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CHAPTER 3 DEVELOPMENT AND IMPLEMENTATION OF A MONITORING PROGRAM (LEVEL 1)

BACKGROUND

Monitoring is critical element in cyanotoxin risk management. The goals of a monitoring program to support risk management are three-fold: to measure cyanobacteria concentrations in source and final drinking water, to measure the concentrations of cyanotoxins in source and final drinking water and to measure source water constituents and conditions that promote or inhibit cyanobacterial growth. Accurate and precise data in these three areas, collected on a regular basis and carefully tracked over time, will help water supply managers to achieve the greatest reduction of risk.

The design of an effective long term monitoring program requires that water supply managers ask, and answer, the following questions: (1) What analytes do I sample for and how do I measure them? (2) Where do I sample for these analytes? (3) How often do I sample for these analytes? (4) How much replication do I build into a sampling event?

Monitoring can be defined as including two components, sampling of the water body and analysis of the samples. Together they provide the information for early warning and tracking the development of cyanobacterial blooms [1]. An overview of recommendations for design of a monitoring and sampling program for cyanobacteria is given later in this section (see Table 3-2).

When choosing an organisation to sample and/or analyse cyanobacterial samples it is recommended that the testing laboratory selected is accredited to carry out these particular analyses by a national laboratory accreditation authority. For example in Australia the National Association of Testing Authorities (NATA) accredits and recognises facilities that are competent in specific types of testing, measurement, inspection and calibration. Not all accredited laboratories use the same methods for testing and this is not important provided the individual methods are accredited. It may however, make it difficult to compare results when samples are analysed by more than one laboratory. Where an accredited laboratory is not available it is important to ensure the analyses are undertaken according to the highest standards, and inter-laboratory testing has shown the validity of testing procedures.

VISUAL INSPECTION

One of the simplest and most important forms of monitoring of a water body is regular visual inspection for water discolouration or surface scums of cyanobacteria. This can be a secondary form of surveillance for higher classes of monitoring, or if few other resources are available, the principal form of surveillance used for remote sites or non-specialised field personnel. However some cyanobacteria, for example *Cylindrospermopsis,* do not form scums and a slight green discolouration of the water may be indicative of dangerously high cell numbers. In situations where non-bloom forming cyanobacteria are present it is essential that samples are collected for analysis to determine the abundance of cyanobacteria in the water body.

When bloom-forming cyanobacteria are present, a qualitative assessment through visual inspection can be a useful indicator of water quality and the relative hazard posed by the presence of cyanobacteria. The frequency of visual inspections may vary depending on seasonal and weather conditions. If visual inspection is the only monitoring being carried out, the position and extent of scum formation should be recorded on a dedicated report sheet.

The first visual indication of cyanobacteria may be the presence of small green particles in the water that may be more obvious by holding a jar of the water up to the light. Scum formation will not normally be observed until open water concentrations of cyanobacteria exceed 5,000-10,000 cells/mL, but exceptions are possible. Blooms or scums are usually most apparent early in the morning following calm days or nights, but as cell concentrations increase, or during prolonged periods of calm weather, scums may persist at the surface for days or weeks. Scum accumulations will normally be observed at the downwind end of a reservoir, lake or river reach and also in sheltered back waters, embayments and river bends.

In general, a healthy cyanobacterial scum will appear like bright green or olive green paint on the surface of the water. Scums only look blue in colour when some or all of the cells are dying. As the cells die, they release their contents, including all their pigments, into the surrounding water. Cyanobacteria have three main pigment types: chlorophyll, phycobiliproteins, and carotenoids. In healthy cells, the green chlorophyll colour normally masks the other pigments, although these other pigments may give blooms a more yellow-green or olive-green colour in some cases. When the cells die, the chlorophyll is rapidly bleached by sunlight, while the blue phycobiliprotein pigment (called phycocyanin) persists. Figure 3-1 shows some examples of cyanobacteria in concentrations that will cause a water quality problem for water suppliers.

Figure 3-1 Cyanobacteria blooms and scums

Cyanobacterial scums should not be confused with scums or mats of filamentous green algae, which appear like hair or spider web material when a gloved hand is passed through the water. There are blooms of other phytoplankton that look very similar to cyanobacterial scums, but these cannot be readily distinguished without a microscope. Scums or mats of filamentous green algae are more common in slow flowing, shallow streams and irrigation channels and drains.

Figure 3-2 shows some examples of green algae similar in appearance to cyanobacteria. The major point of visual differentiation is the bright green colouring of the green algae, compared with a more olive- or blue-green for cyanobacteria.

Figure 3-2 Examples of green algal blooms common in slow flowing streams

Benthic cyanobacteria are usually submerged, and are difficult to monitor. Visual inspection is a very important way to identify an issue with benthic cyanobacteria as they will often break free of the surfaces to which they are attached, and float to the surface. Figure 3-3 shows some examples of attached benthic cyanobacteria and detached floating mats that may cause water quality issues.

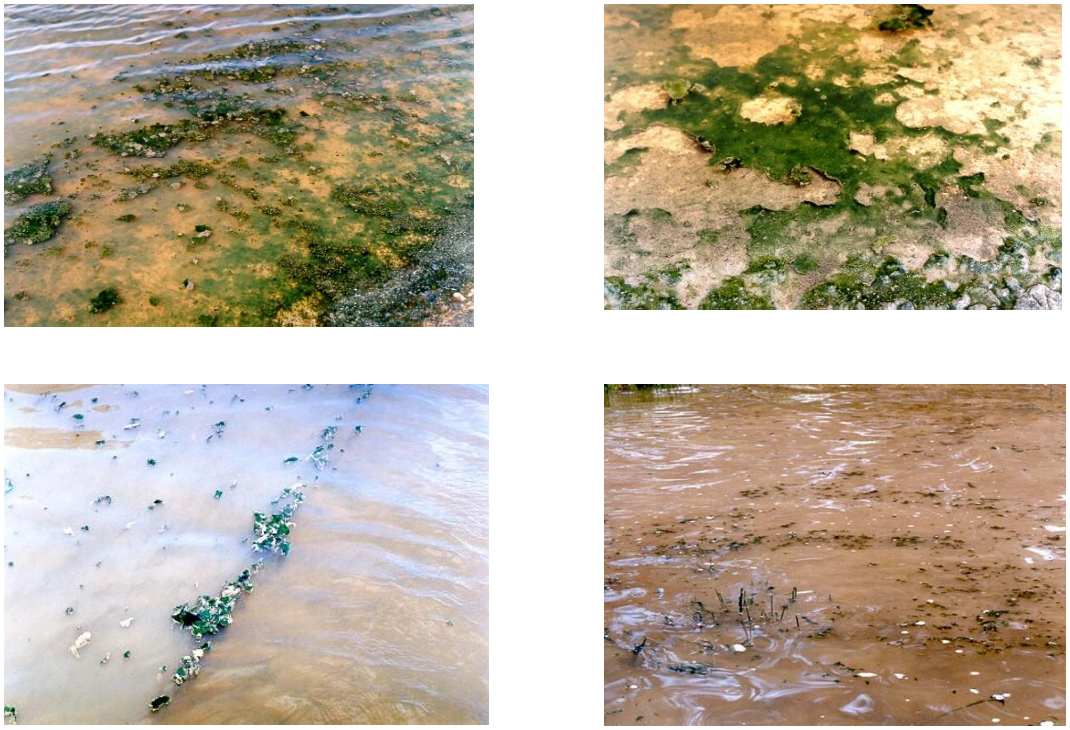

Figure 3-3 Benthic cyanobacteria attached to sediments and rock surfaces, and floating on the surface after breaking free from the substrate

Another tell-tale sign of cyanobacterial blooms is their odour. Some cyanobacteria produce a distinctive earthy/musty odour that can often be smelt at some distance before the bloom/scum can be seen. Therefore it is useful to conduct 'odour surveillance' in conjunction with any visual inspection program.

For an example of a recording sheet for a visual inspection, click here

SAMPLING PROGRAM DESIGN

The development of an appropriate sampling strategy will depend upon the primary objective of the monitoring program. The objective will be determined by the immediate use of the water, which in turn determines the level of confidence required in the monitoring results. For example if the water is being used directly to supply consumers, i.e. is in service, then you will want a very high degree of confidence in the monitoring result for any potential hazards from the occurrence of cyanobacteria. However if the reservoir is not directly in service or is a bulk water storage, then you may have less need for a high degree of confidence in the results. This objective-based approach can be used to design a program based upon the level of sampling effort which translates to resource needs and cost for the program.

For most purposes, the aim should be to obtain samples that are representative of the water body as a whole, or the part of a water body that is in use (e.g. near the water treatment plant offtake). Once the aim of the monitoring program is established the required level of sampling effort described as high, moderate or low, is determined by combinations of the following components:

- Type of access required for sample collection
- Sample type or the method used to collect a sample
- Number of samples collected at any one time
- Frequency of sampling

These components, which are given in Table 3-2 are discussed in more detail below.

ACCESS FOR SAMPLE COLLECTION

Cyanobacteria tend to be extremely patchy in distribution, both vertically and horizontally within the water body. Vertical patchiness results from the development of a stratified water column in warm calm weather, allowing buoyant cyanobacteria to maintain their position at the surface for extended periods. Horizontal patchiness is common for most phytoplankton, but can be particularly pronounced in cyanobacteria due to the effect of prevailing winds, which cause accumulation downwind along shorelines of reservoirs or bends in river reaches.

Depth integrated sampling in open water provides, in general, a better representation of the 'true' or average cyanobacterial population in a water body and is therefore the preferred option. Open water and mid-stream sampling is normally undertaken from a boat, but can also be achieved in some circumstances from a bridge over a river, or from an open water structure such as a reservoir offtake platform. For drinking water supplies, sampling the appropriate depth next to, or from, the water offtake tower is recommended. Due to the resources required for open water sampling (i.e. boat and two people), it is often reserved for high priority public health surveillance.

If open water sampling is not possible, the second option for monitoring drinking water supplies is to sample from reservoir/lake shorelines or riverbanks. Such samples may not be representative of the 'true' cyanobacterial population due to the bias in spatial distribution discussed above and the limited choice of suitable locations. In choosing a location for sampling the likely effects of the prevailing winds and water currents should be taken into account.

Benthic cyanobacteria are also known to cause problems associated with water quality so sampling of the sediments and attached growth, and therefore a different approach to sampling, may be required.

SAMPLE COLLECTION METHODS

The methods used for sample collection will depend on whether the sites require access by boat, shore or platform and will include integrated water column (hosepipe) sampling, discrete depth (grab) sampling, grab sampling from an extension pole, sediment sampling by grab or corer for benthic cyanobacteria and sampling from a pipeline. Different methods are used to collect samples for cyanobacterial identification, for toxin analysis or for assessing benthic cyanobacteria. In addition different techniques may be used to collect these samples from a boat, from depth, from the shoreline or a pipeline.

It is important to be aware of the safety issues involved in sampling for cyanobacteria, whether from the shore or a boat. Samplers should be fully trained and aware of all aspects of sampling including:

- potential environmental hazards (e.g. submerged logs and branches, mosquitoes, crocodiles, UV radiation)
- location and use of safety equipment (e.g. life vests, hats, sunscreen)
- standard safety procedures for use of equipment and vehicles
- the requirement for current qualifications to drive appropriate vehicles (e.g. off-road 4-wheel-drive vehicles, bikes, tractors or boats)
- qualifications in advanced first aid

Once training has occurred, hazards or risks involved with field sampling must be identified and documented on a site- and sampling- specific basis.

SAMPLES FOR BENTHIC CYANOBACTERIAL SURVEYS

In some instances it may be necessary to collect benthic samples for identification of cyanobacteria, particularly if high levels of taste and odour compounds are detected but few, or no, cyanobacteria are present in water samples. In most cases benthic samples are not collected routinely and are generally for qualitative analysis only. The most convenient way to sample benthic cyanobacteria is from any mats that have become detached from the substrate and are floating on the surface. In the absence of floating mats a representative assessment of numbers and distribution of benthic cyanobacteria is difficult. Samples should be collected from a number of transects throughout or around the perimeter of a reservoir. Particular attention should be paid to shallow protected bays and any areas where benthic mats have been observed in the past. Samples at varying depths may be required down to approximately 5 metres, although this will depend upon light attenuation in the water body. Samples can be collected using a benthic sampler such as an 'Eckman' grab or a rigid plastic corer (e.g. PVC or polycarbonate pipe). A transect in a shallow, protected bay should be chosen to sample. Duplicate samples of sediment at varying depths are collected either by grab or hosepipe and emptied into a container with a fitted lid. If large quantities of sediment are collected, a subsample can be taken and stored in a smaller specimen jar. Visual observations of the sediment surface can also provide very useful information on the distribution of benthic cyanobacteria. More detailed surveys can be conducted using underwater cameras or divers. This requires access to relatively sophisticated expertise and resources.

Benthic cyanobacteria may also be found attached to dam walls or offtake structures. Cyanobacteria attached to these structures can be scraped off, most easily when water levels drop.

WATER SAMPLES FOR CYANOBACTERIAL IDENTIFICATION AND COUNTING

RESERVOIR/RIVER SAMPLING BY BOAT

The preferred method for sampling a reservoir or river is by boat, which should always be stationary while sampling proceeds. The sampling stations, or locations, in a reservoir should preferably be chosen randomly within several defined sectors, representing the entire water body. For boat sampling the use of permanent moorings with marker buoys placed in each of the sectors is the most practical approach and makes open water sampling easier, especially in windy weather. Having permanent sampling sites also gives consistency which enables the comparison of results at each site over a given time frame. If it is not possible to place permanent marker buoys in a water body, a global positioning system (GPS) should be used to ensure the consistency of sampling points over time. One way to introduce randomness when boat sampling is to move sampling station moorings within sectors on a yearly basis. For monitoring rivers, randomness of sampling sites is less critical due to instream flow.

Go to Level 2 for more information on open water sampling

Integrated water column samples

Discrete depth samples

SURFACE GRAB SAMPLES FROM SHORELINE

Sampling from a bank or shoreline is comparatively simple, but introduces a risk of excessive bias of samples from patchy shoreline accumulations. A 'pole-type' sampler can be used, where the bottle is placed in a cradle at the end of an extendable pole of 1.5-2 metres length. This procedure is depicted in Figure 3-4. Alternatively, a spear sampler as described in [2] is a useful sampling device for collecting an integrated depth water sample when standing on the bank or shoreline. It is also important to note that in using either the pole or spear sampler, scum accumulations near to the shoreline will not be sampled. A separate dip sample of any accumulations may be needed for toxin analysis.

Figure 3-4 Taking grab samples from the shoreline with an extension pole.

SAMPLES FOR TOXIN ANALYSIS

QUALITATIVE

Qualitative toxin analysis is done by mouse bioassay and is usually carried out either when more sophisticated techniques are unavailable, or the identity of the toxin is initially unknown. These samples are generally collected from dense accumulations of scum along shorelines and riverbanks if these are present. Alternatively, cells may be concentrated by either trailing a phytoplankton net (25-50µm nylon mesh) from a boat or from the shoreline, or by collecting a large volume of water that can be concentrated in the laboratory. Figure 3-5 shows sampling from a shoreline with a net-tow sampler to concentrate the cyanobacteria.

Figure 3-5 Net sampling is a simple method for concentrating cyanobacteria for further analysis

The volume of sample required depends upon the concentration of scum or cyanobacteria collected. Up to 2 litres of sample may be required if cyanobacterial concentrations are low, or if species present are small enough to pass through a phytoplankton net and samples therefore need concentration by other means such as filtration or centrifugation.

This test should be used as a screening tool only. If a mouse bioassay proves positive, quantitative methods are then required to determine the type of toxin, and concentrations present.

QUANTITATIVE

Quantitative toxin analysis is performed using a variety of methods suited to the type of sample and toxin present (see following sections). Samples are collected in the same manner as those taken for phytoplankton identification and enumeration and the volume of sample required is dependent upon the type of analysis to be used. In general, at least 500 mL of water should be collected.

SAMPLING FREQUENCY

For monitoring trends in cyanobacterial abundance, an indication is required of the 'true' cyanobacterial population, representing the entire water body. This can be achieved by collecting a suite of discrete samples from different sampling sites, which are counted separately and then may be averaged. As an alternative to undertaking separate counts on samples collected at several sites, samples may be pooled or composited. These samples are collected at three or more individual sites and pooled into one container. The sub-sample

for counting is then taken from the container after its contents have been thoroughly mixed. If composite samples are made, the individual samples must be of equal volume to prevent bias. An alternative to pooling samples in the field is to send discrete samples to a laboratory, where they can be sub-sampled, pooled and analysed. Using this process, a portion of the original discrete sample can be retained for further analyses if required. The trade off from compositing is a decrease in statistical power for subsequent data analysis against a three-fold or greater reduction in counting costs.

The number of sampling sites in a water body is chosen to determine the spatial variability of the cyanobacterial population and will also be influenced by time and cost considerations. It is recommended that a minimum of three sites be used when cyanobacterial counts exceed 2,000 cells mL^{-1} for both open water sampling and shoreline sampling, or sampling should be undertaken according to the appropriate cyanobacteria incident management plan (see Chapter 6). For lakes and reservoirs the sampling stations should be at least 100 m apart (where possible), while for rivers replicate samples should represent different 'parcels' of water. When sampling from a boat, replicate samples should preferably be taken at the downstream end first to avoid re-sampling the same 'parcel' of water.

The appropriate frequency of sampling will be dictated by a number of factors including the category of use, the current alert level status (see Chapter 6), the cost of monitoring, the season and the growth rate of the cyanobacteria. Apart from cost, the underlying consideration in operations monitoring is the possible health consequences of missing an early diagnosis of a problem. Cyanobacterial growth rates are generally related to seasonal conditions and previous studies have shown that cyanobacteria in the field can exhibit growth rates from 0.1-0.4 d^{-1} (equivalent to population doubling times of nearly a week to less than two days respectively). These estimated growth rates can be used to construct a set of theoretical 'growth curves' for a population of cyanobacteria starting from an initial count of either 100 or 1,000 cells mL^{-1} (Table 3-1). Historical data should be used as an indicator of likely rates of increase in cyanobacterial numbers.

Table 3-1 Cyanobacterial concentrations that can be achieved from an actively growing population by applying two different growth rates and initial starting concentrations.

