GLOBAL WATER RESEARCH COALITION

WATER QUALITY RESEARCH AUSTRALIA

INTERNATIONAL GUIDANCE MANUAL FOR THE MANAGEMENT OF TOXIC CYANOBACTERIA

International Guidance Manual

for the

Management of Toxic Cyanobacteria

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DISCLAIMER

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The Global Water Research Coalition (GWRC) is a non-profit organisation that serves as a collaborative mechanism for water research. The benefits that the GWRC offers its members are water research information and knowledge. The Coalition focuses on water supply and wastewater issues and renewable water resources: the urban water cycle. GWRC was officially formed in April 2002 with the signing of a partnership agreement and a partnership agreement was signed with the U.S. Environmental Protection Agency in July 2003. GWRC is affiliated with the International Water Association (IWA).

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- EAWAG – Swiss Federal Institute for Aquatic Science and Technology
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PREFACE

Cyanobacteria, also known as blue-green algae, are a primitive group of organisms which, according to fossil records, have existed for approximately 3.5 billion years. Cyanobacteria have evolved to allow the efficient utilisation of many environments, including marine and freshwater sources.

Cyanobacteria are a concern for water authorities worldwide as their persistence in water supplies causes numerous problems for water treatment plants. However, the major concern associated with the presence of cyanobacteria is the metabolites they produce, taste and odour compounds, particularly 2-methyl isoborneol and geosmin, and a range of toxic compounds known collectively as algal toxins, or cyanotoxins. The first recorded stock death due to the presence of cyanobacteria was reported in South Australia in 1878, and since that time cyanotoxins in drinking water have been implicated in a range of adverse health effects on the communities receiving contaminated water. As a result, the management of cyanobacteria, in source water and by treatment, has been an ongoing focus of water industry research and over several decades hundreds of journal articles, reports and fact sheets have been published on these topics. Several years ago, a research project was developed through the Cooperative Research Centre for Water Quality and Treatment to consolidate that wealth of knowledge into a practical, user-friendly manual that could be used by Australian water quality managers and operators to help manage cyanobacteria in source waters. During the following years, manuals with similar aims were developed in South Africa and Europe.

The management of cyanobacteria and cyanotoxins is one of the priority issues in the research agenda of the Global Water Research Coalition. In 2007 a GWRC expert workshop was held in South Africa, attended by those responsible for the development of the three regional manuals, with the aim to consolidate the available knowledge and know-how and to develop an international guidance manual incorporating the most important aspects of the different manuals to enable its application worldwide.

SCOPE OF THE GUIDANCE MANUAL

The international manual covers information required to:

- Understand the importance of cyanobacteria and the toxins they produce
- Assess the risks associated with a particular water source
- Develop a monitoring program and incident management strategies consistent with the WHO Water Safety Planning process
- Instigate management procedures both in the source water and treatment plants to mitigate the risks posed by the presence of toxic compounds in drinking water.

It is hoped that the level of information presented in the guide will be appropriate for most readers wishing to learn more about such an important topic.

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CHAPTER 1 INTRODUCTION

CYANOBACTERIA

Cyanobacteria, also known as blue-green algae, blue-green bacteria or cyanophytes, are part of a primitive group of organisms which, according to fossil records, have existed for approximately 3.5 billion years [1, 2]. They are not true algae, they are gram-negative bacteria which contain chlorophyll and perform photosynthesis. Many cyanobacteria have a characteristic bluish-green colour because of phycocyanin pigment contained in the cells and hence the name blue-green algae, while some species may appear red due to the presence of the carotenoid and phycoerythrin pigments [3].

COLONY

Microcystis

SINGLE CELLS
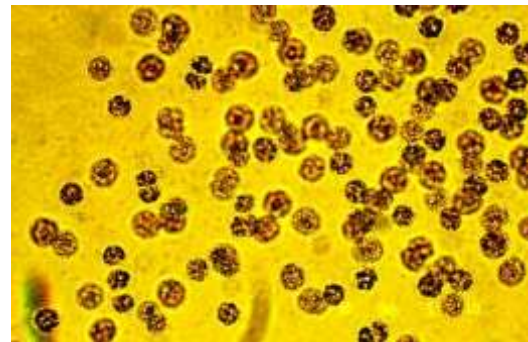
Microcystis

STRAIGHT FILAMENTS

Phormidium

SPIRALING

Cylindrospermopsin

Coiled *Anabaena* showing heterocytes and akinetes

Coiled *Anabaena* showing heterocytes and akinetes

Figure 1-1 Different morphological cell forms of some cyanobacteria (photographs from AWQC photo collection, and 4, 5).

