

Cyanobacteria, cyanotoxins and potential health hazards in small tropical reservoirs

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Scope: questions/ challenges the tool addresses

Cyanobacteria, photosynthetic prokaryotes also called blue-green algae, are said to have been the first oxygen releasing organisms of our planet, two and a half billion years ago. As such, they created the atmosphere that further allowed the development of life. Today, both the beneficial and detrimental features of the cyanobacteria are of considerable significance. The nitrogen-fixing species contribute globally to soil and water fertility. They are also important primary producers and their general nutritive value is high (e.g. *Spirulina*).

Cyanobacteria may however be a source of considerable nuisance in many situations, particularly when proliferating as ultimate state of the eutrophication process in water masses. The building of dams and regulation of rivers has created more habitats suitable for cyanobacteria. The general opinion now is that “cyanobacterial blooms” are increasing in frequency worldwide.

There is also a growing concern related to the development of toxic cyanobacterial populations. Twenty genera and more than 40 species of cyanobacteria are known for their potential toxicity. The cyanotoxins involved are categorized according to their chemical structure and their pathological impacts, e.g. dermatotoxins, hepatotoxins, cytotoxins, and neurotoxins. Exposure to hepatotoxins (microcystins, nodularins and cylindrospermopsins) has been reported to induce several health disorders depending on the route of exposure, the quantities absorbed and the toxicity of the cyanobacterial strain. Harmfulness ranges from minor disorders (headaches, nausea, diarrheas) to lethal deterioration of hepatic functions. It is also thought that chronic exposure to low concentrations can promote liver cancer. In 1996, 60 patients died in Brazil after haemodialysis with contaminated water (Pouria et al. 1998). WHO considers that freshwater contamination by cyanobacteria, and the toxins they synthesize, constitutes a major worldwide threat that can limit utilization of water resources (Chorus & Bartram 1999). A

threshold of $1\mu\text{g eq MC-LR L}^{-1}$ (Microcystin-LR, the most toxic and widespread hepatotoxin) is indicated for drinking water: higher thresholds are allowed for recreational water and other uses (Chorus & Bartram 1999).

Cyanobacterial growth is constrained by low levels of light, temperature, and nutrients. In tropical areas, the first two of these are rarely limiting so nutrient availability is usually the key determinant of their proliferation (Dufour et al. 2006). Hydrological disturbances often appear as initiating factors (Arfi et al. 2001). Anthropogenic factors, particularly the use of pesticides, are also suspected to facilitate cyanobacterial growth (Ma 2005, Lürling & Roessink 2006).

Cyanobacteria are not always visible on the water surface. Generally, their visibility increases when present in large numbers in a particular area (Figure 1). Visibility typically takes the form of a blue-green coloration in the water and/or at the surface. (Color may vary from olive-green to red.) Eventually, foam can form on the water surface. High concentrations of algae and toxins are often found on the shoreline: this is where most contact with water users takes place.



Figure 1: *Microcystis* bloom (April 2004, Burkina Faso, Céline Berger)

Cyanobacteria issues have been addressed in many developed countries, but the impact of algal proliferation remains largely neglected in developing nations. The main causes for this are lack of expertise and inefficiency of monitoring programs – when they exist at all. For Africa, less than 20 references are currently available (Figure 2).

The tool documents the situation as recently observed in Burkina Faso, in the Volta Basin. It aims to contribute to a better understanding of this issue in tropical basins of developing countries. It is of particular importance where goods and services are associated with freshwater resources, especially small reservoirs.

