WaSAf

Good practices for the monitoring of cyanobacteria and cyanotoxins in freshwater ecosystems

Policy Brief n°3

Good practices for the monitoring of cyanobacteria and cyanotoxins in freshwater ecosystems

Monitoring cyanobacteria in aquatic ecosystems used for the production of drinking water or other activities is difficult to implement due to the heterogeneity in the spatial distribution of cyanobacteria and to the changing population dynamics of these microorganisms. For these reasons, sampling strategies that consider these constraints at a reasonable cost are required to make the monitoring sustainable.

I. Why is it necessary to monitor cyanobacteria?

Proliferations of cyanobacteria occur mainly in freshwater ecosystems enriched with phosphorus and nitrogen (eutrophication process). These blooms are frequently harmful to human populations due to the ability of cyanobacteria to produce various toxins. For this reason, numerous developed countries and few developing countries have implemented monitoring programs of cyanobacteria, particularly in ecosystems used for the production of drinking water. The two main goals of these programs are (i) to improve the management of health risks due to cyanobacteria by limiting the exposure of human populations to cyanotoxins and (ii) to provide data on the mid- and long-term evolution of the ecological and sanitary quality of the water resources.

II. Main challenges in the monitoring of cyanobacteria

The main challenge for the monitoring of cyanobacteria is related to the heterogeneous spatial distribution of cyanobacteria in freshwater ecosystems. Specifically,

- At the horizontal scale, accumulations of cyanobacteria frequently occur in some areas due to the direct effect of wind and/or wind-induced currents (as illustrated in **Figure 1**).
- At the vertical scale, some species accumulate at the surface of the lakes, while others are distributed along the water column. Moreover, the vertical distribution of cyanobacteria can change during the day for some colonial genera (e.g., Microcystis), depending on the size of the colonies, their buoyancy abilities and the turbulence induced by winds.

Because of the high variations in the spatial distribution of cyanobacteria, accurate monitoring of these microorganisms requires sampling at several stations and, depending on the species, performing integrated sampling of the first meter of the water column or samplings at different depths.

As illustrated in **Figure 2**, the temporal evolution of the cyanobacterial abundances and/or biomasses may differ significantly during bloom events. The main consequence of these variations in cyanobacteria population dynamics concerns the frequency at which sampling must be performed. In **Figure 2**, monthly sampling was sufficient to follow *Microcystis* dynamics, while weekly monitoring was necessary to detect the two peaks of biomasses for Aphanizomenon.

Fig 1. Accumulation of cyanobacteria (green area indicated by the arrows) in a freshwater ecosystem (Photography D. Latour, UCA)

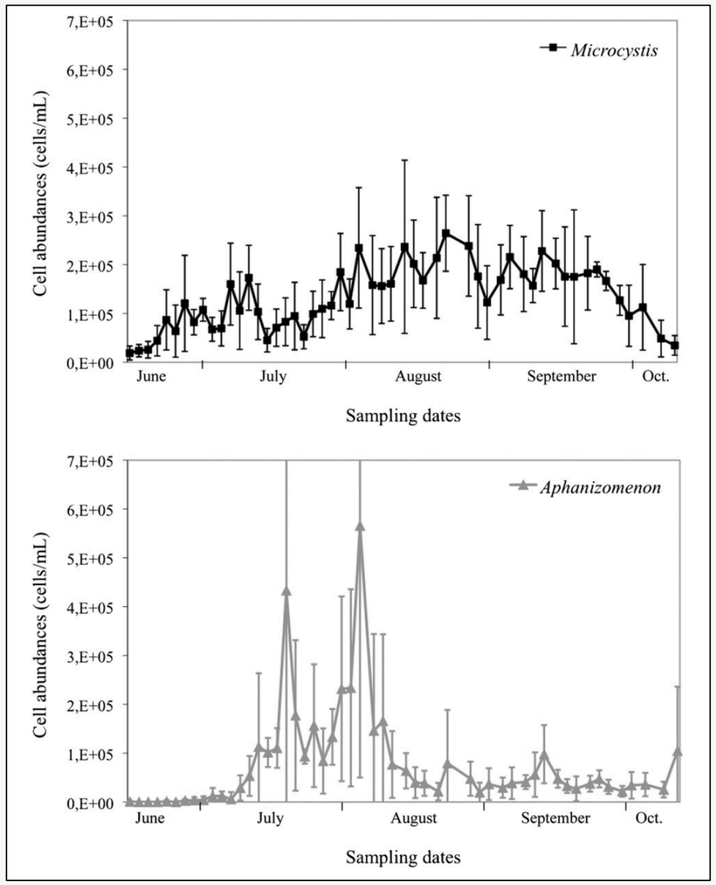

Fig 2. Variations in the cell abundances of two cyanobacterial genera (Microcystis and Aphanizomenon) blooming concurrently in a small French pond. The monitoring was performed in six sampling points every two days, during the summer season (from Pobel et al., 2011)

