributing to the cyanobacterial and harmful algal blooms along with their effects on human health and the environment are also discussed in order to promote awareness in the public about cyanobacteria and harmful algae. The vigilance and alert levels proposed in this technical report by the JRC to guide the assessment of potentially toxic cyanobacterial blooms have been reported in this guidance together with the values recommended by the World Health Organisation (WHO).

## Abbreviations

AGES	Austrian Agency for Health and Food Safety
AIC	Akaike Information Criterion
ANN	Artificial Neural Network
ASP	Amnesic Shellfish Poisoning
ATP	Adenosine Triphosphate
ATX	Anatoxin-a
AZA	Azaspiracid
AZP	Azaspiracid Shellfish Poisoning
BMAA	$\beta$ -N-Methylamino-L-Alanine
BTX	Brevetoxins
BWD	Bathing Water Directive
CFP	Ciguatera Fish Poisoning
Chla	Chlorophyll <i>a</i>
CMEMS	Copernicus Marine Environment Monitoring Service
CTX	Ciguatoxins
CYN	Cylindrospermopsin
DA	Domoic Acid
DIN	Nitrate, Ammonium, Nitrite
DIP	Dissolved Inorganic Phosphorus
DSP	Diarrheic Shellfish Poisoning
ELISA	Enzyme-Linked Immunosorbent Assay
GC-MS	Gas Chromatography-Mass Spectrometry
GV	Guideline Value
HAB	Harmful Algal Bloom
HAEDAT	Harmful Algae Event Database
HPLC	High-Performance Liquid Chromatography
ISO	International Organisation for Standardisation
LC	Liquid Chromatography
LC/MS	Liquid Chromatography-Mass Spectrometry
LC/MS-MS	Liquid Chromatography tandem Mass Spectrometry
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
LWT	<i>Lyngbya wollei</i> Toxins
MAC	<i>Microcystis aeruginosa</i> complex
MC	Microcystins
MC-LR	Microcystins-Leucine Arginine

MIP	Molecularly Imprinted Polymer
MS	Member States
ND	Nitrates Directive
NMDA	N-Methyl-D-Aspartate
NMR	Nuclear Magnetic Resonance
NSP	Neurotoxic Shellfish Poisoning
OA	Okadaic Acid
OD	Optical Density
PCR	Polymerase Chain Reaction
PITX	Polytoxins
PSP	Paralytic Shellfish Poisoning
RBMP	River Basin Management Plan
SERS	Surface-Enhanced Raman Scattering
SPE	Solid-Phase Extraction
SST	Sea Surface Temperature
STX	Saxitoxin
TLC	Thin Layer Chromatography
UBA	German Environment Agency
UHPLC	Ultrahigh Performance Liquid Chromatography
UWWD	Urban Waste Water Directive
WFD	Water Framework Directive
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
YTX	Yessotoxin

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