Initial Concentration (Cells/mL)	Growth Rate -Population doubling time (days)	Cyanobacteria Concentration			
		at 3 days	at 7 days	at 14 days	at 28 days
100	6.93 (μ=0.1) - *slow*		200	400	1500
100	1.73 (μ=0.4) - *fast*		800	6400	
1000	6.93 - *slow*		2000	4000	>15000
1000	1.73 - *fast*	3500	16000	>250000	

Based on this assessment, it is recommended that sampling for high risk/high security supplies (i.e. drinking supplies) should occur on at least a weekly basis and probably twice-weekly when cyanobacterial count of > 2,000 cells mL^{-1} is reached. It is important to understand that frequency of sampling is determined by the need to detect real changes in population numbers and significant upward trends in growth, data collected will inform changes to treatment plant operations, and the application of cyanobacteria management plans, discussed in Chapter 6.

For supplies where the public health risk is deemed to be low (i.e. low cell counts in non-supply reservoirs), fortnightly sampling may be adequate, but caution is advised given the rate at which the cyanobacterial population may increase.

The timing of sampling for buoyant cyanobacteria can be important during calm, stratified periods especially if depth integrated samples are not collected. Buoyant cyanobacteria tend to accumulate near or at the water surface overnight, which can result in an over-estimation of cell concentration in surface samples collected early in the morning or an under-estimate in those collected at depth at the same time. Temporary surface

scums may be observed early in the morning, but they tend to disperse as winds increase and may even be mixed back into the water column during the day. Thus, a sample that is less biased by scum formation is, on average, more likely to be obtained later in the day. If the option exists, it is preferable to delay sampling to later in the day, but whatever time is chosen it is best to adhere to the same sampling times for each location on each sampling occasion if possible.

SAMPLING REPLICATION

At some point, analytical results from a monitoring program may be compared with a fixed standard, set internally by a drinking water provider, or externally by a regulatory agency. Because crossing a regulatory threshold often involves significant consequences, it is critical that water providers understand the degree of statistical uncertainty that is associated with an analytical result. Collecting single samples has the lowest short term cost. However it is impossible to characterize the uncertainty associated with a given sampling event. Moving to duplicate sampling allows characterization of the uncertainty. Triplicate sampling in turn permits a more precise estimate of the confidence interval surrounding the "true" value of the analyte of interest. As a result, it is recommended that, budgets permitting, some degree of replication be practiced in the sampling of critical analytes. A popular compromise is to collect replicate samples at some fraction, such as 30%, of all sampling events. With careful record keeping, it will be possible to develop a feeling for the statistical uncertainty associated with the sampling and analysis of a given analyte.

For an example of statistical analysis of replicate samples, click here

Table 3-2 Recommendations for design of a monitoring and sampling program for cyanobacteria based upon the required purpose of the monitoring and type of water body. The scale of sampling effort and procedures for monitoring are determined by the purpose for the monitoring

Purpose of monitoring	Confidence required from results	Water body type	Sampling effort required	Access required for sampling	Sample type (method)[1]	Number of samples[2]	Frequency of sampling[3]
Public health surveillance of drinking supplies: *in direct service*	Very High	Reservoirs & lakes	High	Supply offtake *and* Open water by boat	Discrete sample at offtake depth *and* Integrated depth	Both offtake location and multiple open water sites	Weekly or 2x-weekly
		Rivers and weir pools		Mid-stream by boat; from bridge or weir	Integrated depth		
Public health surveillance of drinking supplies: *bulk water storage / not in service*	High	Reservoirs & lakes	Moderate	Supply offtake location *and/or* Open water by boat	Discrete sample at offtake depth *and/or* integrated depth	Multiple sites	Weekly or 2x-weekly
		Rivers and weir pools		Mid-stream by boat; from bridge or weir	Integrated depth		
Public health surveillance of recreational water bodies & non-potable domestic supplies	Moderate	Reservoirs & lakes	Low	Shoreline	Surface Sample	Limited number of sites	Weekly or fortnightly
		Rivers and weir pools		River bank	Surface Sample		

1. Integrated depth samples are collected with a flexible or rigid hosepipe, depth (2-5m) depending on mixing depth; surface or depth samples are collected with a closing bottle sampler (van Dorn or Niskin sampler); shoreline or bank samples collected with a 2m sampling rod which holds a bottle at the end.
2. Multiple sites should be a minimum of 100m apart (except in smaller water bodies such as farm dams), including one near the offtake. Multiple samples can also be pooled and one composite sample obtained. River monitoring should include upstream sites for early warning. Samples from recreational waters should be collected adjacent to the water contact area.
3. Frequency of sampling is determined by a number of factors including the category of use, the current alert level status, the cost of monitoring, the season and the growth rate of the cyanobacteria being tracked. Sampling should be programmed at the same time of day for each location. Visual inspection for surface scums should be done in calm conditions, early in the morning.

TRANSPORT AND STORAGE OF SAMPLES

SAMPLES FOR CYANOBACTERIAL IDENTIFICATION AND ENUMERATION

Samples should be preserved as soon as possible after collection by the addition of 1% acid Lugol's iodine preservative. Hötzel & Croome [2] detail the recipe and instructions for the preparation of this iodine solution. It is sometimes useful to retain a portion of sample in a live (unpreserved) state as cyanobacteria are often easier to identify in this way. This may be the case when a new water body is being sampled or a new problem occurs in an existing site. To ensure reasonably rapid turn-around time for reporting results of monitoring, samples should be received at the analytical laboratory used for cyanobacterial counting within 24 hours of collection. If received on the same day as collection, the receiving laboratory may assume responsibility for preservation of samples. In remote rural areas, it is sometimes advantageous to avoid sampling on Thursdays and Fridays so that samples do not remain in a courier or mail sorting depot over the weekend.

The preserved cyanobacterial samples are reasonably stable as long as they are stored in the dark. If samples are unlikely to be examined microscopically for some time, they should be stored in amber glass bottles with an airtight seal or PET plastic (soft drink) bottles. Polyethylene (fruit juice) bottles tend to absorb iodine very quickly into the plastic and should not be used for long term storage. Live samples will begin to degrade quickly especially if there are high concentrations of cyanobacteria present. These samples should be refrigerated and examined as soon as possible after collection.

SAMPLES FOR TOXIN ANALYSIS

Careful handling of samples is extremely important to ensure an accurate determination of toxin concentration. Microcystin and cylindrospermopsin toxins are degraded microbially and to a lesser extent photochemically (i.e. in light). Samples should be transported in dark cold conditions and kept refrigerated and in the dark prior to analysis. Samples should be analysed as soon as possible or preserved in an appropriate manner [3].

A case study of a sampling program for a reservoir that has regular populations of the cyanobacterium Anabaena circinalis can be found here.

ANALYSIS FOR CYANOBACTERIA AND THEIR TOXINS

CYANOBACTERIA

Cyanobacteria concentrations are determined directly, through microscopic examination and enumeration, or indirectly, through the measurement of the concentrations of constituent pigments such as chlorophyll-*a* and phycocyanin. Results are usually given as cells mL^{-1} for a genus/species with an estimated confidence limit. However, cell numbers alone cannot represent true biomass because of considerable cell-size variation among algal species. If, for instance, a mixture of *Microcystis* sp. and *Euglena* sp. is present in a sample, the cell count of *Microcystis* sp. may be higher than that of *Euglena* sp. However, as the *Microcystis* cells are smaller they may contribute a lower biomass than the larger cells of *Euglena* sp. Cell volume (biovolume) determination is one of several common methods used to estimate biomass of algae in aquatic systems.

In the event of a risk to water quality posed by the presence of cyanobacteria, information required by the water manager includes:

- *Identification of the cyanobacteria to species level* - This information is necessary to determine if the cyanobacteria have the potential to be toxic, and the type of cyanotoxins that are likely to be produced. The latter information can be used to determine the degree of risk associated with the presence of the cyanobacteria in the inlet to the treatment plant, and the analytical technique appropriate for determining toxin levels.
- *The concentration of cyanobacteria* – The concentration of cells, either as number per mL, or biovolume, can be used to estimate the potential concentration of cyanotoxin present in the raw water by using a table similar to Table 2-4, (Chapter 2), or in the implementation of the cyanobacteria incident management plans (Chapter 6).

DIRECT CELL COUNTING AND IDENTIFICATION

Direct cell counting involves flooding a transparent chamber with a known volume of sample. The chamber is placed under an inverted microscope, and the cyanobacteria are visually identified and counted by the microscopist. The results are usually expressed in terms of cells per unit volume. Another widely used cell counting procedure involves the filtration of a known sample volume onto a nitrocellulose filter. The filter is mounted with immersion oil on a microscope slide, placed under a microscope and the cyanobacteria are visually identified and counted by the microscopist. Once the analysis is complete, the cell numbers can then be converted to biovolume if required for the application of the incident management plans (Chapter 6).

An extra level of quantification can be added to the procedure through the use of digital cameras inserted into the light path of the microscope. Images collected with the camera can be processed with commercially available image analysis software (e.g. Soft Imaging System – analySIS). The use of images and software has two advantages: 1) an extra level of documentation, and 2) easing the quantification of cyanobacterial biomass when the dominant species is filamentous. The primary advantage of direct counting is that quantification and identification occur simultaneously. The primary disadvantage of the procedure is that it is laborious and must be performed by highly trained and experienced analysts. As a compromise, direct cell counting may be performed in conjunction with, and as a check on, faster and cheaper indirect methods that measure the concentrations of cyanobacterial pigments. However, digital counting methods are not routinely used as a monitoring tool due to the errors involved when analyzing cyanobacteria with a complex three dimensional geometry (eg spiral filaments of *Anabaena*)

Visual taxonomic identification to species level (eg *Microcystis aeruginosa, Anabaena circinalis*) requires an experienced, skilled analyst. Differentiation between toxic and non-toxic strains of the same species, which is very important from a water quality management perspective, is not possible from visual identification. Figure 3-6 shows a range of toxic and non-toxic strains of *Anabaena circinalis*, illustrating the difficulties in identifying cyanobacteria accurately. Expert visual microscopic identification of cyanobacteria can be supplemented/confirmed by molecular biology methods. These methods involve the extraction of DNA, RNA or proteins from cyanobacteria. The extracted material can be amplified and sequenced, and the sequences can be compared against published genetic databases to confirm the identity of the cyanobacteria, often to species level [4, 5, 6].

Genetic techniques can also be used to determine the presence of toxic cyanobacteria within a bloom. The genes responsible for the production of the major toxins have now been identified and it has been found that, in the majority of samples, the presence of the gene is an indicator of toxicity of cyanobacteria [7, 8, 9, 10]. With the rapid advancement of techniques such as real-time PCR and microarray technology, these methods

may eventually prove to be a quick, effective way to determine the identification and toxicity of a bloom in the field, or in the laboratory with a rapid turn-around time [11]. As only approximately 50% of blooms of potentially toxic cynaobacteria prove to be toxic, this could have important implications for the management of treatment and the implementation of cyanobacteria incident management plans.

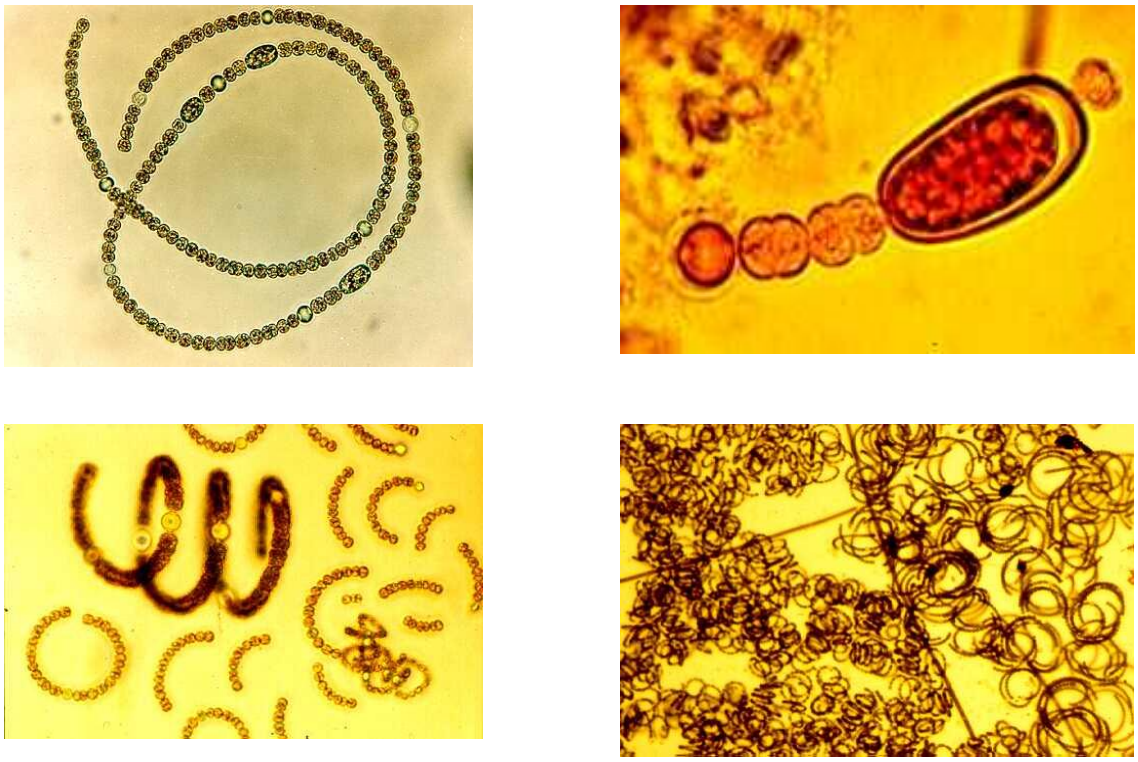

Figure 3-6 Different strains of the same cyanobacterium, *Anabaena circinalis*, several of which are toxic. This figure illustrates the difficulties inherent in microscopic identification for the determination of toxicity.

PRECISION OF CELL COUNTING

Counting precision is an indication of variability about the mean value when repeated measurements (counts) are made. The precision is a function of the number of organisms counted, their spatial distribution in the counting chamber and the variability of cells within a colony or trichome of the population. Many types of cyanobacteria form trichomes and the number of component cells may vary from two to more than two thousand. In the case of colony forming cyanobacteria the precision or reliability of the count is determined by the total number of units (colonies or trichomes) directly counted, not by the total number of cells counted.

Obtaining reliable estimates of abundance for the colonial cyanobacterium *Microcystis* can be particularly difficult due to the tendency of several species to form dense three dimensional aggregates of cells. Problems also arise when counting filamentous cyanobacteria such as *Aphanizomenon, Cylindrospermopsis, Arthrospira (Spirulina), Planktolyngbya, Limnothrix* and *Planktothrix,* where cells in trichomes are poorly defined (Figure 3-7). More information about the counting and identification of a range of cyanobacteria can be found in [2, 12].

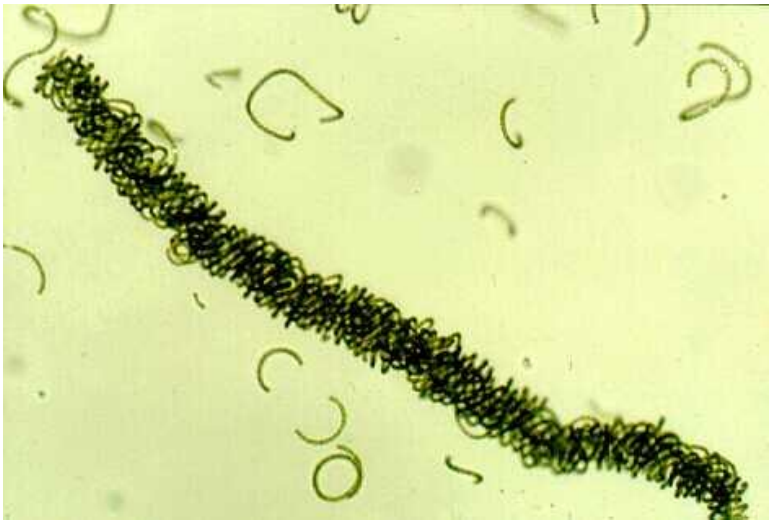

Figure 3-7 Uncertainty of enumeration of cyanobacteria is largely attributable to the clumped distribution of cells in colonies and filaments

The counting precision can be defined as the ratio of the standard error to the mean for replicated counts and assumes a Poisson distribution of counting units (cells, colonies or trichomes) in the counting chamber [13]. An acceptable level of precision for cyanobacterial counting is considered to be in the range of ± 20-30%. A precision of ± 30% enables a doubling of a population in successive samples to be detected while a precision of ±20% will enable a statistically significant change to be detected. This level of precision can only be obtained if high analytical effort is employed in the laboratory.

For more details on the calculation of cell enumeration precision, follow this link.

Level 3 detailed analytical techniques:

cell counting procedure using the sedimentation technique,

cell counting procedure using the filtration technique

calculation of biovolume

MEASUREMENT OF PIGMENT CONCENTRATIONS

Chlorophyll-a is a pigment present in cyanobacteria and eukaryotic algae. Phycocyanin is a pigment specific to cyanobacteria. These pigments can be analysed either by filtration and extraction of the pigments from the cells followed by measurement in a fluorometer or spectrophotometer (*in vitro*), or by bypassing the filtration and extraction steps and analysing the water sample directly in the fluorometer (*in vivo*). Chlorophyll-a has excitation and emission maxima of 436 and 680 nm, respectively. Phycocyanin has excitation and emission maxima of 630 and 660 nm, respectively. The turn-around time on the *in vitro* method is approximately 24 hours because extraction is generally allowed to proceed overnight. Results from the *in vivo* fluorescence methods are instantaneous. Several companies manufacture *in vivo* fluorescence instruments with flow through sample cells for real-time fluorescence measurement. These instruments can be installed at various locations in a water treatment facility, or suspended in probes from boats or buoys in a reservoir. A recent

publication has described the utilisation of a flow-through fluorescence probe to aid in the implementation of a cyanobacteria incident management framework [14]. There are two major disadvantages of using the flow-through instruments to capture real-time data compared with *in vitro* measurement methods. The *in vitro* methods are significantly more sensitive. The increased sensitivity can, in turn, lead to earlier detection of changes in cyanobacterial concentrations. The *in vitro* methods also relate the observed fluorescence in unknown samples to the fluorescence or absorbance of known standard compounds, yielding at least semi-quantitative concentration estimates. *In vivo* and flow-through measurements do not permit identification or direct quantification of the compounds responsible for fluorescence.

