Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management
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Chapter 11. FIELDWORK: SITE INSPECTION AND SAMPLING

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Fieldwork, including site inspection, sample collection and, in most programmes, some on-site analysis, determines to a large extent the quality of information obtained and represents a significant proportion of the total cost of a cyanobacteria or cyanotoxin monitoring programme. A well-designed and implemented fieldwork programme enhances the quality of the data obtained and may assist greatly in containing overall costs. The importance of careful programme design has been emphasised in Chapter 10, together with the need for pilot testing to refine programmes and to assess logistics.

Properly trained field workers are the backbone of effective sampling and inspection programmes; aspects of their professional development and training are described in Chapter 7. Inclusion of members of the public as active participants in monitoring programmes is rapidly gaining acceptance and can contribute significantly to the quantity and quality of information obtained from a monitoring programme. Special interest groups (such as non-governmental organisations and user associations) and also concerned local persons in sensitive or affected areas can provide useful information. Proper orientation and training, for example in site inspection, in flexible responses to the results of inspections, and in conducting basic tests such as transparency, can assist in providing valuable additional data.

Further information concerning planning and performing fieldwork is presented in *Water Quality Monitoring* (Bartram and Ballance, 1996), a companion volume in this series.

11.1 Planning for fieldwork

The principal components of effective planning for fieldwork comprise:

- Timing and preparation (in the context of the monitoring programme plan and information needs for management).

- Prior liaison with the laboratory that will receive and process samples.

- Logistic preparation.

- Local co-ordination as required.

- Prior liaison with other information recipients (local or central).

Preparation of the monitoring programme principally concerns the timing of visits and careful cataloguing of sampling sites. The timing of sampling and inspection visits is described in Chapter 6.

Before routine field visits are performed, a period of pilot testing should be implemented. This will help ensure that time requirements for inspection and sampling are reconciled, and that activities are planned to make the best use of staff time and other resources (e.g. vehicles). Realistic estimation of travelling time is important to avoid exceeding allowable sample storage times prior to analysis. Pilot testing should lead to the development of a detailed inventory and description of sampling sites. If changes in water quality with time are to be interpreted with confidence, samples must be taken consistently from the same locations and/or from precisely identified locations. Pilot testing also provides an opportunity for training personnel and allows their familiarisation with particular aspects of the monitoring programme itself.

Co-ordination with the laboratory is an important aspect in determining the final value of the sampling expedition. In some cases, the laboratory will be responsible for preparation of sample containers and chemical additives for sample preservation, and may also be responsible for the provision and maintenance of equipment for on-site testing.

Laboratory capacity is an important area of concern which should be addressed in programme design and in pilot testing. It is essential that the workload generated by a sampling expedition is properly managed within the laboratory; it is therefore vital that analysts know how many samples will be arriving, the approximate time of arrival and the analyses that are to be carried out. Excessive delays before sample processing and analysis may render the sample results invalid (and thereby useless) for the management purposes for which they had been collected. Therefore, the timing of sample delivery to the laboratory and the workload management within the laboratory should be co-ordinated prior to fieldwork.

Good logistical preparation prior to fieldwork requires that equipment is checked to ensure that it is functioning properly (e.g. electrodes tested and calibrated, batteries charged). The correct number, size and type of sample containers must be prepared and transport must be arranged, ensuring permission and local access to any restricted sites. It is essential to prepare a sampling checklist which includes maps of sampling site locations, a list of equipment required and a detailed explanation of the methods for sample collection. The checklist should also include lists of the types and numbers of samples to be taken at each site, as well as of the required volumes. Good preparation for sampling involves previous labelling of sampling containers with at least the site, date and depth from which the sample is to be taken.

11.1.2 Frequency of field visits

The frequency of site inspection and sampling must be adapted to the local situation as described in Chapter 10. Key criteria are:

• Potable water supply reservoirs may need to be monitored regularly throughout the year if perennial persistence of cyanobacteria cannot be ruled out. This applies particularly to warm climates and in temperate zones to water bodies populated by certain taxa such as *Planktothrix.*

• Monitoring of recreational lakes in tropical countries may cover the whole year, while in temperate zones it can be focused on the warm season from early summer to autumn.

• The frequency of site inspection and sampling should be increased during development of cyanobacterial populations or when persistent blooms occur.

11.1.3 Safety

Caution and attention are appropriate while working with cyanobacteria, particularly when they are highly concentrated in scums. It is wise to treat all blooms as highly toxic. Contact with water should be minimised during sampling and gloves and rubber boots should be worn because cyanobacteria might contain toxins and could also have a high allergenic potential.

In some areas of the world other water-based hazards such as schistosomes (the cause of schistosomiasis or bilharzia) may also be present. In such circumstances water contact should be minimised and following contact the skin should be vigorously dried.

Caution during fieldwork should also apply during the use of boats or other vessels and whilst wading, especially in waters with low transparency where underwater hazards may not be readily visible.