III. Main challenges in the monitoring of cyanotoxins

The first challenge for the monitoring of cyanotoxins concerns the fact that cyanobacteria can produce a large variety of toxins. The same toxin can be produced by several species/genera, and some species/genera can produce several toxins (see Table 1). Finally, for a species known to be able to potentially produce a given toxin, populations are composed of toxic cells, which are able to produce the toxins (have the gene cluster allowing toxin production), and nontoxic cells, which are not able to produce this toxin (due to loss of the gene cluster).

Consequently, it is not possible to predict the potential toxicity of a bloom just on the basis of the identification and cell concentrations of the dominant cyanobacteria involved in the bloom. For this reason, the most common strategy used for the monitoring of cyanotoxins is to perform a toxin analysis only once the cell abundances or biomasses exceed a defined threshold. The choice of the targeted toxins will be oriented according to the species of cyanobacteria that dominate the proliferation and the available knowledge on the toxins they are potentially able to produce (Table 1).

Table 1. Potential of toxin production by the main bloom-forming freshwater cyanobacteria

Toxins	Main cyanobacterial genera known to be able to produce the toxins	Other cyanobacterial genera known to be able to produce the toxins
Microcystins	*Microcystis*	*Aphanocapsa*, *Merismopedia*, *Radiocystis*, *Woronichinia*
	Planktothrix	*Annamia*, *Geitlerinema*, *Leptolyngbya*, *Limnothrix*, *Kamptonema/Phormidium/Microcoleus*, *Pseudanabaena*, *Spirulina*, *Trichodesmium*, *Plectonema*
	Anabaena	*Anabaenopsis*, *Calothrix*, *Nostoc*, *Trichormus*
	Hapalosiphon	*Fischerella*
Anatoxin-a	*Anabaena*	*Aphanizomenon, Cuspidothrix, Cylindrospermum, Dolichospermum, Raphidiopsis/Cylindrospermopsis*
	Kamptonema/Phormidium/Microcoleus Oscillatoria	*Pseudanabaena*, *Tychonema*
Anatoxin-a(S)	*Dolichospermum* (*Anabaena*)	
Cylindrospermopsins	*Raphidiopsis/Cylindrospermopsis*	*Aphanizomenon*, *Anabaena*, *Raphidiopsis*, *Dolichospermum*, *Chrysosporum*
	Umezakia	
	Kamptonema/*Phormidium*/Microcoleus *Oscillatoria*	*Lyngbya*
Saxitoxins	*Aphanizomenon*	*Anaebaena, Dolichospermum, Raphidiopsis/Cylindrospermopsis,, Cuspidothrix, Raphidiopsis, Scytonema*
	Lyngbya	*Hydrocoleum, Trichodesmium*

IV. Global strategy for implementing a monitoring program of cyanobacteria and their toxins

The first key points in the implementation of a monitoring program are described as follows:

1. The choice of the sampling stations in the ecosystem. As previously explained, sampling at several points is necessary to assess the distribution of cyanobacteria at the scale of the whole ecosystem. Sampling stations have to be generally disposed along a transect oriented on the main axis of the lake. To reduce the number of analyses and consequently their cost, samples can be pooled together before the analyses (integrated estimation). Supplemental water samplings can also be performed near the water intake of drinking water plants or bathing areas.

2. The choice of the sampling frequency. At the minimum, monitoring must be performed with a monthly frequency, but during bloom events, bimonthly or weekly monitoring is recommended.

3. The choice of the material for the water sampling. For most cyanobacterial species/genera, integrated (tubular) sampling in the first meter of the water column is recommended. For some cyanobacteria living in deep ecosystems, the use of a sampling bottle (for example, Van Dorn or Niskin) is required for water sampling at different depths in the water column to obtain the vertical distribution of cyanobacteria.

Particular attention should be given to sample storage and transportation knowing that for the identification and quantification of cyanobacteria, subsamples should be preserved preferentially in Lugol's iodine solution in the dark, while for toxin analyses, live subsamples will be transported in dark, cold conditions and then kept refrigerated before analysis.

After defining the sampling strategy, the second step in preparing the implementation of a monitoring program is the choice of the analyses to be performed on the samples. The most common analyses in this framework are described as follows:

1. The estimation of chlorophyll-a biomass provides an estimation of the total biomass of the phytoplankton community (including cyanobacteria).