These methods do not allow the identification of cyanobacteria and cannot be used to replace the identification and enumeration methods. Rather they can be used as a low level monitoring tool in conjunction with the above methods.

Level 3 detailed analytical techniques:

spectrophotometric technique for the determination of chlorophyll-a

CYANOTOXINS

When potentially toxic cyanobacteria have been identified in a water source toxin analysis is required to determine if the cyanobacteria are, in fact, a toxic strain, and if so what concentration of cyanotoxin is likely to reach the treatment plant inlet water.

There is an increasing range of analytical methods available for the detection and quantification of cyanotoxins, and they vary in their manner of detection, the information they provide and level of sophistication [15]. For a complete overview and review of methods please refer to the report "Evaluation of Analytical Methods for the Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines" [16], together with a more recent international review [17]. A comprehensive discussion of the range of cell-based screening assays used to detect cyanotoxins is given in CRC for Water Quality and Treatment Research Report 60 [18]. A list of analytical methods commonly used for cyanotoxin detection and analysis can be found in Table 3-3.

The techniques available for cyanotoxin analysis include immunological or biochemical screening techniques based on enzyme-linked immunosorbent assays (ELISA) and enzyme activity (protein phosphatase inhibition, PPI) assays respectively, to quantitative chromatographic techniques based on high performance liquid chromatography (HPLC) and more sophisticated (and expensive) liquid chromatography-mass spectrometry (LC-MS, LC-MS/MS). Animal bioassays (mouse tests), and in some cases assays based on isolated cell lines, are also available for screening the entire range of toxins.

The method most commonly used to monitor microcystins is high performance liquid chromatography with photo diode array detection or mass spectral detection (HPLC-PDA or HPLC-MS). The analytical methods available for saxitoxins are continuously evolving and are based upon either high performance liquid chromatography and fluorescence detection or mass spectral detection (HPLC–FD or LC-MS/MS). Internationally the only technique recognised by the Association of Official Analytical Chemists (AOAC) for analysing saxitoxins from shellfish (where they are commonly found) other than mouse bioassay is a technique based upon liquid chromatography with pre-column derivatisation [19], although this technique is not yet widely used for analysis of cyanobacterial material. The method recommended for cylindrospermopsin is liquid chromatography with tandem mass spectrometry (LC-MS/MS), although this toxin can also be analysed using a HPLC method similar to microcystin. The method usually applied for the analysis of anatoxin-a is hydrophilic interaction liquid chromatography coupled with mass spectrometry (HILIC-MS).

For more information on various aspects of cyanotoxin analysis, follow these links:

ELISA

Protein phosphatase inhibition assays (PPIA)

Instrumental analysis

While the ELISA and PPI assays are so sensitive that the more concentrated scum samples may require dilution, most instrumental techniques require a pre-concentration step prior to quantification.

For more information on sample concentration follow this link

Another important aspect of the analysis of cyanotoxins is the percentage of the toxin that is found within the cell. Cyanotoxins can be in the dissolved state, after release from the cyanobacteria, or within the cell, or intracellular. The percentage of the toxin in each state will depend on the species, the state of health, and the period in the growth cycle of the cyanobacteria. For example, a healthy *Microcystis aeruginosa* cell during the exponential growth phase will probably contain around 98-100% of the toxin in the intracellular form while during bloom collapse most of the toxin might be released into the dissolved state. In contrast cylindrospermopsin can be up to 100% extracellular even in a healthy cell. This has important implications for risk mitigation through water treatment processes (Chapter 5) and should be an integral part of the monitoring program if high concentrations of toxic cyanobacteria are likely to enter the treatment plant.

For more information on the measurement of total, intracellular and extracellular cyanotoxins follow this link

A summary of analytical techniques that are available for different classes of toxins, their detection limit and other issues to consider when using them are given in Table 3-3.

For the techniques described in Table 3-3 the detection limits may vary depending upon standards available and instrumentation used. The availability of certified standards for toxin analysis is an issue worldwide and can impact on the accuracy and dependability of the results from some of these techniques.

A range of other methods used for screening and analysis includes neuroblastoma cytotoxicity assay, saxiphilin and single-run HPLC methods for saxitoxins and protein synthesis inhibition assays for cylindrospermopsin.

Table 3-3 Analytical methods commonly used for cyanotoxin detection and analysis. Abbreviations: HPLC – high performance liquid chromatography; LC – liquid chromatography; PDA – photodiode array; MS – mass spectrometry; PPIA - protein phosphatase inhibition assay; ELISA - enzyme-linked immuno-sorbent assay; HILIC - hydrophilic interaction liquid chromatography

TOXIN	ANALYTICAL METHOD	DETECTION LIMIT (µg/L)	DESCRIPTION
Microcystins	HPLC – PDA LC-MS	0.5 < 1.0 for individual microcystins	• Detection of microcystins by HPLC/PDA provides a spectrum of a separated analyte and attains a detection limit of considerably less than 1 µg/L for individual microcystins with appropriate concentration and cleanup procedures. • LC-MS is the method of choice, if available, for the measurement of toxins in drinking water
	PPIA	0.1	• Useful as a screening tool, relatively simple to use, highly sensitive, with low detection limits relative to guideline values.
	ELISA	0.05	• Detection of microcystins by ELISA provides semi-quantitative results
	Mouse bioassay	N/A	• Qualitative, screening assay
Nodularin	HPLC – PDA LC-MS PPIA ELISA Mouse bioassay	0.5 < 1.0 0.1 0.05 N/A	• Same as for microcystins (HPLC/PDA), • Commercially available protein phosphatase and ELISA assays for detecting microcystins are also useful for screening for nodularin. • Qualitative screening assay
Cylindrospermopsin	HPLC – PDA LC-MS, LC-MS/MS ELISA Mouse bioassay	Around 1.0 0.05 µg L^{-1}	• Cylindrospermopsin can be detected using LC/MS/MS (without the sample requiring extraction/reconcentration step) • Semi-quantitative screening assay capable of detecting low toxin concentrations • Qualitative screening assay
Anatoxin-a	HILIC/MS/MS	< 0.5 µg L^{-1}	• Sample concentration by SPE carbographs eluting with methanol /formic acid
Saxitoxins (paralytic shellfish poison – PSP's)	(HPLC) with post-column derivatisation and fluorescence detection ELISA Mouse bioassay	Depends upon the variant 0.02 µg L^{-1}	• Detection limits of saxitoxins (from Australian neurotoxic *A. circinalis*) have been determined using HPLC with post-column derivatisation and fluorescent detection and without sample concentration. • Semi-quantitative screening assay. Has advantage of detection of low levels STX. Poor cross reactivity to some analogues. • Qualitative screening assay

MEASUREMENT OF PARAMETERS INFLUENCING THE GROWTH OF CYANOBACTERIA

TEMPERATURE

Cyanobacterial growth rates are temperature dependent. There is significant potential for growth above about 15°C and maximum growth rates are attained by most cyanobacteria at temperatures above 25°C; however growth can also occur at low temperatures [20]. It has been suggested that these temperature optima are higher than for green algae and diatoms, and this allows cyanobacteria to dominate water bodies in warmer temperatures. However there is an argument that the belief that cyanobacteria prefer high temperatures is based mainly upon results from field studies where high temperatures are usually associated with thermal stratification, which may be the more important variable favouring the growth of cyanobacteria [21]. As a result, operational monitoring should include measurement of temperature at different depths to allow the determination of the degree of stratification of a water body. This should occur during routine sampling but thermistor strings are available that can be deployed remotely, collect data at much more frequent intervals and relay this data back to the operator. These systems can be coupled to meteorological stations to measure wind, solar insolation, temperature and humidity to gather the data required for hydrodynamic modelling. When used with phytoplankton cell counts and nutrient data the information of reservoir hydrodynamics is very useful in identifying the conditions that gave rise to increases in cyanobacterial abundance.

Level 3 detailed analytical techniques:

determination of temperature in the field

PHOSPHORUS

Phosphorus is an essential and limiting ingredient for cyanobacterial growth, and its levels are important for determining potential risks associated with toxic cyanobacteria (Chapter 2). Phosphorus is also an essential target variable in any long-term reservoir management plan to reduce the probability of future bloom formation (see Chapter 2 for more detail). Phosphorus in water sources is in the form of phosphate, and it can be measured as total phosphorus, or dissolved phosphate (filterable, or soluble, reactive phosphate, determined from filtered samples).

Level 3 detailed analytical techniques:

flow injection analysis and photometric detection of ortho-phosphate

SECCHI DEPTH

The amount of light received by cyanobacteria in a water body is influenced by turbidity, stratification, colour and ultraviolet transmission (determined by the types and concentration of the natural organic material). The light conditions in a given water body determine the extent to which the physiological properties of cyanobacteria will be of advantage in their competition against other phytoplankton. Light penetration into a water body is also important for growth of benthic cyanobacteria, the greater the light penetration the deeper benthic cyanobacteria can grow.

Generally, the zone in which photosynthesis can occur is termed the euphotic zone. By definition, the euphotic zone extends from the surface to the depth at which 1 % of the surface light intensity is measured. The euphotic zone can be estimated by measuring the transmittance of the water with a 'Secchi' disk and multiplying the Secchi depth reading by a factor of approximately 2-3. Those cyanobacteria that can regulate their buoyancy via gas vesicles are able to overcome these problems by moving to water depths with optimal light conditions.

Level 3 detailed analytical techniques:

for a procedure on Secchi depth measurements, click here

PH AND DISSOLVED OXYGEN

The measurement of pH and dissolved oxygen in a reservoir can yield indirect indications of cyanobacterial presence. During daylight hours, the organisms photosynthesise, consume dissolved carbon dioxide and produce oxygen. When cyanobacterial concentrations are high enough, this process can cause diurnal variations in pH and dissolved oxygen.

Level 3 detailed analytical techniques:

determination of pH in the field

determination of dissolved oxygen in the field

TURBIDITY

Turbidity measures the tendency of a water sample to scatter light; the higher the turbidity, the greater the degree of light scattering. This water quality characteristic is positively correlated with the concentration of suspended particles, including, potentially, cyanobacteria. Regular measurement of source water turbidity will allow for the establishment of site specific relationships with other indicators of cyanobacterial bloom formation, potentially leading to the development of early warning indicators.

Level 3 detailed analytical techniques:

determination of turbidity

PARTICLES

Particles are defined as organic or inorganic solid matter suspended in bulk water. Their concentrations can be measured directly by instruments that correlate the degree of light obscuration to the size and number of particles present in a sample. The principal advantage of particle counters versus turbidimeters is that the former are capable of generating detailed size distribution data.

CHAPTER 3 DEVELOPMENT AND IMPLEMENTATION OF A MONITORING PROGRAM (LEVEL 2)

VISUAL INSPECTION

RECORDING SHEET FOR A VISUAL INSPECTION

Sampling Point:……..

Date: …… Time: ……..

Additional site information (ie sketch map on back if algal problem is observed):

……

Colour of water: clear ☐ murky ☐ green ☐
Other ……

Surface scum: No ☐ Yes ☐ colour .....
extent ……

Water plants : No ☐ Yes ☐ floating ☐
submerged ☐
extent ……

Attached algae: No ☐ Yes ☐ on rocks ☐
on mud ☐
on plants ☐
extent ……

Odour from water: No ☐ Yes ☐ earthy/musty ☐
other…….

Algae/plant sample collected: No ☐ Yes ☐

Comments ……

……..

Sampler's name: …….

Received by: …… Date ……..

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SAMPLING FOR CYANOBACTERIAL IDENTIFICATION AND COUNTING

INTEGRATED WATER COLUMN SAMPLES

Integrated water column samples are also called 'hosepipe' samples and are recommended for open water sampling, where a representative sample of the water column over depth is desirable. The samples should be collected using a flexible hose pipe or rigid plastic pipe (Figure 3-1(L2)). A rigid pipe can be fitted with a one way valve, which simplifies the operation of withdrawing the pipe and sample from the water. The depth that the sample pipe is dipped should reflect the approximate depth to which cyanobacterial cells are likely to be mixed. When the stratification status is uncertain, a temperature probe, if available, may be used to determine the depth of any thermocline present. If this equipment is not available, a 5 metre long flexible pipe is recommended, but a 2 metre long pipe may be more appropriate in shallower water bodies (those that are less than 3 metres deep). The inner diameter of the pipe should be at least 2.5 cm and flexible pipes are probably more practical than rigid pipes for pipe lengths greater than two metres. The recommended method of obtaining a 'hosepipe' sample is shown in Figure 3-1(L2).

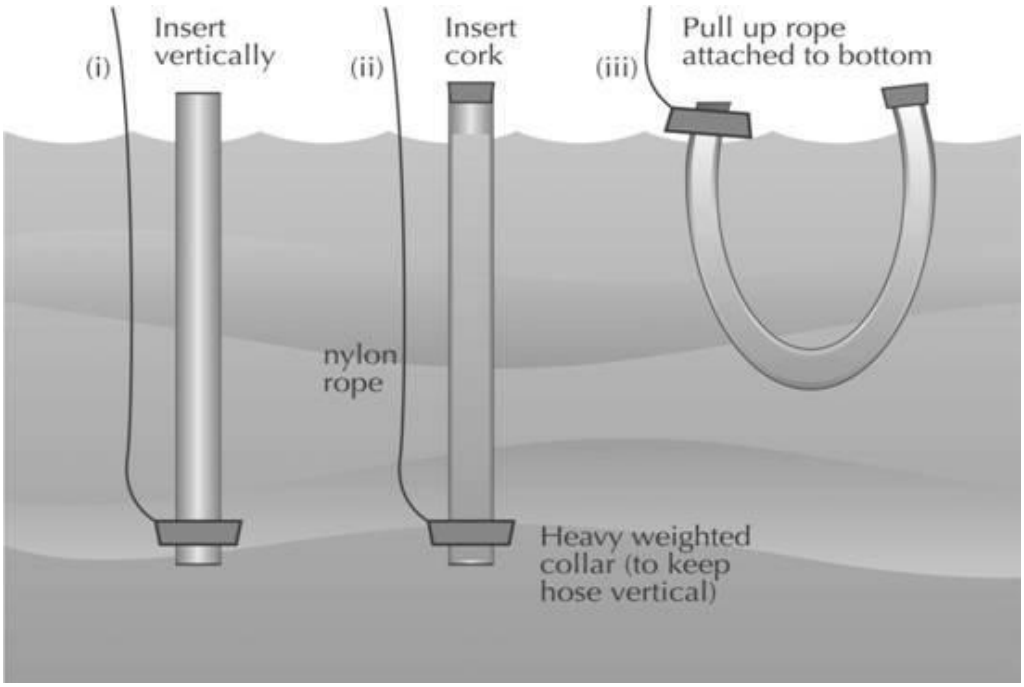

Figure 3-1 (L2) Using a hosepipe sampler to collect an integrated water column sample.

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DISCRETE DEPTH SAMPLES

Water sampling for public health surveillance is often required at the raw water abstraction point for reticulation to a drinking water treatment plant. For this purpose discrete depth samples or 'grab' samples are often collected with a sampling bottle apparatus (e.g. 'Van Dorn' or 'Niskin' samplers), that can be triggered to be filled at a specific depth below the surface corresponding to the offtake depth (Figure 3-2(L2)). The rationale for this is to determine the total load of cyanobacteria (and their toxins) to the water treatment plant. In addition, the degree of cell lysis and toxin release through the reticulation system can be measured from an accurate assessment of intact cells at the offtake point. This is important information for determining the appropriate strategy for cell and toxin removal in the treatment plant. When choosing a sampling site near the water abstraction point in a reservoir the size of the offtake and the abstraction pumping rate should be considered. If pumping rates are high, vortices may occur around the offtake or abstraction valves which indicate that surface water is being drawn down into the offtake. If this situation is present in the reservoir, a number of samples at depths ranging from the surface to the offtake depth should be taken to determine the total load of cyanobacteria cells and toxins entering the water treatment plant. The method for collecting a water sample at depth is depicted in Figure 3-2(L2).