11.2 Site inspection

Sites used for drinking water abstraction or recreation should be subject to programmed inspection by trained professional staff, and preferably in conjunction with sampling expeditions. Careful inspection can assist interpretation of results from sample analysis. Moreover the development of personal expertise in relation to specific water bodies can provide the best form of early warning system.

A protocol for site inspection should be established (see Box 11.1) that includes the ambient data of temperature, wind and other weather conditions as well as an estimate of the situation during the previous 24 hours.

High nutrient input from, for example sewage outlets or run-off from excessively fertilised areas favours the development of cyanobacteria (Chapter 8). Site inspection protocols should also address the identification of sources of nutrient input and significant land uses, as well as land use changes that may assist in the interpretation of findings. Such assessment is not necessary during every sampling tour but is particularly recommended during the pilot phase, and at moderate intervals, such as annually, when changes in the catchment area are suspected.

Box 11.1 Example protocol for site inspection and follow-up

1. Note ambient and weather conditions and, if possible, also those conditions during the past 24 hours, especially wind direction and velocity.

2. Assess the areas most likely to be affected by cyanobacterial blooms first, i.e. the downwind shores.

3. Determine if:

- The bottom of the lake is clearly visible at approximately 30 cm depth along the shore line.

- Note any distinct green or blue-green discolouration of the water; if a Secchi disc is available, note the transparency.

- Note if cyanobacteria can be seen as green or blue-green streaks on the surface, or as accumulations in bays and along shorelines.

- Note whether green or blue-green scums affect large parts of the water surface.

4. If cyanobacteria are present (according to Item 3 above):
- Initiate monitoring, if not already in place.

- Initiate an inspection of the catchment area for sources of nutrient inputs.

- Initiate temporary intensification of monitoring, if necessary, for safeguarding healthy use for drinking water or recreation (see Chapter 10).

5. If heavy blooms or scums are observed, immediately:
- Inform other parties concerned (water suppliers, health authorities, operators of recreational sites).

- Inform public and consider posting warning notices at bathing sites, and intervening against use for water contact sports (see sections 5.2.2 and 6.2.2).

6. If cyanobacteria or dense algal growth is a problem, check whether nutrient pollution sources are apparent, or whether a specific catchment inspection tour should be initiated (see Chapter 8).

When scums appear on the water surface, cyanobacteria may be present in densities hazardous to human health, and thus appropriate responses should be initiated quickly (see sections 11.3 and 6.4) and samples for further analysis should be taken. Sampling of scums outside designated or habitual bathing sites is also of great value for determining and predicting further risk, e.g. for when wind directions change during the following days.

11.3 Sampling

Sampling may address both cyanobacterial population development and hazardous accumulations. Informed on-site decision-making is necessary to refine sampling

programmes. Samples addressing population development as a basis for assessing the potential for hazardous concentrations are usually taken at one or several points in the water body. These points should be representative for the whole water body and are often where it is deepest. For assessing hazards, samples must also be taken in areas where accumulations of cyanobacteria can affect both humans and livestock, or they should be taken at the raw water intake of drinking water reservoirs. The potential for spatial heterogeneity demonstrated by some species (see section 2.2), i.e. horizontal and vertical variations in both cell numbers and toxin content, must also be considered when selecting the number and location of sampling sites.

Sample collection should always be accompanied by a site inspection (see section 11.2) because the data obtained during the inspection will be important in the interpretation of the results of sampling.

Sample collection and storage procedures differ depending on the type of analysis which will be carried out. The three principal categories of analysis usually performed are:

- Nutrient analyses (phosphorus and nitrogen).
- Cyanobacterial identification and quantification.
- Cyanotoxin analysis, e.g. toxicity testing and analysis of cell-bound and dissolved toxin.

11.3.1 Sample containers

Containers and bottles for the transport of samples should ideally be provided by the laboratory that will conduct the analyses. This helps to ensure that they are of a suitable volume, are properly prepared and that due consideration has been given to the need for pre-treatment and chemical addition. Field work is easier if bottles are pre-labelled and well-arranged in suitable containers (in climates where storage in insulated containers is not necessary, soft-drinks crates with subdivisions for each bottle are cheap and practical). For routine sampling of the same sites, it is advisable to always use the same bottle for each site and each parameter. This avoids cross-contamination, which is a particular concern for phosphorus analyses. For most samples, glass bottles are appropriate but often plastic containers can be used that are considerably lighter and unbreakable. It should be decided in advance whether it is more practical to subdivide a water sample into aliquots for each analysis prior to transportation, or whether a single sample can be divided on receipt in the laboratory. The following containers are recommended for the transport of cyanobacteria and related samples:

- *Phosphorus analysis.* Use 100 ml glass bottles pre-washed with and stored containing sulphuric acid (4.5 mol l^{-1}) or hydrochloric acid until usage. Phosphate is indicative of the potential for cyanobacterial growth even when it is at very low concentrations (µg l^{-1}) and, therefore, special care must be taken to avoid contamination of samples. Contamination may arise from phosphate-containing detergents or from previous storage of samples with very high phosphate concentrations. Phosphates are easily adsorbed to glass surfaces and may be released later when the bottle is filled with a new sample with low concentration (see Chapter 12).