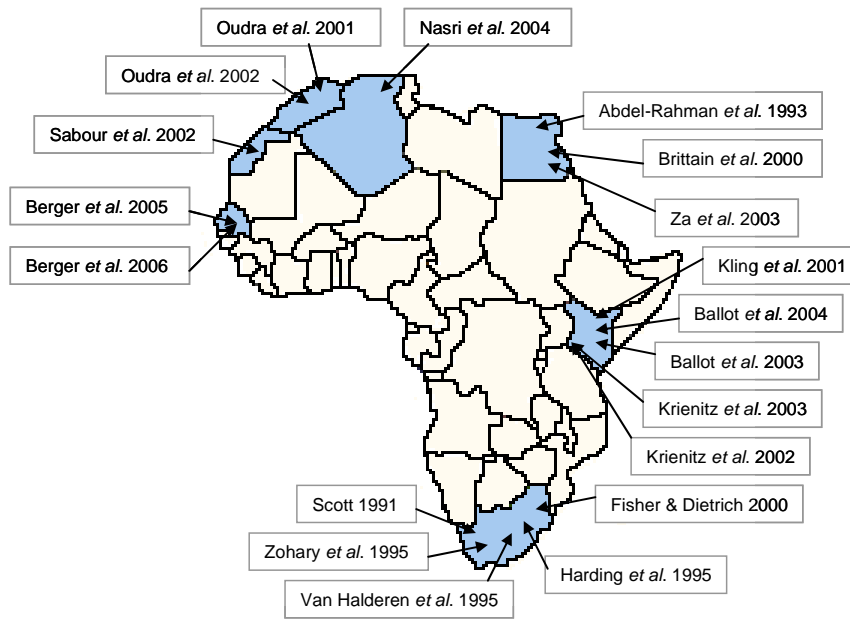


Figure 2: Reports of cyanotoxins in Africa

Target group of the tool

Planners and managers of small multi-purpose reservoirs, and local scientists

Requirements for tool application

There are two main types of methods for detection of cyanobacterial hazards: those focused on cyanobacteria and those focused on cyanotoxins. The latter are implemented when algal proliferation represents a recognized threat, for example, when shellfish in coastal areas may be contaminated. Information collection and dissemination are fundamental. Users of small reservoirs should be provided with basic and practical knowledge (*“How to recognize an algal bloom?”* *“What to do – or not do – in such case?”*). In many developed countries, web sites provide such information. Booklets, movies, radio and television can also be used to disseminate knowledge.

Local scientific communities need to provide regular, validated data. This situation is particularly critical in West African countries, where specialists are rare and scattered. Logistical constraints are often formidable and international partnerships may be required to conduct regular scientific work. Accumulated limnological information on water bodies is needed for a comprehensive assessment of their functioning. Such background information enables a suitable targeting of critical sites and periods in long-term monitoring programs (Codd *et al.* 1999). International partnerships can contribute to focused, short term studies that culminate in consistent monitoring strategies driven by national political and economic issues. The private sector may also play a role, for example, with regard to water quality issues associated with treated tap water production.

Fundamental questions regarding harmful cyanobacterial blooms often cannot be readily answered: *“Where will a bloom take place? Which species will be involved? Which toxins will be produced?”* Prevention is fundamental. When toxicological expertise is not locally available,

detection of cyanobacteria becomes paramount. Cyanotoxin analyses may be conducted in due course, but such analyses are most appropriate when a cyanobacterial threat has previously been clearly identified.

This approach has its limits. Cyanobacterial blooms are not systematically toxic, even if potentially harmful taxa are involved (Carmichael 2007). In addition, toxic metabolites can remain active in the water long after the disappearance of their cyanobacteria, so programs that only monitor algae are not entirely adequate. Ideally, both methods should be implemented simultaneously to efficiently forecast toxic cyanobacterial events and immediately implement remedial measures. In many developing countries, however, this is not feasible because of a lack of economic resources and technical expertise.

The emphasis in this tool is on cyanobacteria detection. Required material ranges from relatively simple inverse microscopes to rather sophisticated equipment such as fluorescent printers, chromatographers, multi-parameter probes, fluorometers and specific software, depending on the methods selected.

Tool: description and application

In Burkina Faso, man-made lakes and small reservoirs (there more than 1,500) play a fundamental role in harvesting water used for drinking by urban and rural populations (see the separate tool on creating an atlas of lakes and reservoirs in Burkina Faso). Conserving water resources and maintaining their purity are national strategic issues. However, the health hazards associated with potentially toxic cyanobacterial proliferation remain largely unrecognized. There have been relatively few studies in this country on phytoplankton populations or cyanobacteria.