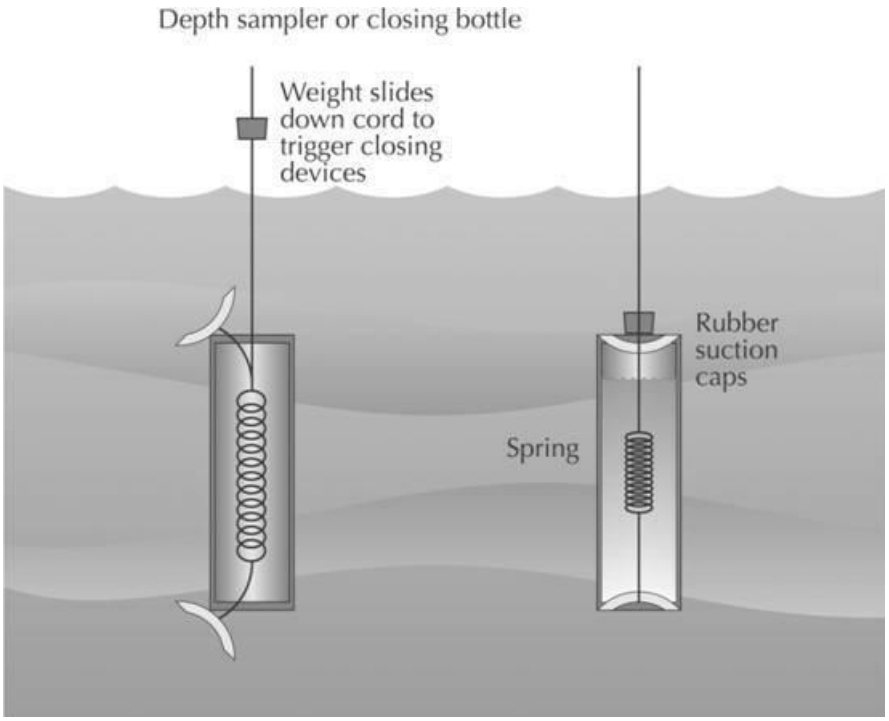

Figure 3-2(L2) Using a depth sampler or closing bottle to collect a grab sample at a discrete depth

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WORKED EXAMPLE ILLUSTRATING THE IMPACT OF SAMPLE REPLICATION

Duplicate samples are collected from the effluent of a drinking water treatment plant. They are analysed by LC-MS for microcystin-LR (MC-LR), with the following results:[1]

Sample 1: 1.12 µg L^{-1}
Sample 2: 1.27 µg L^{-1}

The estimated sample mean **m** is:

$$m = \frac{1.12\ \mu g/L + 1.27\ \mu g/L}{2} = 1.20\ \mu g/L > 1.0\ \mu g/L\ \text{WHO standard for MC-LR}$$

The observed mean of 1.20 µg L^{-1} is an estimate of the true MC-LR concentration in the effluent. At first inspection, the effluent MC-LR concentration appears to exceed the World Health Organization provisional MC-LR standard of 1.0 µg L^{-1}. However, given the observed variability in these two observations, how confident can water supply managers be about their estimate? In order to quantify the level of uncertainty, the following information is needed:

The sample standard deviation, Φ_{n-1} = 0.106 µg L^{-1}
The number of observations in the sample, **n** = 2
The degrees of freedom, **d.f.** = n – 1 = 1
Student's t statistic for 95% confidence, **d.f.** = 1, $t_{(1-\alpha = 0.95,\ d.f. = 1)}$ = 6.31

These values are used to calculate a one-sided 95% confidence interval and establish a lower confidence level (LCL). A one-sided confidence interval was chosen because the primary question is whether or not the true MC-LR concentration exceeds a regulatory threshold.

$$LCL = m - t \bullet \frac{\sigma_{n-1}}{\sqrt{n}} = 1.20 - 6.31 \bullet \frac{0.106}{\sqrt{2}} = 0.727 \approx 0.73$$

The calculated LCL of 0.73 is less than the 1.0 µg/L WHO provisional standard. Based on this data, a decision is made to resample the treatment plant effluent in triplicate, with the following results:

Sample 1: 1.11 µg L^{-1}
Sample 2: 1.21 µg L^{-1}
Sample 3: 1.27 µg L^{-1}

From this raw data, are calculated the following:
m = 1.20 µgL^{-1}
Φ_{n-1} = 0.0808 µg L^{-1}
n = 3
d.f. = n – 1 = 2

[1] This example assumes that the underlying distribution from which the data were sampled is normal.

$t_{(1-\alpha = 0.95,\ d.f. = 2)} = 2.92$

$$LCL = 1.20 - 2.92 \bullet \frac{0.0808}{\sqrt{3}} = 1.06$$

The second round of sampling, with the same mean as the original sampling event, yielded an LCL of 1.06 µg L^{-1} at the 95% level of confidence, which is greater than the 1.0 µg L^{-1} WHO standard. The standard deviation decreased by 24% versus the first round of sampling. More importantly, increasing the number of samples from 2 to 3 increased the degrees of freedom from 1 to 2. Increasing the degrees of freedom by 1 caused the critical t statistic to drop by more than one half. The combination of increased sample size and slightly lower standard deviation led to the calculation of a smaller one-sided confidence interval.

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A CASE STUDY OF SAMPLING PROGRAM DESIGN FOR CYANOBACTERIA FOR MYPONGA RESERVOIR, SOUTH AUSTRALIA.

Myponga Reservoir is a moderate-sized drinking water reservoir that has regular growth of the nuisance cyanobacterium *Anabaena circinalis* each summer. The reservoir is used directly for drinking water supply after water treatment with conventional treatment plant incorporating dissolved air flotation (DAF), and the capacity to dose with powdered activated carbon (PAC) for taste, odour and toxin control.

SITE DESCRIPTION

Myponga Reservoir (S 35° 21' 14", E 138° 25' 49") is located 70 km south of Adelaide in South Australia. The reservoir has a capacity of 26,800 ML at a full supply level of 211.7 m AHD (Australian Height Datum), an average depth of 15 m, a maximum depth of 36 m and a surface area of 2.8 km^2. The mean retention time based on abstraction is approximately 3 years. Water is removed from the reservoir via an offtake valve located on the dam wall at 195.2 m AHD.

ROUTINE SAMPLING PROGRAM

Samples are collected weekly in winter and twice-weekly in the summer growth season for identification and counting of phytoplankton from up to 10 separate locations. Sampling is concentrated at the offtake site where 4 separate samples are collected: a 0-5m integrated surface sample (Location 1221) and three discrete depth samples at 10, 20 & 30m (Locations 1222, 1223 & 1230). Spatial variability is assessed by collecting integrated column samples (0-5m) at 6 locations (Locations 1224-1229) spaced throughout the reservoir. The winter sampling frequency is weekly for 6 months from April - September which then increases to twice-weekly from October - March inclusive. The sampling program in winter incorporates a process of collecting and 'pooling' samples from the 6 reservoir locations which are then processed for a single cell count. If cyanobacteria are recorded in this pooled sample above a certain threshold (200 cells mL^{-1}) the individual sites will be re-assessed individually. Note that this pooling is only used in winter and all locations are sampled and counted individually in summer.

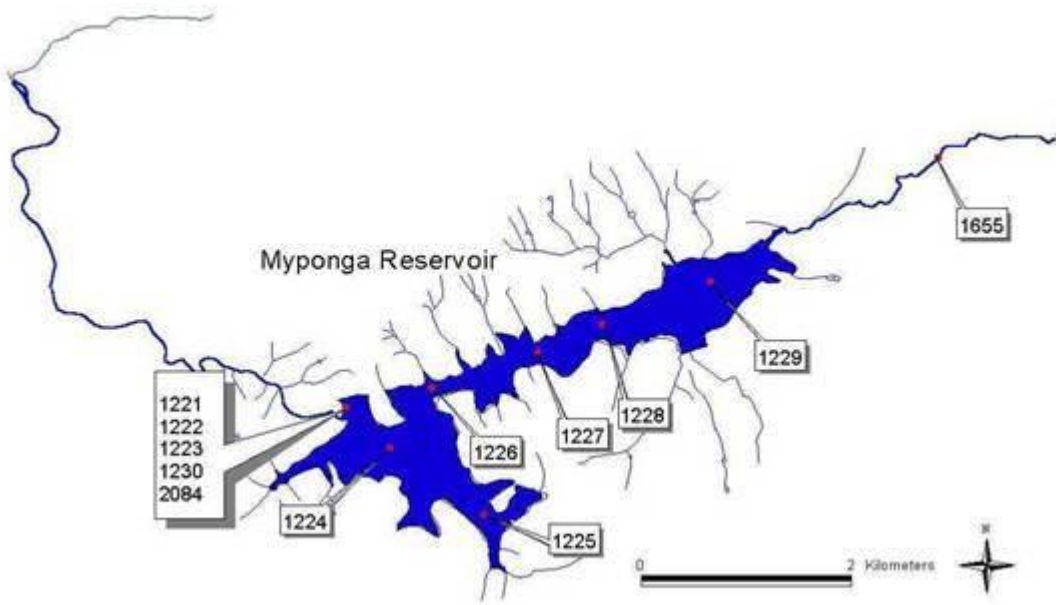

Figure 3-3(L2) Sampling Locations in Myponga Reservoir

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PRECISION OF CELL COUNTING

The precision (counting error) can be calculated from the total number of units counted (n) using the simple formula derived by Laslett et al. [13]:

Counting error (± %) = $100\sqrt{(2/n)}$ (1)

Equation 1 accounts for the variability of cells in a counting unit and assumes that the number of cells in a colony or trichome is always counted. Although it would be unusual for an analyst to count all cells in all trichomes or colonies as this equation assumes, it is still recommended as the benchmark method for enumeration of filamentous cyanobacteria. This is due to the fact that it takes into account the sometimes large variability in trichome/colony size when calculating counting error.

Higher precision will require a higher analytical effort and generally a higher cost. The relationship between counting error and counting effort is shown in Figure 3-4(L2). There is a very strong 'law of limited returns' applying for increased effort beyond about 50 colonies or trichomes counted.

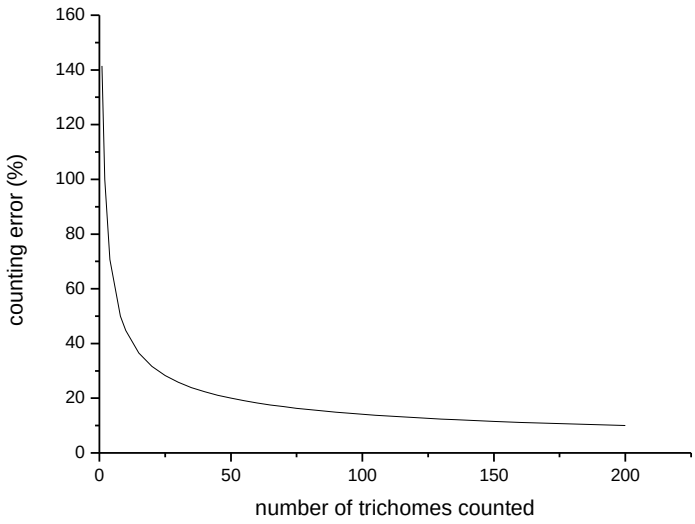

Figure 3-4(L2) Variation of counting error as a function of number of tricomes, or colonies, counted

At the completion of a count, the counting precision should be calculated using Equation 1. This figure could be reported in the following format for each taxon and/or for total cyanobacterial abundance:

XXXX cells mL^{-1} (minimum counting error = ± YY %)

Due to the fact that some of the counting errors may be very large, it is important to accompany any reporting of errors with some clarification and interpretation of these errors.

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ANALYSIS FOR CYANOBACTERIA AND THEIR TOXINS

ELISA

Enzyme linked immune substrate assays (ELISA) can be used for the analyses of several cyanobacterial toxins. The method is based on the coating of well plates or test tubes with toxin antibodies. The antibodies are molecules with a shape that specifically matches the structure of a toxin molecule. This specificity allows the antibodies to bind to, and immobilize, toxin molecules in solution. The number of antibody molecules that can be coated onto a given surface area is controllable and repeatable. Toxin molecules in solution will bind to coated antibodies when an unknown sample is added to a well or tube. Following the sample addition step, a solution containing a known concentration of enzyme-conjugated toxin molecules is added to the well or tube. Toxin molecules in this solution will occupy any binding sites left unoccupied after addition of the unknown sample. The enzyme in turn catalyzes a reaction that yields a color change that is inversely proportional to the concentration of toxin in the unknown sample.

ELISA assays for microcystins, nodularins, saxitoxins and cylindrospermopsins are commercially available as kits containing all of the necessary reagents. The advantage of these assays is that they are relatively inexpensive, simple and rapid to run, and the samples do not require pre-concentration. The total turnaround time is less than half a day. The assays can be performed in any laboratory equipped with multi-well pipetters and a spectrophotometer. The major disadvantage of the assays is that they cannot distinguish between toxin variants. This can complicate risk management decisions because of inter-variant toxicity differences. As a result, ELISA assays are ideal screening tools. They can be incorporated into a suite of routine analyses used to pinpoint the initial stages of a bloom event, and to determine when it is necessary to begin more expensive and time-consuming analyses capable of resolving toxin variants.

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PROTEIN PHOSPHATASE INHIBITION ASSAYS

The mode of toxicity of microcystins involves the inhibition of certain enzymes, the protein (serine/threonine) phosphatases, responsible for the dephosphorylation of intracellular phosphoproteins. The phosphatase PP2A is the most susceptible to inhibition by microcystin toxins. The basis of the PP2A inhibition assay is the measurement of phosphate release from a suitable substrate in the presence of a phosphatase enzyme preparation and an inhibitor such as microcystin. The most commonly used PP2A assay utilises *p*-nitrophenyl phosphate (pNPP) as substrate. In the presence of PP2A *p*-nitrophenol is released from the pNPP and can be measured photometrically. In the presence of an inhibitor such as microcystin, the release of the *p*-nitrophenol is reduced, and the difference between the sample and the control (in the absence of an inhibitor) can be calibrated to microcystin-LR concentration [22]. The assay does not discriminate between the different microcystin variants, and as mLR shows the greatest inhibition effect the result is usually described in terms of mLR toxicity equivalent concentration [23].

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INSTRUMENTAL ANALYSIS

Liquid chromatography (LC) is the technique used to separate mixtures of dissolved compounds (including toxins) prior to some form of instrumental detection. The sample for analysis is injected into a column packed with solid media. Different components of the mixture pass through the column at different rates due to differences in their partitioning behaviour between the liquid and solid phases. Using appropriate combinations of media, mobile solvent, temperature, and flow rate, unknown compounds will exit the column as discrete slugs whose presence in the effluent stream can be detected with ultraviolet (LC-UV), photodiode array, where a UV scan is taken for each peak, rather than an absorbance measurement at one wavelength only (LC-PDA), fluorescence (LC-FD) and mass spectrometric (LC-MS) detectors. When UV and FD response is plotted versus time, the separated compounds will show up as discrete peaks. Identification and quantification of the unknown is accomplished by comparing the timing and size (or scan) of an unknown peak exiting the column with the timing and size of peaks from calibration standards.

Microcystins and cylindrospermopsins can be analyzed by LC-UV. Ultraviolet detectors are more economical than MS and require less skill to maintain and operate. However, UV detectors are not capable of distinguishing among co-eluting toxin variants, or among toxins co-eluting with background organic material.

Saxitoxins can be analysed by LC-FD. Fluorescence detectors are less expensive than MS detectors and have the advantage of greater sensitivity. The primary disadvantage of fluorescence-based detection methods for cyanobacterial toxins is that they require additional reagents added to the LC column effluent. These compounds react with eluted saxitoxins to form a fluorescent end product. This post-column derivatisation procedure adds an additional level of complexity and cost to the analysis.

Microcystins, cylindrospermopsins, saxitoxins and anatoxins can all be analysed by LC-MS. In this technique, a portion of the flow exiting the chromatography column is routed through an MS detector, which generates mass spectra. A mass spectrum shows the relative distribution of components in a sample by their mass to charge ratios. Because cyanotoxin molecular weights are known with great precision, MS detectors allow the analyst to resolve co-eluting toxin variants with small molecular weight differences. MS detectors are also very sensitive, allowing analysts to achieve lower detection limits. The disadvantage of LC-MS systems is that they are complex, expensive, and the interpretation of their results requires a high level of experience.

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SAMPLE CONCENTRATION

Sample concentration and clean-up is a critical step in toxin analysis by instrumental techniques. The toxin of interest is often present at such low concentrations that an unprocessed sample may not generate a quantifiable signal when injected into an analytical instrument. Sample concentration involves passage of a known volume of raw water through a solid adsorbent material to which the toxin preferentially partitions. The adsorbent material is then treated with much smaller volumes of a second solvent, such as methanol, in order to remobilise the toxin into the liquid phase. Ideally, all of the toxin originally partitioned on to the solid phase will desorb into a volume of solvent that is orders of magnitude smaller than the original, thus yielding a concentrated sample. The ratio of the original sample volume to the second solvent volume is the concentration factor. This liquid phase, containing the desorbed toxin, is then subjected to further analysis.

The specifics of the concentration procedure described above vary somewhat from toxin to toxin. Variations in the procedure include the type of sorbent and the eluent solvent. The decision to use a procedural variation is driven not only by the type of toxin, but also by the concentration and nature of the background organic material present in the original water sample. As a result, it may take some time to optimise the concentration procedure when toxin analyses are initiated on samples from a new source. This will be the case whether analyses are performed in house or by a contract laboratory. The uncertainty is exacerbated by the fact that standardised analytical procedures do not yet exist for many of the cyanobacterial toxins in many countries. As a result, the most prudent course of action for water supply managers may be to negotiate the desired quality control criteria (internal standard recovery, surrogate recovery, duplicate reproducibility, etc.) and allow the laboratory to choose the method that best meets the contractual requirements.

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MEASUREMENT OF TOTAL, INTRACELLULAR AND EXTRACELLULAR CYANOTOXINS

In a raw water sample, total toxin concentration is measured after all the cyanobacteria in the sample have been lysed to release the toxin into the dissolved state. The most appropriate technique for liberating intracellular toxin is freeze/thawing in the presence of a solvent appropriate for the particular toxin. If dissolved toxin concentration is also required, two samples should be taken, one treated as for total toxin analysis (above). The other should be gravity filtered through glass fibre filter, to avoid damage to the cells, and the filtrate analysed for toxin concentration. The difference between the total toxin concentration and the filtrate, or dissolved toxin concentration, is the intracellular concentration.

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CHAPTER 3 DEVELOPMENT AND IMPLEMENTATION OF A MONITORING PROGRAM (LEVEL 3: DETAILED EXPERIMENTAL PROCEDURES)

ANALYSIS FOR CYANOBACTERIA AND THEIR TOXINS

Please note: It is recommended that methods are chosen on a case by case basis, depending on the equipment and expertise available. The methods detailed in this section are not necessarily those recommended for each water authority and are mainly for illustrative purposes. Some of the following methods are specific to a particular instrument so will not be transferable to other instruments.

Many of the methods given in Level 3 can also be found in Standard Methods for the Analysis of Water and Wastewater [24]. A recommended text for the analysis of cyanotoxins is [17]

CYANOBACTERIA IDENTIFICATION AND ENUMERATION BY MEANS OF A SEDIMENTATION METHOD.

This method is suitable for raw water samples containing phytoplankton which includes samples from dams, lakes, rivers and streams (spanning all trophic states).

PRINCIPLE OF THE METHOD

Phytoplankton samples are fixed using a suitable preservative (formaldehyde, Lugol's or glutaraldehyde). The sample is then pressurised to rupture gas vacuoles present in cyanobacteria, after which a sub-sample of known and appropriate size (1-6mL) is transferred to a sedimentation chamber. The sample is left to settle for a certain period of time. After this period of time, phytoplankton taxa are identified, as far as possible, to species level and enumerated simultaneously. The results of the enumeration are expressed as a concentration of cells per volume of water (cells mL^{-1}).