- *Nitrate, ammonia and total nitrogen.* Use clean 100 ml glass or polyethylene bottles.

- *Microscopic identification of cyanobacteria.* Wide-mouth polyethylene bottles are appropriate for studying living material in a fresh grab or net sample (see below).

- *Microscopic identification and quantification of cyanobacteria.* Brown glass bottles (100 ml) are preferable. These should already have about 1 ml of Lugol's iodine or formaldehyde solution added, or the preservative may be added immediately after filling the bottles with the sample (see below). Clear glass bottles may be used but must be stored in the dark because sunlight destroys iodine, thereby allowing the sample to degrade.

- *Cell material for analysis of cell-bound toxin concentrations.* Use 1 litre (minimum volume) containers, preferably made of glass although good results have also been obtained with plastic. Containers should be pre-cleaned to prevent contamination.

- *Cell material in large amounts (usually enriched with a plankton net) for structural identification or some toxicity assays.* Use plastic containers with wide necks to facilitate filling. If samples are to be freeze-dried, the sample must be frozen in a layer not thicker than 1-2 cm. Specimen containers (100 ml) for urine samples (easily obtained from medical suppliers) are particularly suitable. Well-sealed, heavy-duty household plastic bags can also be used, but care must be taken to avoid puncture because highly concentrated cyanobacterial material can present a safety hazard. Bags should be placed in watertight ice boxes for cool storage and as a precaution against leakage.

- *Dissolved cyanotoxins.* Use 1 litre (minimum volume) containers, preferably made of glass although good results have also been obtained with plastic. Containers should be pre-cleaned to prevent contamination.

- *Chlorophyll* a *analysis.* Brown glass bottles of 1 litre capacity are recommended to avoid degradation of chlorophyll by sunlight. Clear bottles may be used if the samples can be stored in the dark.

Preparation of Lugol's iodine solution for preserving phytoplankton samples Dissolve 20 g of potassium iodide in 200 ml of distilled water, mix thoroughly and add 10 g of sublimated iodine. The solution must not be supersaturated with iodine because this can result in crystal formation which interferes with cell counting. Supersaturation can be tested by diluting 1 ml of stock solution to 100 ml with distilled water to give concentrations similar to those used for preserving samples. If iodine crystals appear after standing, more potassium iodide (approximately 5 g) should be added and the test repeated. If no crystals appear, 20 ml of glacial acetic acid is added. Use about 1 ml of Lugol's iodine to preserve 100 ml of phytoplankton sample. The resultant sample should be the colour of whisky).

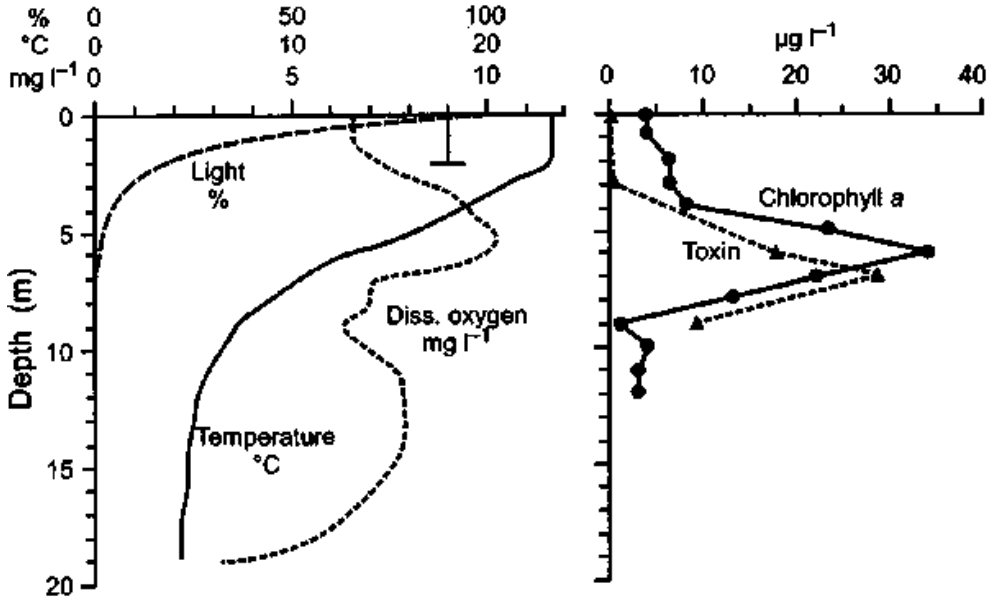
Dense scum samples may rapidly consume the iodine from the Lugol's solution. If samples lose the whisky colour, and particularly if they no longer smell of iodine, they are likely to decay. Samples stored for more than 10 weeks should be periodically checked for sufficient preservation; further drops of Lugol's solution may be added if necessary. If substantially more than 1 ml per 100 ml are required, the volume of Lugol's solution added to the sample should be determined because the sample volume will have changed appreciably and a corrective calculation is required for the cell counts.