In 2004, however, a large field survey was done to sample aquatic cyanobacterial communities in 23 reservoirs scattered across the country (Cecchi et al. 2005). Strains were cultivated to further analyze their toxic profiles at the Museum National d'Histoire Naturelle in Paris (Gugger et al. *in prep*). In following sections, alternative methods for cyanobacteria monitoring are discussed, and the methods used in the Burkina Faso study, and the results of this study, are presented.

Methodologies for Cyanobacteria monitoring

A first step in monitoring can be based solely on visual information provided by volunteers with minimal education. However, the visual detection of blooms often provides information *after* the occurrence of the phenomenon and should be considered more as an alarm than as a monitoring tool.

Taxonomic composition and specific abundances of phytoplankton can be analyzed with an inverse microscope. This standardized method is based on morphological traits of organisms and allows the detection of cyanobacteria before the appearance of blooms. Chorus and Bartram (1999) defined two key threshold levels: a limit of 2,000 cyanobacterial cells.mL⁻¹ for tap water and 100,000 cells.mL⁻¹ as maximum for recreation water.

Quicker screening methods can also be applied, as practiced by the CEAEQ (Centre d'Expertise en Analyse Environnementale du Québec): four main cyanobacterial genera are identified and described in ten classes of relative abundances. Routinely performed in about 75 minutes, this method can provide rapid information useful in taking decisions on water management.

Epifluorescence microscopy (Andersen and Thondsen 2003) allows the detection of low concentrations (10^2 to 10^4 cells.L⁻¹) of cyanobacteria when using fluorescent printers as orange acridin and DAPI.

The knowledge of microscopy and taxonomy required for unambiguous identification is often lacking in most organizations responsible for surveys. Therefore, alternative methods have been developed. The combination of flow cytometry and epifluorescence allows assessment of the size and optical differentiation of phytoplankton populations (Jeffrey 1997). Robust and particularly useful for the discrimination of sub-populations, this method is time-consuming and is difficult to implement for routine purposes owing to the cost of the instrument and the need for skilled personnel to perform the analysis.

Molecular fingerprinting is often proposed as an alternative method for identifying potentially toxic species, but it also involves delays, and requires adequately trained personnel and the discrete sampling of water. The same limitations arise when Liquid Chromatography (which allows the identification of a large panel of pigments) is used in conjunction with software such as CHEMTAX so that the specific biomass of phytoplankton classes including cyanobacteria can be inferred.

Fluorescence properties of phytoplankton are currently used as monitoring tools. Based on the optical properties of their pigments, several methods allow the determination of biomass and the distinction between different groups of organisms. The *in vivo* fluorescence characteristic of pigment-containing microorganisms, such as cyanobacteria and microalgae, thus offers attractive possibilities (Leboulanger et al. 2002, Gregor et al. 2007, this work). *In vivo* fluorescence can be measured on individual samples, with flow-through fluorimeters, *in situ* or remote-sensed. Different materials and instruments are available, with huge variations in their price and sensitivity. Whichever method used, systematic taxonomic (microscope) validations are required.

In situ multi-parameter probes, including *in vivo* fluorescence, constitute today the ideal compromise for rapid and efficient surveys or monitoring. However, their use requires substantial funds (including but not limited to field facilities such as cars, boats, sampling equipment, reagents, calibration, routine maintenance, and laboratory facilities for taxonomic validations).

Results from Burkina Faso

Cyanobacteria are obviously present in most of the reservoirs studied, particularly those in the Volta Basin (blue color dominant on histograms, Figure 3). Its Nakambé sub-basin is particularly affected, also revealing serious erosion in both abundance and diversity of zooplankton populations within related ecosystems (Cecchi et al. 2005).