APPARATUS, MATERIALS AND REAGENTS

INSTRUMENTS AND EQUIPMENT

- Inverted light microscope with a 40x objective and a Whipple grid in the eyepiece (Figure 3-2(L3))
- Dispenser pipette
- Deflation instrument
- Humidifier
- Computer with spreadsheet- and phytoplankton counting software. Other counting devices may also be used
- Calibrated mass balance

GLASSWARE

- Perspex or glass sedimentation chambers
- Cover slips, No. 0 thickness
- Glass beaker

OTHER MATERIALS

- Lens cleaning tissue
- Lens cleaning liquid

REAGENTS

- Formaldehyde solution
- Lugol's iodine solution
- Distilled water

Figure 3-1(L3) Inverted light microscope

PROCEDURE

SAMPLE PREPARATION

- Note that before any work is undertaken, it is imperative that the analyst is familiar with the safety precautions of the hazardous chemicals used.
- The sample should be preserved immediately at the site or in the laboratory when the samples are received. Lugol's iodine solution is added at a ratio of 1:100 to give the sample a weak tea colour. Formaldehyde is added to a ratio of 2:100 [2].
- After preservation, the gas vacuoles of the cyanobacteria need to be pressure deflated to allow these organisms to settle out. Deflating is done by placing a sub-sample in a thick-walled metal container to a volume where there is no air left in the container when it is closed with a rubber stopper. Apply pressure on the rubber stopper with a hammer or similar instrument. However, when Lugol's solution is used as preservative, no deflation is needed.
- The sample is then shaken to ensure the uniform distribution of cells.
- With a calibrated dispenser pipette transfer 1mL of the sample (or sub-sample) into a sedimentation chamber labelled with the sample name and date. Leave it to settle for approximately 30 minutes on a bench free from any vibrations and disturbances. It is important to use a new pipette tip for each sample, as this will reduce the chances of cross contamination.
- Place the sedimentation chamber on the inverted light microscope and briefly examine for turbidity, as well as density and distribution of phytoplankton in the sample.

- In the event of the sample being too turbid or too dense in algal concentration it will need to be diluted. Start by diluting the known volume of the preserved (and deflated) sample to half the volume. This is done by adding one part sample to one part distilled water, giving a dilution factor of 2. Re-examine the chamber briefly for turbidity, if still too turbid or dense in algal concentration, add one part of the diluted sample to one part distilled water, giving a dilution factor of 4. Re-examine the chamber briefly for turbidity. This process is repeated until phytoplankton cells are visible enough to identify and enumerate accurately.
- In the event of the sample being too low in algal concentration, a greater volume can be settled out. This is done by estimating the volume of sample necessary to identify algal taxa without any phytoplankton cells or particles obscuring each other. This would then be the final volume of sample added to the sedimentation chamber. It should be noted that accurate estimation of this volume is gained with experience. For example: After 1mL is added and the sample examined briefly, the analyst feels that more of the sample could be added without hampering the identification process, and an estimate of 4mL is made. An additional 3mL of sample is then added to the 1mL already in the sedimentation chamber. The factor with which the counts are multiplied will then be divided by the amount of sample (mL) present in the sedimentation chamber.
- Make sure that the final volume of sample in the sedimentation tube is recorded on the sedimentation chamber.
- The sedimentation chamber is then filled to the top with distilled water and covered with a cleaned cover slip so that no air is left in the sedimentation chamber.
- Place the sedimentation chamber in a humidifier with water in the bottom section to prevent evaporation of sample water.
- The height of the sedimentation chamber will determine the time necessary for the phytoplankton to settle. For every 1cm of the chamber, the phytoplankton should be allowed to settle for a period of 24 hours.

IDENTIFICATION AND ENUMERATION

- Remove the sedimentation chamber from the humidifier, taking care not to disturb the settled material at the bottom of the sedimentation chamber.
- Place it in the round slot on the microscope table and switch on the inverted light microscope.
- For identification of phytoplankton, 400x magnification is recommended.
- Identify and enumerate the settled phytoplankton to at least genus level, and where possible, to species level. Start counting on the left hand side of the sedimentation chamber on a line running through the centre of the sedimentation chamber. Identify all the phytoplankton taxa in the Whipple grid. Move one grid at a time from left to right, identifying all the phytoplankton species within the grid (Figure 3-2(L3)). Continue counting in this manner until at least one lane is completed. Note that a minimum of 200 cells need to be identified.

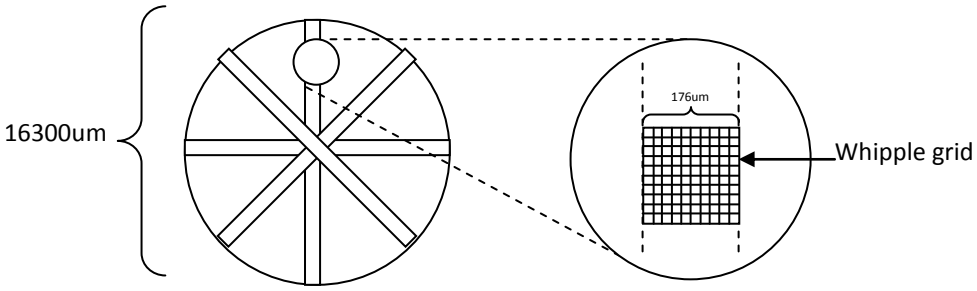

Figure 3-2(L3) Line diagram showing the orientation of lanes and the Whipple grid.

- If the count is less than 200 cells at the end of the first lane, rotate the sedimentation chamber to a cross section that has not yet been analysed and continue as above, this time from right to left. Continue these steps until a total greater that 200 cells is achieved. Do not stop in the middle of a lane if this value is reached, but always finish the lane, so that the exact area analysed is known.
- Every phytoplankton cell is counted as one, whether it is part of a colony/filament or not. The amount of colonies/filament per taxon is also counted.
- If a cell is located on the edge of the Whipple grid, it is only counted if more than half of the cell is located within the Whipple grid. If not, the cell is not counted. When counting cells in a colony/filament, only those cells falling within the Whipple grid are counted.
- Record the counts on a well marked sheet with space for the sample name, date sampled, date of analysis, the amount of lanes enumerated, objective used, the conversion factor, name of the analyst and the count of each species/genus.
- Any of the following literature listed below is recommended for accurate identification of phytoplankton. Some other references not listed, may also be useful.
    - Belcher, H. & Swale, E. 1976. A beginner's guide to Freshwater Algae. Her Majesty's Stationery Office (HMSO). ISBN 0 11 881393 5.
    - Belcher, H. & Swale, E. 1979. An illustrated guide to River Phytoplankton. Her Majesty's Stationery Office (HMSO). ISBN 0 11 886602 8.
    - Bellinger, E.G. 1992. A key to common algae. Freshwater, estuarine and some coastal species. Fourth Edition. The Institution of Water and Environmental Management, London.
    - Entwisle, T.J., Sonneman, J.A. & Lewis, S.H. 1997. Freshwater Algae in Australia. Sainty and Associates Pty Ltd, NSW, Australia.
    - John, D.M., Whitton, B.A. & Brook, A.J. 2002. The Freshwater Algal Flora of the British Isles. An identification guide to freshwater and terrestrial algae. Cambridge: Cambridge University Press.
    - Janse van Vuuren, S., Taylor, J., Gerber, A. & van Ginkel, C. 2006. Easy identification of the most common freshwater algae. A guide for the identification of microscopic algae in South African freshwaters. North West University, Private Bag X6001, Potchefstroom, South Africa and DWAF, Private Bag X313, Pretoria, South Africa.
    - Prescott, G.W. 1951. Algae of the western great lakes area. Wm. C. Brown Co. Publ., Dubuque, Iowa.

- Prescott, G.W. 1978. How to know the freshwater algae. Third Edition. McGraw-Hill, Wm. C. Brown Co. Publ., Dubuque, Iowa.
- Smith, G.M. 1950. The fresh-water algae of the United States. Second Edition. McGraw-Hill Book Company, New York.
- Wehr, J.D. & Sheath, R.G. 2003. Freshwater algae of North America: ecology and classification. San Diego, California: Academic Press.
- Huber-Pestalozzi, G. 1950. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 3. Cryptophyceen, Choromadinen, Peridineen. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1955. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 4. Euglenophyceen. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1961. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 5. Chlorophyceae (Grünalgen). Ordnung: Volvocales. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1962a. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 1. Algemeiner Teil. Blaualgen. Bakterien, Pilze. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1962b. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 2. Diatomeen. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
- Huber-Pestalozzi, G. 1962c. Das Phytoplankton des Süsswassers: Systematik und Biologie. Tl. 2. Hlf. 1. Chrysophyceen, Farblose Flagellaten, Heterokonten. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

SAFETY PRECAUTIONS

HAZARD WARNING

- Formaldehyde – Flammable, irritant liquid. Toxic ☠ by inhalation, contact or ingestion.
- Lugol's solution – for external use only. Do not swallow.
- Ethanol – flammable liquid. Keep away from sources of ignition.

SAFETY INSTRUCTIONS WHEN WORKING WITH FORMALDEHYDE (MERCK, 2004)

- Formaldehyde is toxic by inhalation, in contact with skin and if swallowed it could lead to serious irreversible effects. It could also cause burns, lead to sensitivity during skin contact and there is evidence suggesting carcinogenicity.
- Formaldehyde should always be stored at 15°C - 25°C in a tightly closed container in a well ventilated place.
- When handling this substance, personal protective equipment, such as latex gloves, a laboratory coat and safety glasses, should be used.
- Formaldehyde is heavier than air and should always be used in a suitable extraction cabinet, that is, one with a down flow extraction system.

- Never inhale the substance and avoid any generation of vapours of this substance. The inhalation of fresh air is best after inhalation of formaldehyde.

- After contact with the skin or the eyes, the affected area should be washed thoroughly with plenty of water. Contaminated clothing should be removed. Immediately call a physician/ophthalmologist.
- Should swallowing occur, drink plenty of water and call a physician.
- Formaldehyde vapours are combustible, as it forms explosive mixtures with air at ambient temperatures. In the case of fire, extinguish with water, CO_2, foam or powder, whilst remaining at a safe distance.
- Formaldehyde, and solutions containing formaldehyde, should always be disposed of using a proper waste disposal system.

SAFETY INSTRUCTIONS WHEN WORKING WITH ETHANOL (MERCK, 2006)

- It should be noted that this colourless liquid forms highly combustible vapours, as it mixes with air at ambient temperatures and backfiring could occur. Measures should also be taken to prevent electrostatic charging.

CALCULATIONS AND EXPRESSION OF RESULTS

CALCULATION OF THE PHYTOPLANKTON BIOMASS AS CELLS/Mℓ

Phytoplankton biomass is expressed as the amount of phytoplankton (or cyanobacteria) cells per millilitre (cells mL^{-1}). This value is calculated below (values used in the calculation are for example purposes only).

- Calculate the area of the circular sedimentation chamber floor:

$$\text{Sedimentation chamber floor area} = \pi r^2$$
$$= \pi \times (8150\mu m)^2$$
$$= 208\ 672\ 438\mu m^2$$

- Calculate the area of one rectangular lane:

$$\text{Lane area} = \text{diameter of sedimentation chamber} \times \text{width of Whipple grid}$$
$$= 16\ 300\mu m \times 176\mu m$$
$$= 2\ 868\ 800\mu m^2$$

- Calculate the conversion factor:

The conversion factor is calculated by dividing the total sedimentation chamber floor area by the total lane area. Note that the total lane area is the area of one lane multiplied by the amount of lanes analysed. For this example 1 lane was analyzed.

$$\text{Conversion factor} = \frac{\text{Sedimentation chamber floor area}}{\text{Total lane area}}$$
$$= \frac{208\ 672\ 438\mu m^2}{(2\ 868\ 800\mu m^2 \times 1)}$$
$$= 72.739$$

At this stage it is important to remember the volume of the original sample that was sedimented. The conversion factor is divided by the volume (mL) of sample that was used.

$$\text{Final conversion factor} = \frac{\text{Conversion factor}}{}$$

$$\text{Volume of sample used} = \frac{72.739}{3\text{m}\ell} = 24.246$$

- Calculate the biomass as cells mL^{-1}

The biomass, expressed in cells mL^{-1}, is calculated by multiplying the count of each taxon with the final conversion factor.

Biomass	=	Count x Final conversion factor
	=	78 x 24.246
	=	1891.188
	≈	1891 cells mL^{-1} (rounded to the nearest integer)

Calculating the percentage composition of a taxon

$$\%\ \text{composition} = \frac{(\text{biomass concentration of the taxon in cells mL}^{-1}) \times 100}{\text{Total biomass concentration in cells mL}^{-1}}$$

REPORTING PHYTOPLANKTON RESULTS

- Phytoplankton concentration is expressed as cells mL^{-1} and is rounded to the nearest integer. It is recommended that results be reported to genus level, except when the analyst is a qualified taxonomist and has the skill to identify phytoplankton to species level.
- Percentage composition may be useful to determine the dominant species.
- Phytoplankton biomass can also be better expressed in terms of biovolume that takes the size, shape and volume of each organism into account.

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PHYTOPLANKTON IDENTIFICATION AND ENUMERATION BY MEANS OF THE FILTRATION METHOD

BACKGROUND

This method is suitable for all types of freshwater including dams, rivers and treated drinking water.

PRINCIPLE OF THE METHOD

A known volume of sample is filtered through a nitrocellulose membrane filter. The filter is mounted on a microscope slide with immersion oil and placed under a microscope and the cyanobacteria are visually identified and counted by the microscopist.

By using this method, the analyst will be able to identify and quantify algae in very low (e.g. final drinking water) or high concentrations (e.g. raw water) where additional blending and/or dilution steps are included for very dense algae populations.

APPARATUS, MATERIALS AND REAGENTS

INSTRUMENTS AND EQUIPMENT

- Microscope with a mechanical stage, 10x, 40x and 100x objective lenses and preferably also with a Plan-Neofluar 63x oil immersion lens or other similar lenses (refer to Figure 3-3(L3)).

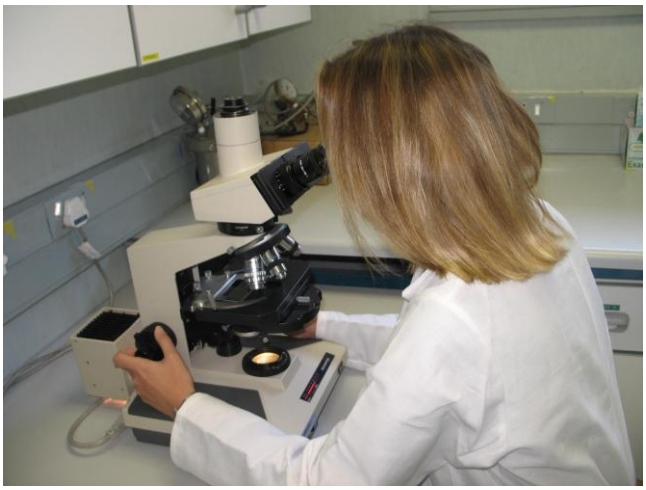

Figure 3-3(L3) Compound light microscope

- Vacuum manifold fitted with membrane filter holders capable of holding 47mm diameter or other similar membrane filters (refer to Figure 3-4(L3)). Vacuum pump with a vacuum gauge and adjustable vacuum connected (via a collection vessel) to the vacuum manifold.
- Homogeniser, with variable speed (Figure 3-5(L3)).

Figure 3-4(L3) Vacuum manifold fitted with 47mm membrane filter holders

Figure 3-5(L3) Homogeniser with variable speed.

GLASSWARE

25mL, 50mL and 2000mL measuring cylinders.

OTHER MATERIALS

0.45µm filters of appropriate quality.

REAGENTS

- Lugol's solution - 20g potassium iodide (AR) with 10g iodine crystals (AR) in 200mL water with 20Ml glacial acetic acid (minimum assay 98% m m^{-1}). Store in a dark glass bottle. The solution is stable for 3 years.
- Buffered formalin - 20g sodium borate (AR) in 1L formaldehyde (minimum assay 37% m m^{-1} AR). This solution is prepared fresh as required.

PROCEDURE

SAMPLE PREPARATION

- Samples should be filtered on the day of collection. Where necessary, bottled samples may be stored between 1 - 8°C for a maximum of three days. In special circumstances, whole samples may be preserved by adding 40mL L^{-1} buffered formalin or 3mL L^{-1} Lugol's solution. Dried filters may be kept in the dark at room temperature for a maximum of 20 days but only if unavoidable.
- Ensure all taps on the vacuum apparatus are turned off.
- Ensure the filter holder is clean. Squirt sufficient water onto the filter holder to wet the surface to prevent the formation of air bubbles. Place the numbered filter onto the filter holder and position the graduated filter funnel.
- Mix the sample well by inverting and shaking the sample bottle several times (See *Note 1*). Using a measuring cylinder, measure a predetermined volume of sample into the graduated filter funnel for filtering (See *Note 2*). The use of the measuring cylinder is more accurate than the use of the graduated filter funnel. The volume will depend on algal densities and also turbidity but commonly falls between 20mL for dam water and 1200mL for potable water. (Previous volumes used may give an indication of the volume needed). See *Note 3* for highly turbid and algal dense samples.
- The tap on the filtering apparatus is turned on and the sample allowed to filter under suction. The suction must not exceed 80kPa.
- Once the sample has nearly finished filtering through, turn off the suction at the tap and let the remainder filter through passively. Never suck the filter dry using suction as this distorts cells and breaks colonial forms.
- Remove the membrane filter and place on a clean surface or tray and leave to dry in the dark at room temperature.
- The sample number and the volume of sample filtered are entered into the relevant laboratory record book.
- The graduated filter funnels must be rinsed thoroughly between samples to avoid contamination. The funnels must be washed with detergent, cold water and a brush once a week or whenever a deposit is noticed or when extremely dense samples are filtered.
- Clean or replace the plastic filter holder grid if it becomes blocked. This will be evident by an uneven distribution of sample on the membrane filter.

- A check must be kept on the water level in the reservoir to prevent water from being drawn into the vacuum manifold. When the water level is high the vacuum must be closed and the reservoir drained.