11.3.2 Types of sample

Two different types of sample can be taken from lakes or similar surface waters. The simplest, a "grab" sample, is a discrete volume of water taken at a selected location, depth and time. Grab samples are also known as "spot" or "snap" samples (see Bartram and Ballance (1996) for different sampling devices and a description of available methods). In contrast, composite or integrated samples are made up of several sub-samples from different parts of the water body. These are aimed at representative sampling of a water body. Whereas grab samples are suitable for analysing situations at specific sites (e.g. maximum density of cyanobacteria or cyanotoxins at a bathing beach), composite samples are preferable for assessing the water body's total content of a substance (e.g. total phosphorus potentially available for phytoplankton growth) or the population of an organism (e.g. the size of a cyanobacterial population). Composite samples are particularly important if the variables to be assessed are unevenly distributed. If knowledge of the precise distribution is required, each sample can be evaluated individually. However, the integration of samples prior to analysis is often far more cost-effective.

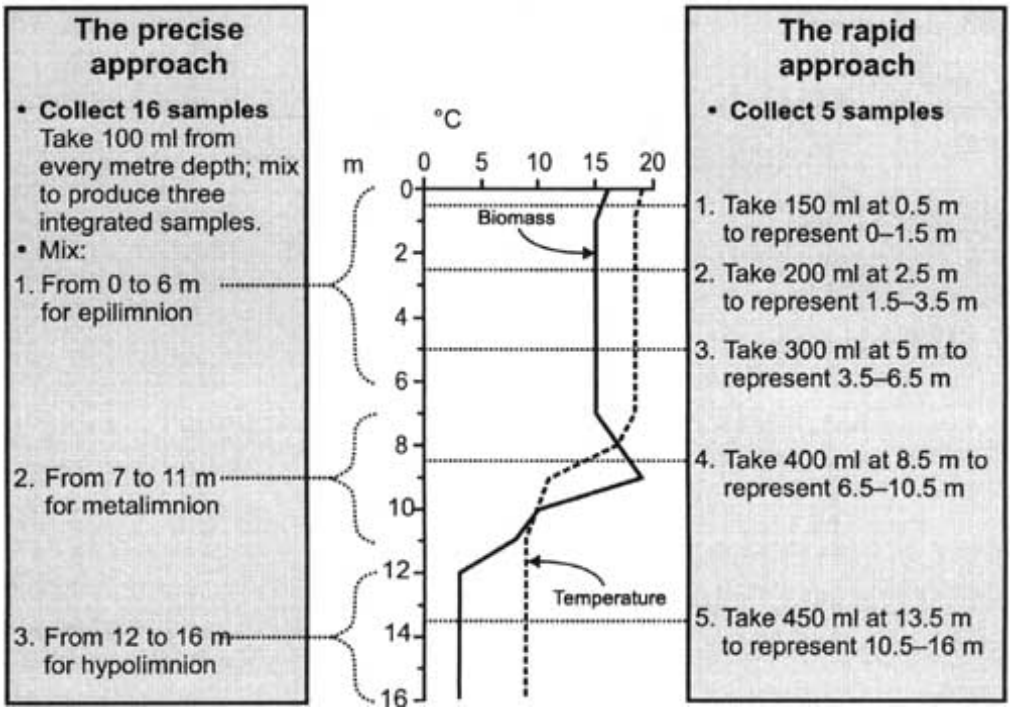
Depth-integrated samples may be obtained either by continuously sampling the total column of water from the surface to just above the sediment, or by discontinuously taking grab samples from representative depths and then mixing them together. The latter is particularly appropriate for deep lakes. However, in order to choose representative depths and to achieve meaningful integration, knowledge of thermal stratification of the water body is necessary.