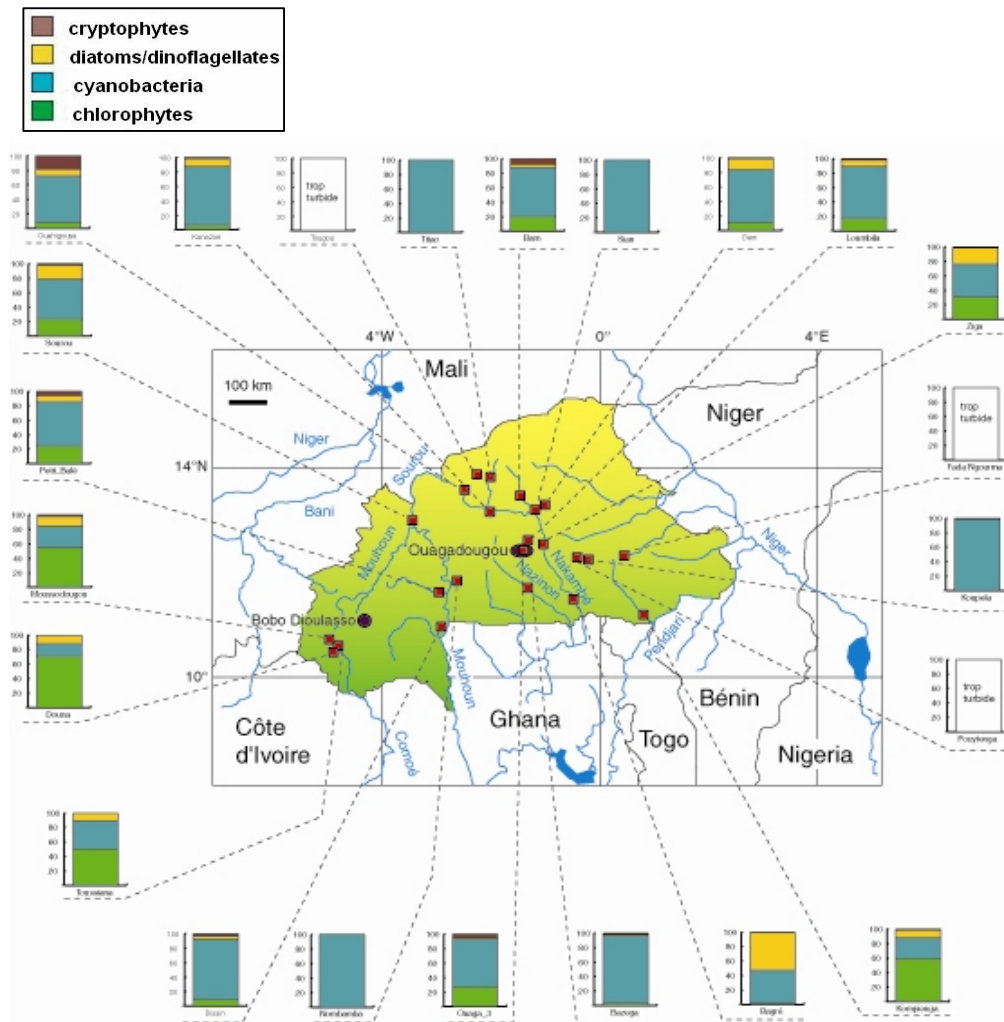


Figure 3: Taxonomic composition of phytoplankton within the 23 lakes sampled in Burkina Faso in 2004. Colors correspond to the contributions (%) of the 4 main taxonomical groups discriminated *in situ* in using an *in vivo* fluorometer (bbe Fluoroprobe moldaenke©). Excessive turbidity (white histograms) was related to a mixture of inorganic particles and tremendous cyanobacteria in 2 on the 3 cases involved.

During taxonomic analysis, 70 morphotypes belonging to three cyanobacterial orders were found: cyanobacteria were identified in all except one sampling site (Gugger et al. *in. prep.*). Among the Chroococcales, nine genera (30 different species) represented by one to nine different species were present at each site. *Microcystis* in particular showed five different species observed at 21 sites. Among the Oscillatoriales (27 different species), ten genera illustrated by one to six different species were identified. Cyanobacteria showing the presence of differentiated cells (as heterocysts) were poorly diversified (eight species only) and all belonged to the Nostocales order. This order was present at only 18 sites and no Nostocales were found in the downstream lakes in the Nakambé catchment area.