> *Note 1: Sample bottles should not be completely filled as this prevents thorough mixing when the bottle is shaken.*
>
> *Note 2: When* Microcystis *is present in samples, it is necessary to break up colonies into individual cells but without destroying the cells. To do this, homogenize approximately 100mℓ sample for approximately 10 seconds using the homogenizer on speed 13 500rpm. Thereafter continue with filtering the sample (adapted from Zohary and Pais-Madeira, 1987).*
>
> *Note 3: If a very turbid sample, or a sample with an exceptionally high algal density is to be filtered, it may be necessary to dilute the sample. The sample is mixed vigorously (especially when buoyant algae are present) and the necessary volume of sample made up to at least 50mL with distilled water using a calibrated measuring cylinder; this ensures an even distribution of sample on the filter.*

IDENTIFICATION AND ENUMERATION

- The membrane filter must be completely dry before being viewed. This is essential if clarity is to be obtained. To test for dryness a small spot of immersion oil can be applied to the edge of the filter. If the filter becomes transparent, then it is dry. If the filter is damp, the oil area will remain opaque.
- Once dry, the filter is placed on a drop of immersion oil on a microscope slide and a second drop of oil placed gently on top of the filter. This will clear the filter enabling light to shine through.
- The slide and filter are then placed on the microscope stage.
- To ensure an even distribution of the sample, the filter is examined briefly under low magnification. The higher magnification oil immersion lens is then carefully swung into position for enumeration.
- Identify and count the algae in a number of fields which must be totally randomly selected. The easiest way of achieving this is to avoid looking down the microscope when the field is moved, or use an accepted random cell selection technique.
- SCS (standard counting software) is available commercially for the enumeration of organisms like invertebrates and phytoplankton (see Addendum A for supplier's details). The SCS has its data storage facility from which results are exported to LIMS (Laboratory Information Management System) once all samples for the day are complete. Throughout the counting, data can be copied to an Excel worksheet on the analyst's C-drive as a temporary file. The SCS will indicate when sufficient fields have been counted to reach a pre-determined level of statistical confidence. This level may only be set by the Section Head and is recorded together with the data. In the event of a failure in the counting software, a manual count can be done using a minimum of 15 fields that would yield a count with acceptable precision.
- In order to identify the algae observed, reference could be made to any applicable phytoplankton identification book (refer to Section 4.6 for a detailed reference list).

- Turbid samples should be read just like the non-turbid samples. If no algae are visible, a comment to that effect should be captured on LIMS.

SAFETY PRECAUTIONS

HAZARD WARNING

- Glacial acetic acid (and thus Lugol's solution) is dangerous and should be handled with care in a fume cupboard. Do not pipette by mouth.
- Ensure that you are familiar with the dangers and treatment associated with each of the substances mentioned above.

CALCULATION AND EXPRESSION OF RESULTS

The actual number of algae observed is converted to numbers per milliliter.

$$\text{Conversion factor (CF)} = \frac{\text{Area of filter}}{\text{Area of view under microscope}}$$

$$\text{Algae number} = \frac{\text{CF x no. of individuals counted}}{\text{No. of fields x volume filtered (m}\ell\text{)}}$$

Under normal circumstances the SCS (algal counting software) performs the final calculation. The conversion factor should be checked and changed if necessary if a new microscope or different optics is used.

The results are expressed as counts per mL.

Sources of error may arise from the following:

- Poor mixing of sample before filtering.
- Incorrect identification to genus level.
- Inadequate selection of random fields.
- Incorrect optics.
- Uneven distribution of algae on membrane filters due to clogged holder.
- Damage to cells during dispersion of colonies.
- Loss of cell detail due to damage/desiccation on filter.
- Incorrect counts due to cells being clumped.
- Very high turbidity/silt obscures algae.

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BIOVOLUME CALCULATION OF PHYTOPLANKTON AND CYANOBACTERIA [25]

Measurement of biovolume as an estimate of biomass of individual taxa in a sample is a determinand that can provide valuable additional information to the usual cell counts. The biovolume of a species can be regarded as more representative of the relative contribution to the total phytoplankton population. Biovolume can also be used as measure or "surrogate" for the potential toxicity or toxin hazard for species and taxa in a sample, where no toxin determinations have been made (Chapter 2).

The method for determining cyanobacterial cell biovolume is:

- Determine the appropriate geometric shape and measure the dimensions of cyanobacterial cells using 1000x magnification with an oil immersion objective. Measurements for the closest geometric shape can generally only be made to approximately 0.5 μm. The majority of cyanobacterial cells have a shape that approximates to either a sphere or a cylinder (cells within a filament).
- Determine the mean cell dimension for the taxa based upon a minimum of 30 cells per sample and up to 100 cells, depending on size variability.
- Calculate the mean cell volume using the mean cell dimension and the appropriate calculation for that shape (Table 3-1(L3)).

The average cell volume is then multiplied by the total cell number to obtain biovolume in cubic microns per millilitre ($\mu m^3\ ml^{-1}$), which should then be converted to cubic millimetres per litre ($mm^3\ L^{-1}$) by dividing by 10^6.

This process should be repeated individually for each cyanobacterial species.

It is recommended that the biovolume of individual species should be determined on the first two consecutive regular samples from a site in a particular waterbody (i.e. weekly samples) and if the variance is not significantly different (determined by *t*-test), it is then only necessary to recheck and compare biovolume for that species at monthly intervals.

Cyanobacterial cells that have been preserved with Lugol's iodine have been shown to shrink (L. Bowling, DIPNR, pers. comm.). The amount of shrinkage may vary from taxon to taxon and therefore may need to be considered in any biovolume calculations.

Table 3-1(L3) Common geometric shapes and formulae for volume calculations

Geometric Shape	Cyanobacteria examples	Measurements	Volume Calculations
Sphere	*Anabaena, Microcystis, Aphanocapsa*	Cell diameter	$4/3\pi r^3$ or $\pi d^3/6$
Ellipsoid	*Anabaena, Anabaenopsis, Coelomoron*	Cell diameter and length	$\pi d^2 L/6$
Cylinder	*Aphanizomenon, Pseudanabaena Cylindrospermopsis Oscillatoria, Planktothrix, Nodularia*	Cell diameter and length	$\pi r^2 L$ or $\pi d^2 L/4$
Cone	*Myxobaktron plankticus*	Cell diameter and length	$* \frac{1}{12} \pi \ Ld^2$
Rod	*Aphanothece,Cyanonephron, Rhabdoderma, Synechococcus*	Cell diameter and length	$\pi dL^2/6$

1. r = radius
2. L = length
3. d = diameter
4. * = diameter at the base of the cone

A tool available for analysts to aid in the determination of biovolume is a freeware biovolume calculation program available on the internet. Its address is http://www.msu.edu/~kirschte/biovol

Another site which provides a spreadsheet file containing average, standard deviation, minimum, and maximum biovolumes (μm^3) of 545 algal taxa commonly occurring in samples collected by the United States Geological Survey, National Water Quality Assessment Program (NAWQA) can be found at http://diatom.acnatsci.org/nawqa

To determine the biomass of a sample of algae consisting of more than one species, the following method is described by Hötzel and Croome [2]. Measure the dimensions of the cells of each species and calculate an average volume for each species as described previously. The average volume for each species is then multiplied by the cell counts for each species and all the products are summed to determine a biovolume per sample in mm^3 per millilitre. The equation for calculating the total wet algal volume is given by APHA, [24]:

$$V_t = \sum_{i=1}^{n} (N_i \times V_i)$$

where:
V_t = total plankton cell volume ($mm^3\ L^{-1}$)
N_i = number of organisms of the *i*th species $m^{-1}L$
V_i = average volume of cells of the *i*th species (μm^3)

Standard calculated cell volumes for a variety of cyanobacterial species found within Australian freshwaters are given in Table 3-2(L3). These volumes were calculated by choosing cell dimensions that were the midpoint of the range of values that were provided by a range of water quality laboratories from South Australia, New South Wales and Queensland that have NATA registration for algal counting and from values provided in authoritative taxonomic guides by Baker [26], McGregor and Fabbro [27] and Baker and Fabbro [28]. The data in the table are specific for those species, and therefore cannot be used for other species within the same genus, as their cell dimensions can vary widely.

Cells described as spherical in this table may on occasions also appear to be ellipsoidal or even hemispherical depending upon the state of cell division. In individual cases, operators should use judgement to choose the most appropriate cell shape to estimate cell volume.

Some filamentous cyanobacteria with attenuated apices contain cells that vary in shape and size from quadrate (midtrichome) to cylindrical towards the apices. Those genera that exhibit distinct attenuated trichomes (e.g. *Gloeotrichia*) are not included in this table.

Some laboratories use published cell volume data to convert their cell counts to biovolumes. These methods are a useful indicator provided that the extent of possible error is known and acknowledged when the data is used, and taken into account when management decisions are made.

For best practice, biovolume should only be used when individual laboratories take the important step of determining their own biovolumes for individual populations.

Table 3-2(L3) Standard reference algal cell volumes for various taxa based upon cyanobacteria from Australian freshwaters.

Taxa	Geometric Cell Shape	Mean Cell Volume (μm^3)
Anabaena affinis	Sphere	76
Anabaena aphanizomenoides	Cylinder	98
Anabaena bergii	Cylinder	85
Anabaena circinalis	Sphere	250
Anabaena crassa	Ellipsoid	330
Anabaena flos-aquae	Sphere	56
Anabaena pertubata var *tumida*	Sphere	270
Anabaena planktonica	Ellipsoid	433
Anabaena inequalis	Sphere	70
Anabaena oscillarioides	Ellipsoid	36
Anabaena smithii	Ellipsoid	433
Anabaena spiroides f. *spiroides*	Sphere	270
Anabaena spiroides var *minima*	Sphere	48
Anabaena torulosa	Cylinder	125
Anabaenopsis arnoldii	Ellipsoid	257
Anabaenopsis elenkinii	Ellipsoid	133
Anabaenopsis tanganyikae	Cylinder	63
Aphanizomenon gracile	Cylinder	49
Aphanizomenon issatschenkoi	Cylinder	57
Aphanizomenon ovalisporum	Cylinder	52
Aphanizomenon volzii	Cylinder	89
Aphanocapsa delicatissima	Sphere	0.1
Aphanocapsa elachista	Sphere	2.1
Aphanocapsa holsatica	Sphere	0.5
Aphanocapsa incerta	Sphere	1.8
Aphanocapsa koordersii	Sphere	7.2
Aphanocapsa nubilum	Sphere	1.8
Aphanothece clathrata	Rod	2.1
Aphanothece stagnina	Rod	86
Arthrospira cf *maxima*	Cylinder	59
Chroococcus dispersus	Sphere	28
Chroococcus limneticus	Sphere	450
Chroococcus microscopicus	Sphere	0.3
Chroococcus minimus	Sphere	6.8
Chroococcus minutus	Sphere	220
Chroococcus turgidus	Sphere	4190
Coelosphaerium cf *kuetzingianum*	Sphere	7.2
Coelosphaerium cf *natans*	Sphere	2.3
Coelosphaerium punctiferum	Sphere	0.5
Coelomoron pusillum	Ellipsoid	14
Coelomoron microcystoides	Ellipsoid	5.2
Cyanodictyon imperfectum	Sphere	0.1
Cyanodictyon planktonicum	Rod	1.1
Cyanonephron styloides	Rod	5.4
Cylindrospermopsis raciborskii	Cylinder	42
Cylindropermum licheniforme	Cylinder	140

Geitlerinema splendidum	Cylinder	22
Geitlerinema unigranulatum	Cylinder	23
Gloeothece subtilis	Ellipsoid	1.3
Limnothrix cf *planktonica*	Cylinder	12
Merismopedia elegans	Sphere	144
Merismopedia glauca	Sphere	33
Merismopedia hyalina	Sphere	8.0
Merismopedia punctata	Sphere	14
Merismopedia tenuissima	Sphere	0.9
Merismopedia warmingiana	Sphere	0.1
Microcystis aeruginosa	Sphere	87
Microcystis botrys	Sphere	113
Microcystis flos-aquae	Sphere	22
Microcystis cf *panniformus*	Sphere	33
Microcystis wesenbergii	Sphere	113
Myxobaktron plankticus	Cone	0.8
Nodularia spumigena	Cylinder	227
Nostoc linckia	Sphere	40
Oscillatoria princeps	Cylinder	4275
Oscillatoria sancta	Cylinder	1134
Phormidium amoenum	Cylinder	212
Phormidium formosum	Cylinder	142
Phormidium retzii	Cylinder	98
Planktolyngbya contorta	Cylinder	1.7
Planktolyngbya subtilis	Cylinder	5.9
Planktothrix agardhii	Cylinder	47
Planktothrix mougeotii	Cylinder	64
Planktothrix perornata	Cylinder	291
Planktothrix cf *planktonica*	Cylinder	396
Planktothrix raciborskii	Cylinder	291
Plectonema tomasinianum	Cylinder	663
Plectonema wollei	Cylinder	9557
Pseudanabaena galeata	Cylinder	14
Pseudanabaena limnetica	Cylinder	11
Rhabdoderma cf *lineare*	Rod	51
Rhabdoglea cf *smithii*	Cone	5.8
Raphidiopsis cf *mediterranea*	Cylinder	59
Romeria elegans	Rod	31
Snowella lacustris	Ellipsoid	9.8
Snowella litoralis	Sphere	8.2
Synechococcus cf *nidulans*	Rod	46
Synechocystis sp.	Sphere	3.6
Trichodesmium iwanoffianum	Ellipsoid	84
Tychonema bornetti	Cylinder	393

Return to level 1

SPECTROPHOTOMETRIC TECHNIQUE FOR THE DETERMINATION OF CHLOROPHYLL-*A* CONCENTRATION

BACKGROUND

Chlorophyll-a is the pigment that gives phytoplankton their green colour and is the major agent in the process of photosynthesis. The expression of phytoplankton (including cyanobacteria) biomass in water is generally in the form of chlorophyll-a concentration. The analysis is relatively easy to perform and is therefore widely used in the analysis of water samples. The downside of chlorophyll-a analysis is that it is not suitable for water with very low chlorophyll content, such as drinking water.

The chlorophyll-a method is used as an indirect quantitative indication of algal biomass in water. This method is suitable for all types of water, such as tap, rivers, dams, industrial and sewage effluents.

Certain interferences with the method have been identified:

- Inorganic turbidity (>100NTU) may block the glass fibre filter (GF/C) through which the water containing phytoplankton and cyanobacteria is filtered. This results in small volumes of water, containing low concentrations of phytoplankton and cyanobacteria being filtered, with consequent low absorbance values.
- Multi-cellular phytoplankton in the form of colonies, filaments or flocs are usually not uniformly distributed through a sample even after proper stirring. This may result in a larger than expected variance (>10%) between replicates.
- Dissolved substances absorbing at the same wavelength.

APPARATUS, MATERIALS AND REAGENTS

INSTRUMENTS AND EQUIPMENT

- Centrifuge
- Filtering apparatus
- Micropipette
- Pipetboy
- Spectrophotometer
- Uninterruptible power supply
- Vacuum pump
- Bottle top dispenser or equivalent pipette
- Vortex shaker
- Water bath

GLASSWARE

- Screw-capped test tubes
- Test tubes - rimless, medium wall (100mm x 14mm)
- Bulb pipettes - 4mL A-grade
- Graduated pipette - 10mL A-grade
- Volumetric flask – 1L A-grade
- Thermometer or thermostat - calibrated (with certificate)

- Measuring cylinders - 100mL 250mL, 500mL, 1000mL

OTHER MATERIALS

- Whatman glass fiber filters (GF/C) - 47mm diameter
- Trace-Klean
- Safety glasses when working with acid

REAGENTS

- Ethanol (95%) - AnalR grade - pro analisi
- Hydrochloric acid (HCl):
  0.3 M hydrochloric acid made up as follows:
  Make up 9.4mL HCl (measured using a 10mL A-grade graduated pipette) to 1L with reagent water. Make up monthly
- Reagent water - Water that has been filtered by reverse osmosis, has a conductivity of less than 6.0mS/m and turbidity of less than 2.0NTU. This reagent water has no detectable salts or impurities

PROCEDURE

- Filter a known volume of sample (in duplicate) using a glass measuring cylinder (0.5L to 2.5L), depending on the density of the phytoplankton, through a glass fibre filter (Whatman GF/C). Before filtration, the sample must be shaken thoroughly to ensure uniformity. The glass measuring cylinder and the filtering cup must also be rinsed thoroughly with reagent water.
- Remove the filter and the entrapped phytoplankton without disturbing the phytoplankton or tearing the filter. Gently roll the filter without applying pressure.
- Place the filter into a marked screw-capped test tube (20 mℓ) and add approximately 10mℓ ethanol (95%), using the ethanol bottle top dispenser or equivalent pipette.
- Place test tubes in the water bath at 78 ±2ºC for 5 minutes prior to placing in the dark at room temperature for 24 ±7 hours.
- After 24 ±7 hours shake test tubes vigorously (using the vortex shaker at setting ±7 for ±15 seconds) before decanting the extract into marked centrifuge tubes.
- Centrifuge the extract for ±15 minutes at ±4800rpm (to clarify the extract) using the centrifuge. Ensure the test tubes in the baskets are balanced.
- Carefully decant the supernatant into marked test tubes.
- Accurately transfer 4mL of the supernatant using a 4mL A-grade bulb pipette into another set of marked test tubes used for the acidification process.
- Read the absorbency of the remaining supernatant, using the spectrophotometer at 665nm and 750nm wavelengths.
- Acidify the 4mL extract by adding approximately 100µL of a 0.3mole L^{-1} HCl solution. Mix the content of the test tube by shaking (using the vortex shaker at setting ±4 for ±5 seconds) and allow standing for approximately 4 minutes. The acidification converts the chlorophyll-a to phaeophytin-a.
- Read the acidified sample.
- The absorbency values obtained are used to calculate the chlorophyll-a concentration (see the section on calculations and expression of results).

SAFETY PRECAUTIONS

HAZARD WARNING

- Ethanol - flammable liquid.
- Hydrochloric acid - corrosive, causes burns and irritation to respiratory system.

CLOTHING

- Always wear a laboratory coat when performing chlorophyll-*a* analysis.
- Always wear protective eye-wear when making up acids.
- Wear gloves when handling water samples, if necessary.

SAFETY INSTRUCTIONS WHEN WORKING WITH ETHANOL

- Highly flammable, keep away from sources of ignition - no smoking.
- Mark all containers very clearly toxic!
- Keep ethanol container tightly closed.

SAFETY INSTRUCTIONS WHEN WORKING WITH ACID

- Always wear an acid-resistance laboratory coat or -apron.
- Always wear protective eye-wear when making up acids.
- Always add acid to water, never water to acid! The density of water is less than that of acid. If water is added to acid the water will collect on the surface, increasing the contact surface and thus increasing the severity of the reaction.
- Wear acid-proof gloves when handling acids.
- Wear protective shoes.