Figure 11.1 Vertical distribution of chlorophyll a and a toxin (desmethyl-microcystin-RR) from the species *Planktothrix agardhii* in Lake Östra Kyrksundet in relation to hydrophysical conditions on 6 July 1988. Light intensity is given as a percentage of surface intensity (Reproduced from Lindholm and Meriluoto, 1991, *Can. J. Fish. Aquat. Sci.,* 48, with permission)



The thermal stratification of a water body is influenced by the morphology of lakes and reservoirs, the latitude, weather conditions and the physical nature of the water. It can be determined by measuring vertical profiles of temperature within the water body. Where thermal stratification occurs, it results in a water body functioning as two separate masses of water (the epilimnion and the hypolimnion) with different physicochemical characteristics and cyanobacterial populations, and with a transitional layer (metalimnion) sandwiched between (Figures 11.1 and 11.2). In temperate climates, thermal stratification generally occurs seasonally in water bodies of appropriate depth, whereas in tropical climates it often follows diurnal time patterns. Thermal stratification has important implications for the depth at which cyanobacteria are likely to be found (some species may accumulate on the surface, some in the metalimnion, see also section 2.2), as well as for concentrations of nutrients and interpretation of phosphorus and nitrogen data. Usually, shallow (2-3 m), wind-exposed lakes are unstratified, whereas in temperate climates deeper lakes usually exhibit a stable stratification from spring to autumn. Lakes of intermediate depth (e.g. 5-7 m) may develop transient thermal stratification for a few calm and sunny days, and which is then disrupted by the next event of rain or wind.

Figure 11.2 Derivation of representative depths for taking discontinuous samples and derivation of the volume of each sample

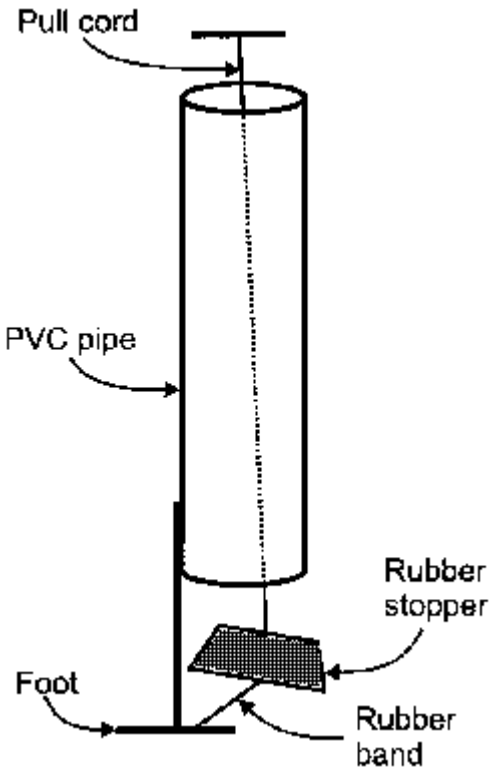


Thermal stratification usually results in inhomogeneous distributions of oxygen, nutrients, and populations of algae, cyanobacteria and other organisms. However, even when temperature is uniform throughout depth, stratification of organisms may develop on calm days. Depth gradients of oxygen concentration and pH are good indicators of this. Depth-integrated samples are more adequate than surface grab samples for the assessment of population size and nutrient concentration in such homothermous situations.

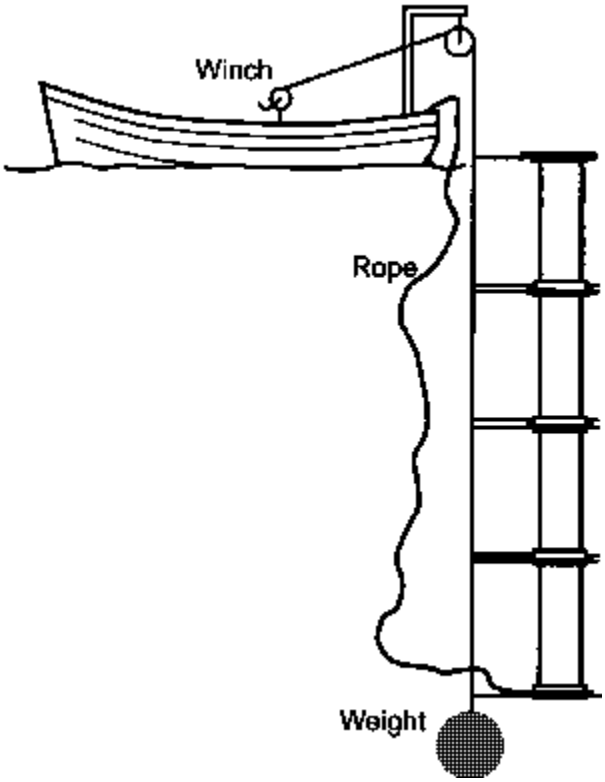
In deeper lakes or reservoirs with thermal stratification, depth-integrated sampling of representatively chosen depths is necessary - taking samples evenly spaced over depth may not yield fully representative results. When background information on the typical stratification characteristics of a given lake is available (e.g. from a temperature profile previously taken with a probe), sample numbers can be reduced by selecting adequate depths to represent specific strata. If depth intervals are unequal and samples are to be integrated, the volume of each sub-sample must be chosen to represent the actual fraction of the vertical gradient it represents (see Figure 11.2).

Figure 11.3 Simple tube devices for taking depth-integrated samples.