Other phytoplankton was observed in most of the surface samples of the 23 lakes. The Chlorophyta with 35 different genera were represented at all except one site. Among the Heterokontophyta, the class of the Bacillariophyceae was the most diverse with seven genera found in 21 lakes, but the Chrysophyceae and the Xanthophyceae was poorly represented with

four and three genera respectively. The four genera of the Euglenophyta were found in 20 lakes, particularly one site with *Trachelomonas* as unique algae identified. The Dinophyta were represented by three genera in only 14 of the sampled lakes.

Nine lakes were sampled for cyanotoxin analyses, after observation of important biomass in the field, and further analyzed using an improved version of the method developed by Gugger et al. (2005). Mass spectrometry screening (Figure 4) of lyophilized samples revealed that two samples contained microcystin (MC).

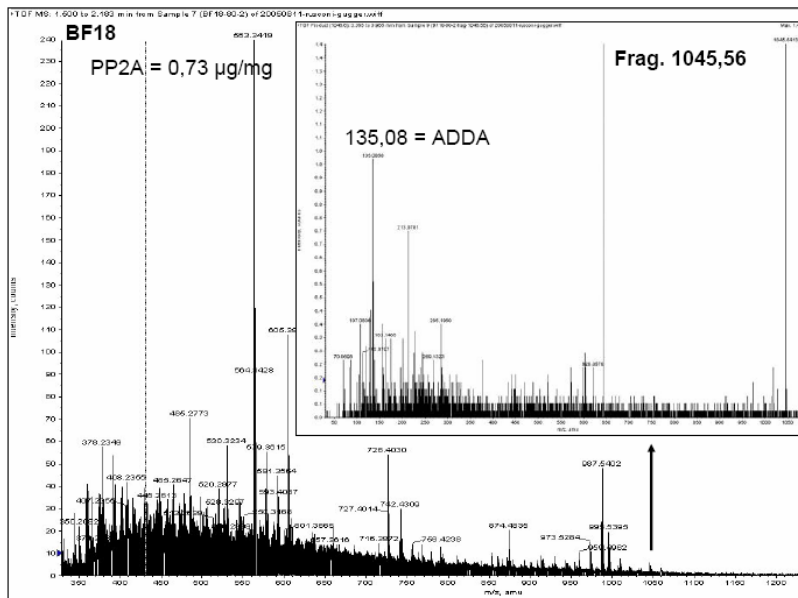


Figure 4: Example of Microcystin screening using Mass spectrometry. Cyanotoxin content quantified using PPEA bioassay is indicated.

Confirmation of the presence of Microcystin and their quantification in these two samples (namely from Nombamba and Dissin lakes) were performed with PP2A inhibition assay (Briand et al. 2002) which revealed 0.55 and 0.73 μg total MC per mg of lyophilized samples respectively. Both correspond to important biomass (see Figure 5). However, strains known to be potentially toxic were identified in almost all samples.



Figure 5: Sample (mesh size of the net: 20 μm) collected in the Nombamba Reservoir (Mouhoun, April 2004, Céline Berger). Microcystin contents exceeded the recommended thresholds in this sample.

This summary of the studies in Burkina Faso highlights the importance of cyanobacteria within the plankton communities of almost all reservoirs sampled. Even if "only" two cases of significant toxicity were confirmed, the dominance of these taxa in a large majority of reservoirs should alert water managers, and should be kept in mind when additional small reservoirs are developed. Indeed, such high levels of cyanobacteria dominance have never been observed before by the scientific staff involved in the survey in Burkina Faso. Although the determining mechanisms remain to be fully understood, the situation is unambiguous and alarming. Perennial specific monitoring networks have been implemented in countries where risks appear serious (e.g. different states in the USA and in Australia). Such initiatives should not be limited to countries where both economic resources and technical expertise are readily available.