CALCULATION AND EXPRESSION OF RESULTS

- Use the following formula for the determination of chlorophyll-*a*:

$$\text{Chl-a } (\mu g\ L^{-1}) = \frac{[(A_{665} - A_{750}) - (A_{665a} - A_{750a})] \times 28.66 \times Ve}{Vm}$$

Where:

A_{665}	=	Absorbance at 665nm before acidification
A_{750}	=	Absorbance at 750nm before acidification
A_{665a}	=	Absorbance at 665nm after acidification
A_{750a}	=	Absorbance at 750nm after acidification
28.66	=	Constant (taking into account: ethanol with its specific absorption coefficient and path length of the cuvette)
Ve	=	Volume of ethanol used for extraction in mL (usually 10mL)
Vm	=	Volume of sample filtered in mL
x	=	Multiplication

- The chlorophyll-a values are “rounded off” as follows:

0 < Result < 1	Report to 2 decimal places
1 ≤ Result < 10	Report to 1 decimal place
10 ≤ Result	Report to the nearest integer

Note: It is important to note that rounding off should only occur in the final step (presentation phase) of calculation and not in the analytical phase.

Return to level 1

FLOW INJECTION ANALYSIS AND PHOTOMETRIC DETECTION OF ORTHO PHOSPHATE.

BACKGROUND

Inorganic phosphate has a very low toxic potential, however phosphate can interfere with flocculation processes and also stimulate algal growth. Phosphate is known to be the primary limiting nutrient in most cases where cyanobacteria blooms occur, especially with the occurrence of nitrogen fixing cyanobacteria species.

This method covers the determination of orthophosphate, also called Soluble Reactive Phosphate, in water samples using Flow Injection Analysis followed by photometric detection. The method is based on the reactions that are ion specific. The results are expressed in mg L^{-1} P. This method is fit for the purpose of, and is suitable for the determination of orthophosphate in drinking, ground, catchment and surface waters, from the range of 0 - 10mg L^{-1}.

PRINCIPLE OF THE METHOD

The orthophosphate ions react with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a phospho-molybdic complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880 nm.

The absorbance is proportional to the concentration of orthophosphate in the sample.

Orthophosphate forms a blue colour in this test. Polyphosphates and organic phosphorus compounds do not react. The sulphuric acid in the molybdate reagent does not have enough contact time with polyphosphates to hydrolyse them.

Two important sources of interferences have been identified in this method:

- Silica forms a pale blue complex, which also absorbs at 880nm. This interference is generally insignificant as a silica concentration of approximately 30mg L^{-1} would be required to provide a 0.007mg L^{-1} P positive error in orthophosphate.
- Concentrations of ferric iron greater than 50mg L^{-1} will cause a negative error due to the precipitation of, and subsequent loss, of orthophosphate. Samples high in iron can be pre-treated with sodium bisulfite to eliminate this interference. Treatment with bisulfite will also remove the interference due to arsenates.

APPARATUS, MATERIAL AND REAGENTS

APPARATUS

- Flow Injection Analyzer (e.g. Lachat Quickchem 8000)
- Autosampler
- Multichannel proportioning pump

- Reaction unit or manifold
- Colorimetric detector
- Data system

MATERIAL:

- Glassware/Plasticware
- Volumetric Flasks:
  - 1000mL A-grade flasks
- Pipettes
  - 1mL Bulb pipette A-grade
  - 5mL Bulb pipette A-grade
  - 10mL Bulb pipette A-grade
  - 20mL Bulb pipette A-grade
  - 50mL Bulb pipette A-grade
  - 100mL Bulb pipette A-grade
  - 25mL Bulb pipette A-grade
- 50mL Polyethylene bottles for standards
- 100mL Polyethylene bottles for samples
- 10mL Borosilicate sample tubes
- 1000mL Polyethylene bottles for reagents

REAGENTS:

It should be noted that alternate volumes of reagents, standards and Q.C. verification standards may be prepared if desired. The concentrations however shall conform to those specified. This shall not constitute a method deviation.

- Ammonium Molybdate
- Antimony Potassium Tartrate
- Ascorbic Acid
- Sodium Dodecyl Sulphate
- Sodium Hydroxide
- Titriplex IV
- Potassium Dihydrogen Phosphate
- Sulphuric Acid
- Helium Gas
- Potassium Dihydrogen Phosphate
- Reagent Water (<0.1mS/m)

PROCEDURE

PREPARING OF SOLUTIONS:

AMMONIUM MOLYBDATE SOLUTION

$(NH_4)_6$ Mo_7 $O_{24}.4H_2O$ 40 g

Preparation: Dissolve 40g ammonium molybdate to ± 900mL of water. Dilute to a final volume of 1000mL and mix. The solution is stable for 1 month.

POTASSIUM ANTIMONY TARTRATE SOLUTION

Dissolve 3.0g potassium antimony tartrate in ± 800mL water. Dilute to a final volume of 1000mL and mix. The solution is stable for 1 month.

MOLYBDATE COLOUR REAGENT

Dilute 500mL water with 35mL concentrated sulphuric acid (H_2SO_4) (± 96% acidity). Cool the solution. Then add 213mL ammonium molybdate ($[NH_4]_6$ $Mo_7O_{24}.4H_2O$) solution and 72mL potassium antimony tartrate ($K_2Sb_2NO_3$) solution. Dilute to a final volume of 1000mL and mix. This solution must be degassed with argon gas for ± 15 minutes. This solution is stable for one month.

ASCORBIC ACID REDUCING SOLUTION

Dissolve 60.0g ascorbic acid ($C_6H_8O_6$) in ± 700mL water and then add 1.0g sodium dodecyl sulphate. Dilute to a final volume of 1000mL and mix. The solution is stable for one month.

SODIUM HYDROXIDE - TITRIPLEX RINSE

Dissolve 65.0g sodium hydroxide (NaOH) and 6.0 g titriplex ($C_{14}H_{22}N_2O_8.H_2O$) in ± 800mL water. Dilute to a final volume of 1000mℓ and mix. The solution is stable for 2 weeks.

CALIBRATION OF STOCK SOLUTIONS:

ORTHOPHOSPHATE CALIBRATION STOCK SOLUTION (1000 MG/L (PPM)) - MERCK

Dry potassium dihydrogen phosphate (KH_2PO_4). Dissolve 4,3940 ± 0.0005g of the dried potassium dihydrogen phosphate (KH_2PO_4) in ± 800mL water. Make up to a final volume of 1000mL and mix. Store solution in a one litre amber bottle. The solution is stable for 1 year.

ORTHOPHOSPHATE WORKING STOCK (100 MG/L (PPM))

Dilute 100mL orthophosphate calibration stock solution to a final volume of 1000mL with reagent water to make up a working stock of 100mg/L. Prepare monthly.

CALIBRATION STANDARDS

Use A-Grade bulb pipettes and prepare these standards weekly from working stock solution.

Standards	Volume (mL)	Concentration ppm (mg L^{-1})
1	100 mL in 1000 mL	10 ppm
2	75 mL in 1000 mL	7.5 ppm
3	50 mL in 1000 mL	5 ppm
4	20 mL in 1000 mL	2.0 ppm
5	10 mL in 1000 mL	1.0 ppm
6	5 mL in 1000 mL	0.5 ppm
7	1 mL in 1000 mL	0.1 ppm
8 Blank (Milli-Q water)	0 mL	0 ppm

SAMPLE PREPARATION:

- Samples should be analysed within three days and kept at 5°C ± 3°C when not analysed immediately.
- All samples must be filtered as soon as possible after reception in the laboratory, before analysis.

OPERATING INSTRUCTIONS OF ANALYZER / DATA SYSTEM:

- Operating instructions for the analyser/data system are presented in the operating manuals of the Flow Injection Analyzer (FIA).

CALCULATION AND EXPRESSION OF RESULTS:

- Calculated data are automatically obtained from the Flow Injection Analyzer.

Return to level 1

SECCHI DEPTH MEASUREMENTS

A method developed in 1865 by an astrophysicist Fr. Pietro Angelo Secchi involves a device called a Secchi disk (Figure 3-6(L3)), to measure the transparency of open waters. It is a quick and inexpensive way to determine transparency of the water.

The Secchi disk with its alternate black and white quadrants are slowly lowered into the water. The depth at which the pattern on the disk is no longer visible is taken as a measure of the Secchi disk depth of that specific water body or part of the water body.

Secchi measurements may be subjective to the operator and more precise measurements should be done with a turbidimeter.

Figure 3-6(L3) Secchi disk measurement for determination of the euphotic depth

PROCEDURE

- Use a disk of the appropriate size for the clarity range (20mm for 0.15-0.5m, 60mm for 0.5-1.5m, 200mm for 1.5-5 , 600mm for 5-15m), painted matte white or in black and white quadrants. Use a graduated line, and attach a weight to hold the line vertical.
  There is some discrepancy in the literature whether it is best to do the measurements on the sunny or the shady side of the boat. It seems however, that the tendency is to do it on the shady side of the boat. What is important though, is to be consistent in the decision and to (where possible) use the same person to take all related readings.
- Lower the disk into the water.
- Allow sufficient time (preferably 2min) when looking at the disk near its extinction point for the eyes to adapt completely to the prevailing luminance level.
- Record the depth at which the disk disappears.

- Slowly raise the disk and record it depth of reappearance.
- The Secchi depth is the average of the depth of disappearance and reappearance
- The readings should be made as near to mid-day as possible.
- The water depth should be at least 50% greater than the Secchi depth so that the disk is viewed against the water background, not bottom-reflected light.

Return to level 1

DETERMINING TEMPERATURE IN THE FIELD

BACKGROUND

A wide variety of field instruments exist for the determination of temperature in the field (e.g. YSI 556 MPS (Multi Probe System) - Oxygen, Conductivity, pH and Temperature). Since field instruments are usually not as sensitive as bench-top instruments it is very important to calibrate/verify the instruments before going out to the field and on return to the laboratory.

Verification is done with a calibrated mercury-filled Celsius thermometer. This thermometer should be calibrated at least once a year by a SANAS / ILAC (or equivalent) accredited testing laboratory. The data is recorded on an applicable form. If the verification data does not fall within the specified limits (±1.0ºC), the analysis should be repeated. If the results are still out of specification, maintenance on the probe should be carried out and the analysis repeated. If the results persist to be out of specification the laboratory supervisor should be informed and he/she should take the necessary action (e.g. to have the instrument serviced and or calibrated by a suitable supplier).

Notes: *1) Temperature readings should be taken to the closest integer, unless specified differently.*

2) When calibrated thermometers are used in a refrigerator, it should be kept in a screw-cap tube filled with glycerol.

APPARATUS, MATERIAL AND REAGENTS

Please note that this procedure applies to the YSI 556 MPS (Multi Probe System - Oxygen, Conductivity, pH and Temperature), but that the overall quality control and other aspects can be applied to any other applicable instruments.

APPARATUS

- YSI 556 MPS(Multi Probe System).
- Appropriate carrying case for the instrument to be taken to the field.
- Magnetic stirrer

REAGENTS

- 0.01M KCl
- Deionised water

PROCEDURE

TEMPERATURE VERIFICATION (QUALITY CONTROL) IN THE LABORATORY

- Turn on the instrument by pressing the ON/OFF key.
- Rinse probe module with deionised water and gently shake off the excess solution.
- Rinse the 100mL graduated calibration cup with deionised water.

- Shake the sample well and pour 30 to 35mL of the sample into the accompanying calibration cup.
- Place a magnetic stirrer bar in the calibration cup and place the calibration cup onto the magnetic stirrer.
- Carefully immerse the sensor end of the probe module into the sample solution. The sensor must be completely immersed.
- Gently rotate and/or move the probe module up and down to remove any bubbles from the sensor.
- Switch on the magnetic stirrer at a low setting.
- Record the temperature reading onto the appropriate form (after it was allowed to stabilize).
- Remove the magnet from the graduated calibration cup.
- Rinse probe module with deionised water.
- Insert a mercury-filled Celsius thermometer into the calibration cup containing the sample.
- Record the temperature reading onto the appropriate form (after it was allowed to stabilize).
- Make sure the YSI reading complies to the ±1.0ºC verification limits of the calibrated thermometer.

TEMPERATURE READING IN THE FIELD

- Before an instrument is taken out of the laboratory, it should be checked that the verification complies with the ±1.0ºC verification limits and that the battery pack is fully charged.
- Make sure the instrument is switched off.
- For the YSI 556, the probe module should be kept in a 0.01 M KCl solution, until the sampling site is reached. *Please note that this step is mainly for protection and stability of the pH probe and may not be necessary when instruments do not have these multi probe modules.*
- When the instrument is used in the field, discard the KCl solution from the calibration cup.
- Switch on the instrument by pressing the ON/OFF key.
- The probe should be immersed in the water directly (e.g. into the dam or river). *Note that the* wrong *procedure is to take a sub-sample into the calibration cup and measure the variables in the calibration cup as would be done in the lab.*
- If the surface water etc. is not naturally flowing over the probe gently swirl it around in the water to ensure that the water is flowing over the probe.
- Make sure the probe is always submerged. For a surface sample, submerge the probe ± 10 – 15cm below the surface.
- Allow the reading on the display to stabilise. The first reading after storage in the KCl may take longer to stabilize (this is especially true for oxygen and pH determinations).
- Record the readings onto the appropriate form.
- Rinse probe module with deionised water.
- Switch off the instrument, especially when readings at other sampling localities still need to be taken (due to limited battery life).
- Replace the calibration cup onto the probe module (not necessary to fill the calibration cup with KCl or anything else).
- When a new sampling site is reached, switch on the instrument and rinse the probe module with the new sample.
- Allow for stabilisation before reading is documented.
- Rinse probe module with deionised water and switch instrument off.

Return to level 1

DETERMINING PH IN THE FIELD

BACKGROUND

A wide variety of field instruments exist for the determination of pH in the field (e.g. YSI 556 MPS (Multi Probe System)). Since field instruments are usually not as sensitive as bench-top instruments it is very important to calibrate/verify the instruments before going out to the field and on return to the laboratory.

Calibration is done with pH Buffer solutions from appropriate suppliers. Verification is done with two a verification buffers, namely potassium tetroxalate dihydrate - $KH_3C_4O_8 \cdot 2H_2O$ - and calcium hydroxide - $Ca(OH)_2$. The data is recorded on an applicable form. If the verification data does not fall within the specified limits (±0.2pH units), the analysis should be repeated. If the results are still out of specification, maintenance on the probe should be carried out and the analysis repeated. If the results persist to be out of specification the laboratory supervisor should be informed and he/she should take the necessary action (e.g. to have the instrument serviced by a suitable supplier).

Although this method is suitable for all aqueous samples (source water, sewage, factory effluent and drinking water), at a pH above 10, high sodium ion concentrations may cause interference. The estimated error measurement for samples is 0.2pH units. The analytical range is 0.5 to 13.5pH units. This range has been validated to be true in Rand Water's hydrobiology laboratory, but validations for any method should be repeated in each laboratory since environmental factors may influence validations.

Notes: 1) pH readings should be taken to two significant digits, unless specified differently.

PRINCIPLE OF THE METHOD

The basic principle of electrometric pH measurement is the determination of the activity of the hydrogen ions by potentiometric measurement using a standard glass electrode. The electromotive force (emf) produced in the glass electrode system varies linearly with pH. This linear relationship is described by plotting the measured emf against the different pH buffers. Sample pH is determined by extrapolation.

APPARATUS, MATERIAL AND REAGENTS

Please note that this procedure applies to the YSI - MPS (Multi Probe System) Oxygen, Conductivity, pH and Temperature System, but that the overall quality control and other aspects can be applied to any other applicable instruments.

APPARATUS

- YSI 556 MPS (Multi Probe System - Oxygen, Conductivity, pH and Temperature).
- Appropriate carrying case for the instrument to be taken to the field.
- Magnetic stirrer

- Teflon sample cups (250mL)

REAGENTS

- 0.01M KCl
- Deionised water (<0.1mS m^{-1})
- pH 4 buffer solution
- pH 7 buffer solution
- BDH pH 9.00 buffer solution
- Verification Standard (pH = 7.00):
  Merck pH 7.00 buffer.
- Verification Standard (pH = 1.68):
  Pre-dry approximately 15g potassium tetroxalate dihydrate ($KH_3C_4O_8$ $2H_2O$) until constant mass. Constant mass means two consecutive masses that do not differ more than 0.001g. Dissolve 12.61g of the pre-dried $KH_3C_4O_8$ $2H_2O$ in reagent water and dilute to 1L.
- Verification Standard (pH = 12.45):
  Prepare a saturated solution (± 2 g in 1 L reagent water) of calcium hydroxide ($Ca[OH]_2$). Filter this solution under suction through a filter paper of medium porosity. Use the filtrate as the buffer solution. Discard the buffer when atmospheric CO_2 causes turbidity to appear.

PROCEDURE

PH CALIBRATION:

- The pH probe needs periodic calibration to assure high performance. (In the Hydrobiology laboratory at Rand Water calibration is done daily before any analysis commences.)
- Check that the battery of the instrument has enough power before starting with the calibration.
- Ensure that the probe is clean and not cracked, and check that the date and the time are correct.
- Ensure that port plugs are installed in all ports where sensors are not installed. It is extremely important to keep these electrical connectors dry.
- The key to successful calibration is to ensure that the sensors are completely submersed when calibration values are entered.
- Turn on the instrument by pressing the ON/OFF key.
- Choose the CALIBRATE function.
- Choose the calibrate pH function.
- Choose the 2-point selection (the instrument is calibrated at two different pH's).
- Remove the calibration cup and discard the storage solution.
- Rinse the calibration cup with deionised water.
- Pour the 30mL of pH 7 buffer solution into the clean pre-rinsed calibration cup. Ensure that the buffers have not expired.
- Place a magnetic stirrer bar in the calibration cup and place the cup onto the magnetic stirrer.
- Rinse the pH probe with deionised water. Shake the excess water off from the probe module.
- Dry the probe module between rinses and calibration solutions with absorbent paper towels. Making sure that the probe module is dry reduces carry-over contamination of calibration solutions and increases the accuracy of the calibration.
- Carefully immerse the sensor end of the probe module into the solution. The sensor must be completely immersed.