A. From shallow lakes or water layers (< 5 m);



B. From deeper layers (< 20 m)



Continuous depth-integrated samples are often quite adequate for shallow and moderately deep water bodies. They can be obtained using a water pump (submersible pumps are available which allow sampling at depth) attached to a garden hose which is operated at a steady pumping rate while the water inlet is drawn upwards between the desired depths at a uniform speed. The sample obtained is therefore representative of the water column. A very simple hose-pipe sampler may be used to depths of 30-35 m. This sampler is made of a piece of flexible plastic piping (a garden hose will do) of about 2 cm diameter and appropriate length; the pipe is weighted at one end and open at both. The weighted end, to which a cord is attached, is lowered slowly into the water so that when the tube is fully extended it encloses a columnar section of the water. Before hauling in the lower end with the attached line, the upper end should be closed in order to avoid loss of water once the lower end emerges from the surface. Hoses need to be stored clean and dry between sampling trips to avoid microbial growth inside the tube.

A simple depth-integrating pipe or tube sampler for shallow water columns (5 m deep) or for the surface layers of deeper water bodies is shown in Figure 11.3A. By combining tube segments as described by Sutherland *et al.* (1992) the sample may be extended to a total length of up to 20 m (Figure 11.3B).

Sampling scums

Scums of cyanobacteria often occur near shorelines at low water depths, and therefore working with a grab-sampler or a plankton-net may be difficult. Sampling scums is carried out more easily with a wide-necked plastic or glass container. When sampling scums, a suitable approach must be developed which allows for their observed heterogeneous density. Two samples can be useful for different purposes. One sample

can be aimed at assessment of the maximum density of cyanobacteria or highest toxin levels by taking a sample from where the scum is thickest (move the bottle mouth along the surface to collect the dense mats of buoyant cyanobacteria). The other sample can aim to simulate conditions where shallow waters are mixed by bathers and playing children (agitate the scum before submerging the bottle). Both types of sample can be used for comparison.

Plankton net sampling

Sampling of cyanobacteria with a plankton net is mainly carried out when large quantities of cell material are required (for example for toxicity testing or for extended chemical analysis) or when only a qualitative analysis of the phytoplankton is necessary. In contrast to the use of depth samplers which quantitatively trap all of the particles in a defined volume, the water volume filtered through a plankton net cannot be determined precisely. Calculations based on the area of the net opening and on the length and the distance it has been hauled are not recommended because they strongly overestimate the amount of water actually filtered (due to clogging of the pores in the net, only a fraction of the water volume actually passes through the net).

The depth at which the plankton net is deployed is dependent on the taxa of algae and/or cyanobacteria present. Floating cells (e.g. *Microcystis, Anabaena)* are harvested within the upper metres of the water column, while the sampling of well mixed or stratified water bodies with distinct depth distributions of cyanobacteria (e.g. *Planktothrix,* see Figure 11.1) may include deeper water layers.

11.4 Nutrients, cyanobacteria and toxins

11.4.1 Nutrient analysis (phosphorus and nitrogen)

Mass developments of cyanobacteria are associated with high nutrient concentrations. Phosphorus is usually the key nutrient controlling proliferation, although the availability of nitrogen may be an important variable to assess because it can influence whether or not nitrogen-fixing species dominate (see Chapters 2 and 8).

Total phosphorus (from unfiltered samples) determines the capacity of a water body to form cyanobacterial blooms. If dissolved phosphate (soluble reactive phosphate (SRP) determined from filtered samples) is detected at concentrations of only a few micrograms per litre, cyanobacterial growth and biomass are not limited by phosphate (see Chapter 8). Because such low concentrations are critical for interpreting the situation, sample contamination (e.g. through a few micrograms of dissolved phosphate leaching from contaminated sample bottles, or through the release of phosphorus by degradation of organic material during sample transport) may very easily lead to misinterpretation. In contrast, samples for total phosphate analysis are less sensitive. If SRP is important in a sampling programme, rapid filtration in the field or even on the boat may be desirable, particularly in warm climates.

Transformations between nitrate and ammonia may occur, if samples are not stored properly (cooled) and analysed rapidly (within 24 hours).

11.4.2 Identification and quantification of cyanobacteria

Cyanobacteria tend to accumulate in layers (at the surface or bottom) of sampling containers. When samples are integrated or when sub-samples are taken, the sample must be mixed immediately before sub-sampling.

In addition to the samples preserved with Lugol's solution, fresh unpreserved samples are useful to aid microscopic identification, because Lugol's solution masks the characteristic colours of the cyanobacteria, making it more difficult to recognise some species. Unpreserved samples can be qualitative and are easily obtained by hauling a plankton net (10 µm mesh) through the water column, or by taking a grab sample if the density of cyanobacteria is high. Unpreserved samples for identification may be stored for up to 24 hours, provided ambient temperature and light conditions are kept similar to those in the field. If longer storage is necessary, preservation with formaldehyde may be useful.