2. The identification of cyanobacteria and cell counting with a microscope allows the identification and quantification of the dominant cyanobacteria and potentially the cyanotoxins they can produce.

3. The identification and quantification of the cyanotoxins potentially present in the water samples knowing that various approaches can be used (ELISA test, HPLC, mass spectrometry) depending on the equipment available at the laboratories in charge of these analyses.

Estimation of the cost of a monitoring program in Uganda

Costs of sampling:
- Fuel for the boat (dependent on the sampling strategy)
- Materials for water sampling
o Phytoplankton net (250 €) and bottle for water sampling (type Niskin: 500 €)
o Cooler box: 100 €
o GPS for localization of the sampling sites: 250 €
o Plastic bottles and tubes for water storage, filters for chl-a analyses: dependent on the number of samples and analyses

Costs of water analyses (per water sample):
- Cyanobacterial cell counting: 10 €
-Chlorophyll-a quantification: 10 €
-Cyanotoxin quantification (ELISA): 20 €
- Nutrient analyses (Ptot, Ntot, PO4, NH4, NO3): 10 €

For these analyses, the following equipment are required:
- Inverted microscope: 5,000 €
- Spectrophotometer: 3,500 €
- ELISA plate reader: 2,000 €

Multiparameter probe (type YSI) for measurements in the water column of temperature, conductivity, turbidity, oxygen concentration, pH, chlorophyll-a, and phycocyanin: 15,000 €.

In addition to these costs, the time spent on the field sampling and laboratory analysis must also be taken into account.
- Field sampling: 0.5 to 1 day/ecosystem
- Phytoplankton counting: 2-3 hours per water sample, 1 hour if only cyanobacteria are counted
- Chlorophyll-a quantification: 0.5-1 day for several samples (1 to 30)
- Chemical analyses: 1-3 days for several samples (1 to 30)

In addition to these analyses focused on cyanobacteria and their toxins, monitoring programs on cyanobacteria are generally described as follows:

1. The main nutrient concentrations in the water (total phosphorus and total nitrogen, soluble reactive phosphorus, NH3 and NH4).
2. The measurement of basic physicochemical parameters such as pH, water temperature, water transparency, oxygen concentrations and turbidity.

All these data will improve the understanding of bloom dynamics and, more globally, the physical, chemical and biological functioning of ecosystems experiencing cyanobacterial blooms. However, to ensure that the monitoring will be efficient

and sustainable, the following parameters should be followed:

1. The definition of the skills necessary to perform the monitoring (from water sampling in the field to data recovery) is key to identifying the actors/stakeholders involved in this monitoring. For middle/long-term monitoring, it is recommended to associate scientists working in universities and/or research institutions because they will be able to perform a deep analysis of the data.
2. There is a need for continuity in (i) the sampling strategy, (ii) the methods of analysis and (iii) the operators for the different steps of the monitoring to ensure middle- and long-term comparisons of the data.
3. Validation of the monitoring outputs is very important. For example, it is well known that data provided by sensors must be carefully checked because these devices may generate false results due to deficient calibration or misfunctioning. More globally, all data deviating from the main trend should be carefully checked.
4. Finally, data storage, after their validation, and the rules defining their access and uses (Who? Why? When?) should be described in detail before the beginning of monitoring.

V. New tools for the monitoring of cyanobacteria and their toxins

Alternative methods avoiding water sampling and analyses have been developed in the past 20 years for the monitoring of cyanobacteria. These methods are based on the autofluorescence characteristics of pigment-containing microorganisms (microalgae and cyanobacteria). Submersible fluorescence sensors can rapidly provide a direct quantification of the biomass of microalgae and cyanobacteria. Different kinds of sensors are available depending on the targeted photosynthetic pigment:

1. Many sensors target chlorophyll-a pigment, which is present in all phytoplanktonic microorganisms. They allow estimation of the total biomass (µg/L) of this community (microalgae + cyanobacteria).
2. Other sensors target the phycocyanin pigment, which is only found in cyanobacteria. They permit the estimation of the biomass of cyanobacteria.
3. A few sensors that are able to work at different wavelengths allow estimation of the biomasses of the main groups in the phytoplankton communities (diatoms, dinoflagellates, cyanobacteria, chlorophytes, cryptophytes).

The main interest of all these sensors is to provide a direct estimate of biomasses in water. They allow to perform, in a short time, vertical profiles of the phytoplankton distribution in the water column and multipoint estimates of the horizontal distribution of the targeted microorganisms in a lake.