Cyanobacteria species display a remarkable diversity in cell morphology or form. The unicellular cyanobacteria have spherical, ovoid or cylindrical cells that can occur single-celled or may aggregate into irregular colonies. A slimy matrix secreted during the growth of the colony holds it together. Some cyanobacteria aggregate into regular colonies, or filaments, also called trichomes. Trichomes can be straight, or coiled (Figure 1-1).

The life cycle of cyanobacteria requires water, carbon dioxide, inorganic substances (such as phosphorus and nitrogen) and light. Although energy metabolism is primarily through photosynthesis where sunlight and carbon dioxide are used to produce energy-rich molecules and oxygen, some species can survive in complete darkness, while others have heterotrophic abilities [6]. Some cyanobacteria species also have specialised cells called heterocytes (formerly called heterocysts, but they aren't cysts at all) which enable them to fix atmospheric nitrogen. These cells are indicated in a filament of *Anabaena circinalis* in Figure 1-1. It is not surprising that cyanobacteria can live nearly anywhere on earth, from freshwater to salt and brackish water, from rainforests to the desert, in the air, in soil and other terrestrial habitats. It is also not surprising that cyanobacteria are adaptable organisms that can thrive under the harsh conditions in many regions affected by drought and climate change.

Although from an operational viewpoint high numbers of cyanobacteria can adversely impact a range of drinking water treatment processes such as coagulation and filtration, the main issue for the water supplier is the production by cyanobacteria of metabolites, in particular the algal toxins, or cyanotoxins.

FACTORS INFLUENCING OCCURRENCE

Cyanobacteria are a natural component of surface freshwater bodies. Their occurrence may vary radically with seasonal changes from only a few per unit volume in the water column to excessive numbers occurring as 'blooms' at the surface of a water body. Their distribution in the water column may vary from the surface of the water column, a few metres below the water surface or at the bottom of the water body.

UTILISATION OF THE AQUATIC ENVIRONMENT BY CYANOBACTERIA

Different cyanobacterial species can display quite different behaviour in their utilisation of the water body. Many cyanobacteria species (e.g. *Microcystis, Anabaena, Aphanizomenon* sp.) possess gas vacuoles that cause them to move up or down in the water column, depending on their stage in the daily photosynthetic cycle. This is illustrated in Figure 1-2 in a stylised cartoon drawing of the daily migration cycle of *Anabaena.* Buoyancy regulation is a mechanism that positions the cyanobacteria at the best depth for capturing light for optimum growth and may also allow them to scavenge nutrients from the water column [7]. This may be a significant advantage over other phytoplankton algae particularly in stratified lakes where turbulence is low and heavy cells tend to sink. This mechanism only works well when the water body is not too turbulent and is also deep. One consequence of this buoyancy regulation mechanism is that cyanobacterial colonies may all become buoyant at night and rise to the surface and form the characteristic surface scums often seen in the morning when a lake is calm.
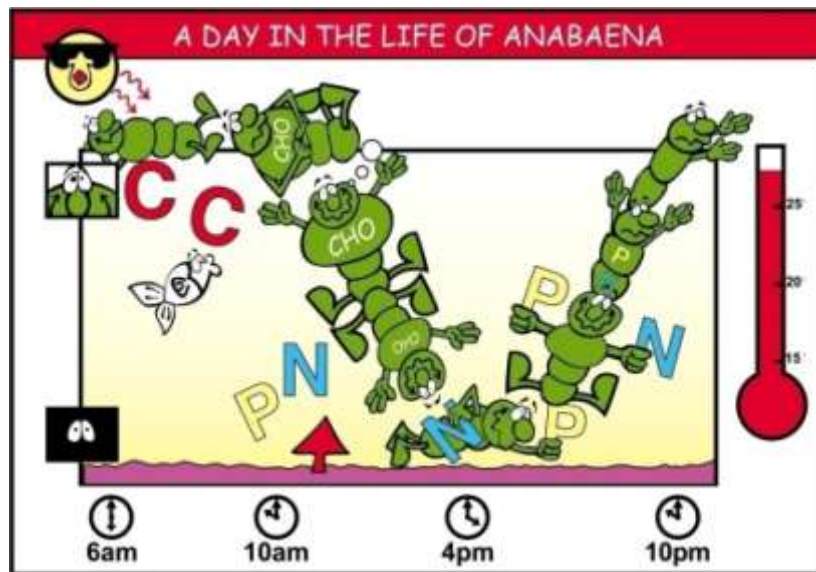
Figure 1-2 A stylised diagram of the daily cycle of buoyancy regulation and vertical migration in a lake by the cyanobacterium *Anabaena*