11.4.3 Samples for chlorophyll *a* analysis

When collecting samples for chlorophyll *a* determination it should be born in mind that this pigment will start to degrade rapidly after collection; therefore it is essential to keep storage times to a minimum. Filtration at the sampling site and storage of the filters in an ice box is recommended for warm climates. If samples are to be filtered and analysed in the laboratory, they should be transported as soon as practically possible (e.g. within 4 hours) and processed immediately on arrival.

Apparatus

- Measuring cylinder
- On-site filtration equipment, including vacuum hand pump and filter cup
- Glass fibre filters, GF/C, 47 mm
- Plastic Petri dishes and/or aluminium foil

Procedure

1. Thoroughly mix the sample and pour immediately into a measuring cylinder. Pass the measured sample through the glass fibre filter with the aid of a hand vacuum pump. If samples are dense, begin by filtering a small volume. If this is easily filtered, add more sample, mixing every time a sub-sample is poured from the bottle. Use as much sample as will easily pass through the filter leaving a distinctly visible greenish layer on the filter. Record the volume of water filtered.

2. Once filtration is complete, carefully remove the filter from the filtration apparatus and either place it in a Petri dish or wrap it in aluminium foil.

3. Fold the filter with the cells innermost because this both protects the pigment from light and also prevents the sample from being dislodged from the filter surface.

4. Keep filters at near freezing temperatures and in the dark prior to their delivery to the analytical laboratory. Deep-freeze filters if storage of several hours or more is necessary.

11.4.4 Bulk samples of cyanobacteria for cyanotoxin detection

For some purposes, it is important to collect a large amount of cell material using a plankton net as described in section 11.3; toxicity tests for example require large quantities because bioassays (e.g. mouse test) are less sensitive than chemical analysis. Large amounts of cell material may also be needed for some detailed chemical analyses (see Chapter 13). If the results are to be related to the dry weight of cellular material, a quantitative sample (i.e. sampling all particles of a defined water volume) is not necessary.

For later expert identification of the cyanobacterial taxa, and particularly for relating toxin concentrations to cell numbers or biomass of specific taxa, a subsample for cell counts may be taken directly from a well-mixed net sample before it is frozen. It is important to realise that plankton net hauls accumulate particles selectively (enriching large cells and colonies more than small or thin ones) and are therefore likely to contain a different quantitative distribution of taxa than the sample used for assessing the composition of cyanobacteria (as described in 11.3.2). Such subsamples for microscopy should be preserved in 10 per cent formaldehyde or Lugol's solution in a separate container; small volumes (e.g. 10 ml) are sufficient.

High biomass densities in such concentrated samples may rapidly consume the preservation capacity of Lugol's solution. If not analysed within several days, samples should be periodically checked for their preservation capacity; if they no longer show a brown colouration, more Lugol's solution should be added. Dilution of the sample may be useful to avoid rapid consumption of preservative (i.e. 1 ml net plankton sample, 8 ml water, 1 ml Lugol's iodine solution or formaldehyde).

11.4.5 Quantitative analysis of cell-bound and dissolved toxins

For recreational sites and for assessment of the total toxin concentration, cell-bound toxins are of primary importance. The concentration of dissolved toxin in the water is of special interest for drinking water reservoirs and where large amounts of cyanotoxin such as anatoxin-a are released into the water (Bumke-Vogt *et al.,* 1996). Large volumes may be required for dissolved toxin analysis (e.g. 1 litre) and this should be checked with the analytical laboratory. If the concentration of cell-bound and dissolved cyanotoxin is to be related to the water volume from which the cells were collected, a defined volume must be filtered. Several methods have been proposed (Coyle and Lawton, 1996; Gjølme and Utkilen, 1994) and are described in detail in section 13.1. Essentially samples are handled in a similar manner as those prepared for chlorophyll *a* analysis. The filtration step can be readily performed at the sampling site but with the following modifications:

- Glass fibre or membrane filters may be pre-weighed so that the amount of material retained (i.e. dry weight of cells) can be determined. Thus, toxin concentration can be related both to dry weight and to water volume.

- The filtrate can be collected, placed in a glass bottle and processed as described in Chapter 13 to enable the dissolved cyanotoxin concentration to be determined.

- Filters and filtrate should be kept cool.

Laboratory studies have shown that the microcystin content of material collected and dried on filters is unaltered for several months, when the filters are stored in the dark at room temperature and low humidity. So far this method has been shown to be useful for microcystin as well as for anatoxin-a.