These tools have many limitations, which may lead to incorrect and misleading estimations. The main limitations are (i) that they must be subject to regular maintenance and to more or less frequent calibrations depending on the device, and (ii) that they tend to underestimate the biomasses of certain genera of cyanobacteria (for example, Microcystis). For these reasons, it is recommended to use them as additional tools for monitoring based on laboratory analyses of water samples.

In recent years, the use of remote sensing techniques for the monitoring of cyanobacteria has become easier to implement (Figure 3). This approach is particularly useful for large lakes where assessment of the spatial distribution of cyanobacteria is time-consuming and costly.

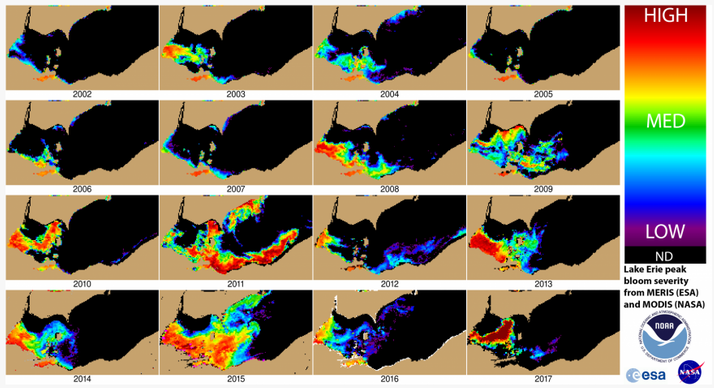

Fig 3. Lake Erie peak bloom severity between 2002 and 2017. Images from MERIS (ESA) and MODIS (Nasa). Image Credit: R. Stumpf, NOAA

To date, there has been no example of cyanobacteria monitoring based on the use of remote sensing approaches for the management of health risks linked to cyanobacterial blooms. In addition, there is no suitable satellite instrument that can accurately monitor cyanobacterial blooms in small inland waters. However, long-term monitoring programs of cyanobacteria by remote sensing approaches have been performed on several large lakes (e.g., Lake Taihu in China; Lake Erie in USA) for scientific purposes with the goal of better understanding the temporal and spatial dynamics of cyanobacterial blooms.

Finally, concerning assessment of the potential toxicity of a cyanobacterial bloom, molecular methods based on qPCR have been developed in the past ten years. These tools allow quantification of the number of copies of various genes involved in the biosynthesis of toxins in a water sample. These data are potentially interesting to obtain at the beginning of the blooms because they allow the identification of cyanotoxins that may be produced during the bloom, but they cannot replace a classical quantification of cyanotoxins based on HPLC, mass spectrometry or ELISA approaches.

VI. Citizen participation in the monitoring of cyanobacteria

Recently, we tested the monitoring of cyanobacteria in freshwater ecosystems through the development of citizen science projects involving the population living around targeted freshwater ecosystems. A first pilot study was performed in the framework of the WaSAf program at the Ivory Coast. Based on the use of a smartphone application, the citizens of the three villages were invited to report changes in water color, as these changes could reflect cyanobacteria proliferation. After a two-year experimentation period, this strategy showed that it was possible to mobilize the local populations to monitor cyanobacterial blooms. The data collected by citizens were consistent with the data obtained by classical monitoring of cyanobacteria. This participatory approach also provided great improvements in the understanding and awareness of local populations regarding water quality issues and cyanobacterial blooms.

Finally, the greatest challenge of the citizen-based monitoring of cyanobacteria, like all participatory approaches, is sustainability, knowing that this issue will be handled differently in developed and developing countries. In developed countries that already implement institutional monitoring of cyanobacteria, the challenge of the sustainability of citizen monitoring is to show how it can contribute to improving the institutional monitoring of cyanobacteria to legitimize its contribution. For most developing countries that lack existing institutional monitoring, the challenge is to coordinate the joint implementation of these two approaches (institutional and citizen) to maximize their efficiency and to minimize the overall monitoring costs, which is a key point for sustainability.

Redactors of this Policy Brief:

JF Humbert, Directeur de Recherche INRAE, iEES Paris, Sorbonne Université, Paris, France (jean-francois.humbert@upmc.fr)
W. Okello, Researcher, NaFIRRI, Jinja, Uganda (wiokello@yahoo.com)
M. Olokotum, Researcher, NaFIRRI, Jinja, Uganda (markolokotum@yahoo.com)
C. Bernard, Professeur, MNHN-UMR MACAM, Paris France (cecile.bernard@mnhn.fr)

For more information:

In French

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