Lessons learned and perspectives

Cyanobacteria were present in almost all of the reservoirs studied in Burkina Faso. Luckily, even with potentially toxic taxa present everywhere, toxic episodes were rare at the time the samples were collected (middle of the dry season). The situation of water bodies in the Nakambé basin appears particularly intriguing, revealing several forms of metabolic disorders (such as impressive cyanobacterial dominance, alteration of zooplankton communities). Classical limnological proxies, alone or in combination, failed to explain the observed situations. Interactions between catchment land use, reservoir use and ecosystem properties are currently being explored.

Convergence of field observations and preliminary experimental assays tends to highlight the very beginning of the rainy season as the most likely time for cyanobacterial proliferation. Nutrient enrichment and simultaneous increases in particle loading of water masses, both associated with runoff and/or rains, may stimulate primary planktonic producers, and favor certain cyanobacteria owing to their physiological fitness. Agricultural intensification and particularly horticulture developing actively around most of reservoirs are known to require important amounts of inputs (fertilizers and pesticides) and are, therefore, also suspected. These scientific hypotheses remain to be formally assessed.

Recommendations

Direct consumption of surface water is undoubtedly a risky kind of behavior (See a separate tool on water quality). When hydrological conditions are favorable, increased nutrient levels result in increased phytoplankton biomass (reflected by chlorophyll and cell concentrations). Eventually, cell numbers may reach "bloom" levels, which can be detrimental for ecosystems and water treatment processes. If cyanobacteria reappear frequently in the same area, the following actions are recommended:

- Avoid all direct contact with the water, e.g., swimming and aquatic activities
- Do not drink the water
- Do not use it to prepare or cook food (boiling the water will not eliminate the toxins)
- Avoid consuming fish or other aquatic species taken from the affected area

- Do not let animals drink or bathe in the water
- Do not use algicides to destroy cyanobacteria (toxins are released more massively when cells die)
- Be informed that toxins can persist after cyanobacteria have disappeared.

Be aware that there are no recognized methods to effectively eliminate cyanobacteria from water masses. Watershed management and nutrient control remain the only options to reduce risks. The problem is best addressed by reducing sources of phosphorous and nitrogen in water, for example, by reducing fertilizer and manure use, and by eliminating wastewater discharge. Most additional nutrients are supplied to water bodies from surface run-off, particularly during high rainfall events. Concentrations of soluble reactive phosphorus less than 0.01 mg.L^{-1} are considered to be limiting for growth and a concentration of 0.1 mg.L^{-1} of soluble inorganic nitrogen is considered the minimum concentration to maintain growth during the growing season.

Catchment management practices to reduce nutrients loading include:

- A reduction in nutrient (fertilizer) application to reduce nutrient inputs
- Development and application of appropriate farming practices to reduce surface run-off, soil erosion, and nutrient leaching, for example, in irrigated vegetable production near small reservoirs.
- Rehabilitation of riparian vegetation, known as buffer zones, to increase nutrient utilization before polluted or “nutrient-enriched” water enters the reservoir. Coincidentally, this also helps to reduce the risk of contamination with *Cryptosporidium* (Atwill et al. 2002).
- Rehabilitation of aquatic vegetation to compete with phytoplankton for nutrients.
- Livestock management to reduce shore line erosion and pollution of the water with animal wastes (e.g. by constructing cattle troughs away from the reservoir).

These strategies are most effective when used in combination.

Limitations of the tool

Information at different levels, economic resources and technical expertise appear to be key issues: absence of only one of them could preclude any kind of attention devoted to cyanobacteria and their potentially harmful consequences.

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WHO web site: http://www.who.int/water_sanitation_health/en/

http://www.who.int/water_sanitation_health/resourcesquality/toxicyanbact/en/index.html

CyanoNet web site: <http://www.cyanonet.org/>

Cyanosite: <http://www-cyanosite.bio.purdue.edu/>