11.5 On-site analysis

Analyses for many variables can be carried out in the field. A significant advantage of on-site testing is that tests are carried out on fresh samples that have not been contaminated or otherwise changed as a result of storage. Variables generally analysed on-site are:

- *Transparency* (with a Secchi disc). Cyanobacteria may occur as floating streaks or mats on the water surface (see Chapter 2) making it difficult to obtain representative transparency data (depending on the measuring site values can vary from 0.2 to 2 m). It may be useful to determine the transparency in areas without floating cells as well as within scums. The Secchi disc has to be lowered very carefully so as not to destroy the formation of accumulated cyanobacterial cells, and before taking the measurement the surface scums should be given time to return to their original position (i.e. their position prior to disturbance by the Secchi disc).
Bathers can easily perform an improvised transparency determination while standing knee-deep in the water and being careful to avoid suspending the sediment. If greenish turbidity obscures their view of their feet, a significant cyanobacterial bloom could be present corresponding to a level at which some authorities advise against bathing (see Chapter 5).
- *Temperature* (with a probe lowered into the water, or a thermometer reading taken immediately from the sampling device).

- *Dissolved oxygen* (electrometrically with a probe or chemically with the Winkler method).

Further details of the methods mentioned above are available in Bartram and Ballance (1996).

11.6 Field records

As with every sampling programme, careful field records are of critical importance for interpretation of results (see Bartram and Ballance, 1996). Field records involve pre-prepared lists and questionnaires, but which also leave plenty of space for additional comment and for the recording of observations. Such observation should include:

- Presence of scums.

- Weather conditions on the day of sampling and, if available, an indication of conditions over the previous 2-3 days, because this affects the accumulation of cyanobacteria.

- Any additional or unusual observations, such as scum distribution, estimation of numbers of people bathing despite the presence of scums, defect warning or prohibition signs regarding bathing in scum areas, suggestions for improvement of the sampling

programme, previously undetected inlets, changes in agricultural practices (such as previously unknown distribution of manure) or other use, etc.

11.7 Sample preservation and transport

As has been emphasised in several sections earlier in this chapter, samples must be clearly labelled with sampling site (station), date and time of sampling and depth of sampling. In general, samples should be stored cool and dark in a storage box, taking into consideration the following criteria:

• Cool storage is most important for all samples to be filtered because biological activity may enhance shifts between particulate and dissolved fractions of the variable to be analysed (biological activity is strongly enhanced by high temperatures). This is particularly important for nutrient samples, chlorophyll *a* samples and samples for cell-bound cyanotoxin analysis (whether collected on filters or with a plankton net). If storage time exceeds 2-3 hours, particularly in warm climates, cooling during sampling and transportation is recommended.

• Unpreserved samples for identification of cyanobacteria, fixed samples for determining cyanobacterial cell numbers and biomass, and samples fixed with Winkler's reagents for oxygen determination should be stored at ambient temperatures but protected from direct sunlight.

• Storage times should be kept as short as possible, generally in the range of a few hours. Storage times over 24 hours should be avoided. Specific descriptions of preservation and transportation of samples for toxicity testing or toxin analysis of cyanobacterial cells are given together with the sampling methods and in Table 11.1.

Table 11.1 Overview of sampling methods for the determination of cyanobacteria, toxicity testing and toxin analysis

Purpose	Equipment	Preservation	Transport	Advantages	Disadvantages
Determination of cyanobacteria					
Qualitative	Plankton net (10 µm); depth sampler[1]	Unpreserved, or formaldehyde solution	Cool and dark	Formaldehyde does not discolour the sample	Caution necessary when using formaldehyde
Quantitative	Depth sampler[1]	Lugol's solution	Cool and dark	Lugol's solution enhances settling in counting chambers	Colour of algae changed by Lugol's solution (identification difficulties for less experienced staff)
Toxicity tests and toxin analysis					
Qualitative, (large amounts)	Plankton net (25-50 µm mesh)	Must be frozen within 24 hours, freeze-drying	Cool up to 24 hours, otherwise frozen or freeze-dried	Provides large amount of cell material for toxicity testing and toxin analyses	No relation to water volume; sampling efficiency less than 100%; selective

Quantitative					
1. Cell-bound toxin Filter method	Depth sampler[1]; hand vacuum pump or filtering device; glass fibre or membrane filters	Directly frozen until analysis or air dried to send to laboratory	Cool up to 24 hours, or send dried filters	Cheap, quick, easy to send; direct relation to volume; biomass estimate possible with pre-weighed filters	No large amounts of cell material for further analysis; amount of toxin on filters may be below detection limit; caution required in humid climates with dried filters
Plankton net with defined water volume	Plankton net (10 µm mesh) graduated bucket	Must be frozen within 24 hours; freeze-drying	Cool up to 24 hours, otherwise frozen or freeze-dried	Provides large amount of cell material for toxin analysis and toxicity testing: relation to water volume	Selective sampling with a net
2. Dissolved toxin	Depth sampler[1]; hand vacuum pump or filtering device; glass fibre or membrane filters	Frozen	Cool up to 24 hours, otherwise frozen	Filters and filtrates can be obtained in one step	

[1] Depth samplers include Ruttner, Van Doom, hose sampler or water pump

11.8 References

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