Other species tend to accumulate in the intermediate region of the water column (or metalimnion, between the warm upper layer and the cooler bottom layer, or hypolimnion). Examples are *Planktothrix* (*Oscillatoria*) *rubescens* and other red cyanobacteria. Under some conditions these cyanobacteria may also form surface scums. Examples of cyanobacteria that are often distributed uniformly through the water column are *Planktothrix (Oscillatoria) agardhii, Limnothrix (Oscillatoria) redekei* and *Cylindrospermopsis raciborskii.*

Non-planktonic, or benthic cyanobacteria can be found attached to sediments or rocks and other surfaces at depths that allow sufficient light penetration for photosynthesis. These cyanobacteria can form thick mats that may break off and float to the surface, particularly when oxygen produced by photosynthesis becomes concentrated within the mats. The *Phormidium* filament shown in Figure 1-1 is a species of benthic cyanobacteria.

THE CYANOBACTERIAL LIFE CYCLE

For one type of cyanobacteria, the filamentous, heterocystous cyanobacteria (Order *Nostocales*), the life cycle involves the planktonic population and benthic resting stages or akinetes. Akinetes are thick-walled reproductive structures that are found in sediments and are thought to provide a resting stage that may enable the survival of a species. They germinate when environmental conditions are appropriate, thereby providing a source of inoculum for subsequent populations, particularly from one season to the next [8]. Several akinetes are indicated in the *Anabaena* filaments shown in Figure 1-1. The life cycle of akinete-producing cyanobacteria can be summarised in a number of steps. First, the filaments of cyanobacteria grow by cell division. Akinete production and release follows, usually for the population to survive over winter. Finally, growth from the akinetes occurs, which is triggered by environmental factors, including light and temperature, with new cyanobacteria maturing and growing by cell division for the new season's population [8,9]. The cycle of akinete formation in the cyanobacterium *Anabaena* is illustrated in Figure 1-3.
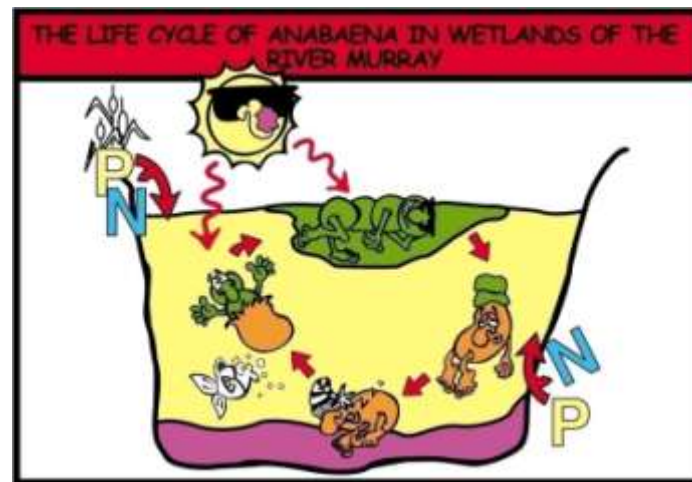
Figure 1-3 The typical life cycle of the cyanobacterium *Anabaena* showing akinete formation and germination

Other filamentous or single cell/colonial cyanobacteria are not known to form akinetes or other resting-stage cellular structures. It has been suggested that some of the normal or regular growth cells called vegetative cells may rest over winter in a state of senescence in the sediment. For example *Microcystis* can 'overwinter' as vegetative colonies on the lake sediments, where they may survive for several years, apparently without light or oxygen [10]. The new population may then appear in spring from the normal growth of these colonies by cell division.