- Gently rotate and/or move the probe module up and down to remove any bubbles from the pH sensor.
- Screw the calibration cup on the threaded end of the probe module. Do not over tighten as this could cause damage to the threaded portions.
- Switch the magnetic stirrer to a low setting.
- Use the keypad to enter the calibration value of the buffer solution (pH 7) you are using at the current temperature.
- Allow at least one minute for temperature equilibrium before proceeding. When the reading shows no significant change for approximately 30 seconds, press ENTER. Record the pH reading on the appropriate form.
- The screen will indicate that the calibration has been accepted and prompt you to press ENTER again to continue.
- Remove the magnet from the calibration cup.
- Rinse the calibration cup and the magnet with deionised water.
- Pour the 30mL of pH 10 buffer solution into the clean pre-rinsed calibration cup.
- Place the magnet in the calibration cup and place the cup onto the magnetic stirrer.
- Rinse the pH probe with deionised water. Shake the excess water off from the probe module.
- Carefully immerse the sensor end of the probe module into the solution. The sensor must be completely immersed.
- Repeat the calibration step, now using the pH 10 buffer solution.
- Allow at least one minute for temperature equilibrium before proceeding. When the reading shows no significant change for approximately 30 seconds, press ENTER. Record the pH reading on the appropriate form.
- Remove the magnet from the calibration cup.
- Rinse the probe module, sensors, magnet and calibration cup with deionised water.

PH READING IN THE FIELD

- Before an instrument is taken out of the laboratory, it should be checked that the instrument has been calibrated and that the battery pack is fully charged.
- Make sure the instrument is switched off.
- For the YSI Model 85 Handheld, the probe module should be kept in a 0.01 M KCl solution, until the sampling site is reached. *Please note that this step is primarily for protection and stability of the pH probe and is therefore important in this procedure.*
- When the instrument is used in the field, discard the KCl solution from the calibration cup.
- Switch on the instrument by pressing the ON/OFF key.
- The probe should be immersed in the water directly (e.g. into the dam or river). *Note that the* wrong *procedure is to take a sub-sample into the calibration cup and measure the variables in the calibration cup as would be done in the lab.*
- If the surface water etc. is not naturally flowing over the probe gently swirl it around in the water to ensure that the water is flowing over the probe.
- Make sure the probe is always submerged. For a surface sample, submerge the probe ± 10 – 15 cm below the surface.
- Allow the reading on the display to stabilize. The first reading after storage in the KCl may take longer to stabilise (this is especially true for oxygen and pH determinations).
- Record the readings onto the appropriate form.

- Rinse probe module with deionised water.
- Switch off the instrument, especially when readings at other sampling localities still need to be taken (due to limited battery life).
- Replace the calibration cup onto the probe module (not necessary to fill the calibration cup with KCl or anything else).
- When a new sampling site is reached, switch on the instrument and rinse the probe module with the new sample.
- Allow for stabilisation before reading is documented.
- Rinse probe module with deionised water and switch instrument off.

Note: It is preferable that pH readings be taken on site at the sampling locality, since pH changes are inevitable when samples are enclosed in a smaller container. If pH readings cannot be taken on site, sample bottles should be filled to the brim and capped tightly after sampling, and transported in a cooler bag with ice bricks. The pH readings should be done within 8 hours of sampling.

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DETERMINATION OF DISSOLVED OXYGEN IN THE FIELD

BACKGROUND

Dissolved oxygen (DO) levels in natural and wastewaters depend on the physical, chemical and biochemical activities in the water body. The analysis for DO is a key test in water pollution and waste treatment process control. Two basic methods for DO analysis are generally available: the Winkler or iodometric method (and its modifications) and the electrometric method using membrane electrodes. The iodometric method is a titrimetric procedure based on the oxidising property of DO while the membrane electrode procedure is based on the rate of diffusion of molecular oxygen across a membrane.

The most common method used in the field is the electrometric method using membrane electrodes. This method is suitable for the analysis of Dissolved Oxygen (DO) of surface water, ground water (e.g. borehole water), drinking water, industrial effluent, sewage water and other fluid substances.

The calibration of the DO meter must be performed every time it is used (e.g. in the morning before it goes out to the field). A dirty DO probe is a common source of incorrect calibration and/or erratic results.

Erratic results may be due to:

- Fouling of the DO electrode by highly organic substances e.g. where the DO of an effluent from a water treatment works is continuously measured over time.
- Plastic films used with membrane electrode systems are permeable to a variety of gasses besides oxygen. Prolonged use of membrane electrodes in waters containing such gases as hydrogen sulfide (H_2S) tends to lower cell sensitivity. Eliminate this interference by frequently changing and calibrating the membrane electrode.
- On-site sample should be flowing past membrane head of probe. If not (stagnant/unmoving water sample) then gently move probe through stationery water sample.

Dissolved oxygen ranges from 0 to 500% air saturation or 0 to 50mg L^{-1}. Dissolved oxygen is very sensitive to air pressure and therefore the specific height above sea level is necessary for correct calibration.

PRINCIPLE OF THE METHOD

Oxygen-sensitive membrane electrodes of the polarographic or galvanic types are composed of two solid metal electrodes in contact with supporting electrolyte separated from the test solution by a selective membrane. The basic difference between the galvanic and the polarographic systems is that in the former the electrode reaction is spontaneous, while in the latter an external source of applied voltage is needed to polarize the indicator electrode.

APPARATUS, MATERIAL AND REAGENTS

APPARATUS

Different instruments exist for the determination of dissolved oxygen e.g.

- YSI 556 MPS Multi Probe System.
- YSI Model 85 MPS (Multi Probe System).
- YSI 6600 MPS Multi Parameter System.

Note: This procedure applies to the YSI MPS (Multi Probe System - Oxygen, Conductivity, pH and Temperature), but the overall quality control and other aspects can be applied to any other applicable instruments.

REAGENTS

- Deionised water
- 0.01M KCL
- 0.4M Na_2SO_3

With the handheld systems, the measurement of dissolved oxygen for field determinations is done on site. The samples cannot be stored for later analysis using the handheld systems.

PROCEDURE

CALIBRATION

- Calibration of any one option (% saturation or mg L^{-1}) automatically calibrates the other.
- If calibration cup is used, ensure to loosen the seal to allow pressure equilibration before calibration process is initiated.
- Access the calibration screen.
- Select the dissolved oxygen option.
- Select the %DO option.
- Remove the calibration cup and discard the storage solution (usually 0.01M KCl).
- Rinse the calibration cup with deionised water.
- Place approximately 3mm of tap water in the bottom of the calibration cup.
- Place the probe module into the calibration cup, but make sure that the DO and temperature sensors are not immersed in the water.
- Engage only 1 or 2 thread of the calibration cup to ensure that the DO sensor is vented to the atmosphere.
- Enter the current local barometric pressure.
- Allow approximately 10 minutes for the air in the calibration cup to become water saturated and for the temperature to equilibrate before proceeding. Ensure that the %DO reading shows no significant change for approximately 30 second and press ENTER. Record the DO reading on the appropriate form.
- Rinse the probe module and sensors with deionised water.

DISSOLVED OXYGEN READING IN THE FIELD

- Before an instrument is taken out of the laboratory, it should be checked that the instrument has been calibrated and that the battery pack is fully charged.
- Make sure the instrument is switched off.
- For the YSI Model 85 Handheld, the probe module should be kept in a 0.01M KCl solution, until the sampling site is reached. *Please note that this step is primarily for protection and stability of the pH probe and may therefore not be necessary with certain instruments. In fact, when it is an oxygen meter only, it is preferable that the probe be kept in a cup with a moist sponge (as to supply a 100% water saturated environment).*
- When the instrument is used in the field, discard the KCl solution from the calibration cup.
- Switch on the instrument by pressing the ON/OFF key.
- The probe should be immersed in the water directly (e.g. into the dam or river). *Note that the* wrong *procedure is to take a sub-sample into the calibration cup and measure the variables in the calibration cup as would be done in the lab.*
- If the surface water etc. is not naturally flowing over the probe gently swirl it around in the water to ensure that the water is flowing over the probe.
- Make sure the probe is always submerged. For a surface sample, submerge the probe ± 10 – 15cm below the surface.
- Allow the reading on the display to stabilize. The first reading after storage in the KCl may take longer to stabilise (this is especially true for oxygen and pH determinations).
- Record the readings onto the appropriate form.
- Rinse probe module with deionised water.
- Switch off the instrument, especially when readings at other sampling localities still need to be taken (due to limited battery life).
- Replace the calibration cup onto the probe module (not necessary to fill the calibration cup with KCl or anything else).
- When a new sampling site is reached, switch on the instrument and rinse the probe module with the new sample.
- Allow for stabilisation before reading is documented.
- Rinse probe module with deionised water and switch instrument off.

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METHOD FOR THE DETERMINATION OF TURBIDITY

BACKGROUND

Clarity of water is important in producing products destined for human consumption and manufacturing uses. The clarity of a natural body of water is a major determinant of the condition and primary productivity of that system. Turbidity in water is caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble coloured organic compounds, plankton and other microscopic organisms. Correlation of turbidity also affects the light scattering properties of the suspension.

The method is suitable for all water i.e. potable water, source water, as well as sewage waters and industrial effluents.

PRINCIPLE OF THE METHOD

The method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity.

Turbidity can be determined for any water sample that is free of debris and rapidly settling coarse sediment. Dirty glassware, the presence of air bubbles as well as vibration that disturb the surface visibility of the samples will give false results. Temperature fluctuations of the sample may alter suspended particle characteristics, which may also interfere with the readings.

APPARATUS, MATERIAL AND REAGENT

APPARATUS:

- A turbidimeter (e.g. Model 2100 AN turbidity meter)

MATERIALS:

- Sample cells fitting the specific turbidimeter
- Lamp replacement kit
- Standards (e.g. Gelex Standards)
- Calibration Kit

REAGENTS:

- Reagent water (<0.1mS m^{-1})

PROCEDURE

CALIBRATION OF INSTRUMENT

(Note that this is the procedure for the 2100 AN turbidimeter, but can be adapted to suit most turbidimeters):

(Calibration procedure must be performed once a month. The new values for the adjusted Gelex standards and the date of calibration must be recorded. Verification checks must be performed daily before proceeding with the measurement procedure. The checks must be conducted with the secondary Gelex standards that were adjusted with the most recent calibration procedure.)

- The suppliers recommend the use of a 20- 200- 1000- 4000- and 7500- NTU Formazin Standards for calibration of the model 2100 AN turbidimeter.
- Invert ampule several times before being used. Take care not to over-do it otherwise bubbles cause a problem.
- Insert the EPA filter module. Handle the ampules by the top and mix well by gently inverting several times. Take care not to over-do it. Bubbles cause problems.
- Press CAL
- Press Enter. The instrument display counts down from 60 to 0 and makes a measurement.
- Wipe clean the Formazin Ampule <0.1. Place in the cell holder. Press ENTER. The instrument display counts down from 60 to 0 then make a measurement. The display automatically increments to the next standard. Remove the ampule.
- Wipe clean the 20NTU Formazin ampule. Place in the cell holder. Press ENTER. The instrument counts down from 60 to 0 then makes a measurement. The display automatically increments to the next standard. Remove ampule.
- Repeat with all the different calibrators.
- If calibration was not acceptable the instrument flashes the CAL mode, then repeat steps.

MEASUREMENT PROCEDURE:

- Collect a representative sample in a clean container. Shake very well. Fill the sample cell to the line (approximately 30mℓ). Take care to handle the sample cell by the top. Cap the sample cell.
- Hold the sample cell at the cap and wipe to remove water spots and finger prints.
- When necessary apply a thin bead of silicon oil from the top to the bottom of the cell - just enough to coat the cell with a thin layer of oil. Spread oil uniformly. Wipe excess.
- Place the cell in the instrument cell compartment and close the cell cover.
- Press ENTER and wait for the reading.
- Press PRINT.
- Read and record result.
- Verify the instrument with secondary Gelex standards after every 10th turbidity sample done. Record these verification standards in the turbidity quality control file and plot the control charts.

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REFERENCES

1 Lawton L., Marsalek B., Padisak J. and Chorus I. (1999) Determination of cyanobacteria in the laboratory. In: Chorus I., Bartram J., Ed. Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. Published by E & FN Spon on behalf of the World Health Organization. pp347-367.

2 Hötzel G. and Croome R. (1999) A Phytoplankton Methods Manual for Australian Freshwaters, LWRRDC Occasional Paper 22/99. Land & Water Resources Research & Development Corporation, Canberra.

3 Nicholson B. and Burch M. (2001) Evaluation of Analytical Methods for the Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines. NHMRC, National Health and Medical Research Council of Australia, Canberra.

4 Rudi K., Skulberg O.M., Skulberg R. and Jakobsen K.S. (2000) Application of sequence-specific labeled 16S rRNA gene oligonucleotide probes for genetic profiling of cyanobacterial abundance and diversity by array hybridization. *Applied Environmental Microbiology*, 66: 4004-4011.

5 Castiglioni B., Rizzi E., Frosini A., Sivonen K., Rajaniemi P., Rantala A., Mugnai M.A., Ventura S., Wilmotte A., Boutte C. et al. (2004) Development of a universal microarray based on the ligation detection reaction and 16S rRNA gene polymorphism to target diversity of cyanobacteria. *Applied Environmental Microbiology*, 70: 7161-7172.

6 Fergusson K. and Saint C.P. (2000) Molecular phylogeny of Anabaena circinalis and its identification in environmental samples by PCR. *Applied Environmental Microbiology*, 66, 4245-4148.

7 Fergusson K.M., Saint C.P. (2003) Multiplex PCR assay for Cylindrospermopsis raciborskii and cylindrospermopsin producing cyanobacteria. *Environmental Toxicology*, 18: 120-125.

8 Foulds I.V., Granacki A., Xiao C., Krull U.J., Castle A. and Horgen P.A. (2002)Quantification of microcystin-producing cyanobacteria and E. coli in water by 50-nuclease PCR. *Journal of Applied Microbiology*, 93: 825- 834.

9 Kellmann R. and Neilan B. (2007) Biochemical characterization of paralytic shellfish toxin biosynthesis in vitro. *Journal of Phycol*ogy, 43: 497-508.

10 Al Tebrineh J. and Neilan B.A. (2009) Multiplex quantitative-PCR determination of toxic cyanobacteria in environmental samples. Cyanobacterial Bloom Management - Current and Future Options. Extended abstract, 12 & 13 August, Parramatta, NSW. http://www.wqra.com.au/temp/Cyano/National_Cyanobacteria_Workbook_web.pdf

11 Pearson L.A. and Neilan B.A. (2008) The molecular genetics of cyanobacterial toxicity as a basis for monitoring water quality and public health risk. *Current Opinion in Biotechnology*, 19: 281–288.

12 Komarek J. and Anagnostidis K. (1986) Modern approaches to the classification system of cyanophytes. *Archiv für Hydrobiologie, Sopplement 73, Algological Studies*, 43: 157-164.

13 Laslett G., Clark R. and Jones G. (1998) Estimating the precision of filamentous blue-green algae cell concentration from a single sample. *Environmetrics* 8: 313-340.

14 Izydorczyk K., Carpentier C., Mrowczynski J., Wagenvoort A., Jurczak T. and Tarczynska M. (2009) Establishment of an Alert Level Framework for cyanobacteria in drinking water resources by using the Algae Online Analyser for monitoring cyanobacterial chlorophyll a. *Water Research*, 43(4): 989-996.

15 Harada K-I., Kondo F., Lawton L. (1999) Laboratory analysis of cyanotoxins. In: Chorus I, Bartram J, Ed. *Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management.* Published by E & FN Spon on behalf of the World Health Organization. pp369-405.

16 Nicholson B. and Burch M. (2001) Evaluation of Analytical Methods for the Detection and Quantification of Cyanotoxins in Relation to Australian Drinking Water Guidelines. NHMRC, Canberra. http://www.nhmrc.gov.au/publications/synopses/_files/eh22.pdf

17 Meriluoto J. and. Codd G. A, Eds. (2005) TOXIC: Cyanobacterial Monitoring and Cyanotoxin Analysis, Turku: Åbo Akademi University Press, 149 pp., ISBN 951-765-259-3.

18 Froscio S. Fanok S., King B. and Humpage A.R. (2008) Screening Assays for Water-borne Toxicants CRC for Water Quality and Treatment Research Report 60. http://www.waterquality.crc.org.au/publications/report61_cylindrospermopsin_toxicity.pdf

19 Lawrence J.F., Niedzwiadek B. and Menard C.(2005) Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: collaborative study. *Journal of AOAC International* 88(6): 1714-1719.

20 Robards R.D. and Zohary T. (1987) Temperature Effects on Photosynthetic Capacity, Respiration, and Growth Rates of Bloom-Forming Cyanobacteria. *NZ Journal of Marine and Freshwater Research*, 21: 391-399.

21 Oliver R. and Ganf G. (2000) Freshwater blooms. in: Whitton, B., and Potts, M. (eds), The ecology of cyanobacteria : their diversity in time and space, The Netherlands: Kluwer Academic Publishers 149-194.

22 An J. and Carmichael W.W. (1994) Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularin. *Toxicon* 32: 1495-1507.

23 Heresztyn T. and Nicholson B.C. (2001) Determination of cyanobacterial hepatotoxins directly in water using a protein phosphatase inhibition assay. *Water Research* 35: 3049-3056.

24 APHA/AWWA/WEF Standard Methods for the Examination of Water and Wastewater (1998), 20th Edition Maryland, USA.

25 Burch M.D., Harvey F.L., Baker P.D. and Jone, G., (2003) National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Fresh Waters. ARMCANZ National Algal Management. Draft V6.0 for consideration LWBC.

26 Baker P. (1991) Identification of common noxious cyanobacteria. Part 1 – *Nostocales*. UWRAA Research Report No. 29, Urban Water Research Association of Australia, Melbourne.

Baker P. (1992) Identification of common noxious cyanobacteria. Part 2 – *Chroococales* and *Oscillatoriales*. UWRAA Research Report No. 46, Urban Water Research Association of Australia, Melbourne.

27 McGregor G.B. and Fabbro L.D. (2001) A guide to the identification of Australian freshwater planktonic Chroococcales (Cyanoprokaryota/Cyanobacteria). Cooperative Research Centre for Freshwater Ecology Identification Guide No. 39.

28 Baker P.D. and Fabbro L.D. (2002) A guide to the common blue-green algae (Cyanoprokaryotes) in Australian freshwaters. Cooperative Research Centre for Freshwater Ecology Identification Guide No.25. Second Edition.