FACTORS INFLUENCING GROWTH

Various cyanobacteria have the capacity to grow at a range of depths; this ability varies with species and is strongly influenced by nutrient and light availability (either the turbidity or the clarity of the water). Many cyanobacteria genera (e.g. *Planktothrix* and *Cylindrospermopsis*) are also adapted to grow in light limiting environments. This enables the cyanobacteria to utilise nutrient rich environments at various depths. For example, bands of *Planktothrix* can occur at a depth of 12m and layers of *Cylindrospermopsis* filament at a depth of 7m. Some cyanobacteria, such as the filamentous *Anabaena* sp., prefer higher light intensities, and *Planktothrix* will form dense bands just below the water surface. The benthic cyanobacteria, (e.g. *Phormidium*, *Pseudanabaena* and *Oscillatoria*) thrive in shallow reservoirs with clear water as they are generally immobile in the water body. They can also colonise the shallow areas of larger reservoirs where they will be attached to rocks, sediment, or larger organisms such as macrophytes.

A complex interaction of environmental factors has been shown to contribute to cyanobacterial growth. These factors include light intensity, water temperature, pH, carbon dioxide concentration, nutrient availability (nitrogen, phosphorus, iron, and molybdenum), physical characteristics of the water body (shape and depth), water column stability, water flow rate (rivers) or horizontal movement due to inflows or wind (reservoirs and lakes) and aquatic ecosystem structure and function. Factors which favour the growth of cyanobacteria will be discussed below. If several of these factors occur simultaneously cyanobacterial growth will be optimised and potential bloom conditions may be present.

NUTRIENTS

Since cyanobacterial blooms often develop in water bodies enriched with nitrogen and phosphorus (eutrophic conditions), it has been assumed that they require high nutrient concentrations. This contrasts to observations that cyanobacterial blooms often occur when concentrations of dissolved phosphate are lowest. Experimental data have shown that the affinity for nitrogen or phosphorus of many cyanobacteria is higher than for many other photosynthetic microalgae. If dissolved phosphate (soluble reactive phosphate determined from filtered samples) is detected at concentrations of only a few micrograms per litre, cyanobacterial growth and biomass are not limited by phosphate availability [11]. Cyanobacteria effectively utilise phosphorus and out-compete green algae, especially in phosphorus-limiting environments, as they (1) have a greater affinity for phosphorus, (2) can store enough phosphorus to perform 2 to 4 cell divisions, which corresponds to a 4 to 32-fold increase in biomass [11] and (3) migrate to areas of higher phosphorus concentration in the water column. Cyanobacteria (e.g. *Microcystis* sp.) can store nitrogen in proteins (cyanophycin and phycocyanin), which can be utilised during nitrogen-limiting conditions. Other cyanobacteria (e.g. *Cylindrospermopsis*) can utilise atmospheric nitrogen and can thus proliferate and out-compete green algae in nitrogen-poor surface water where sufficient light is available. As a simple guide, the influence of nutrient levels on cyanobacterial growth can be measured in terms of total phosphorus levels in the water body. In general, a total phosphorus level of 10–25 μgL^{-1} presents a moderate risk in terms of the growth of cyanobacteria. For levels of less than 10 $\mu g\ L^{-1}$ there is a low risk of cyanobacteria growth, and a level greater than 25 $\mu g\ L^{-1}$ provides high growth potential. However, growth can be maintained at low phosphorus concentrations provided there is rapid recycling of the nutrient. This will be discussed further in Chapter 2.

In the past the ratio of total nitrogen to total phosphorous was thought to be a key parameter in the growth of cyanobacteria compared with other phytoplankton [12]. However, more recent studies have refuted this contention and it is no longer considered a controlling factor [13]. A more important issue is whether either nutrient could be considered limiting for cyanobacterial growth, or growth of other algae.

LIGHT

Cyanobacteria contain the photosynthetic pigment chlorophyll-a, but unlike other phytoplankton they also contain phycobiliproteins. These pigments are able to harvest light in the green, yellow and orange part of the spectrum (500-650 nm). This enables cyanobacteria to utilise light energy efficiently. High phytoplankton density leads to high turbidity and low light availability and under these conditions cyanobacteria can harvest light more effectively and therefore may be able to out-compete other phytoplankton. For example, in light limiting conditions, cyanobacterial growth rates are higher than that of green algae, which allows them to out-compete green algae in highly turbid waters.

Both turbidity and water colour can influence the amount of light received by cyanobacteria in a water body. 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 (see Chapter 3 for more information about Secchi depth measurement). Those cyanobacteria that regulate their buoyancy via gas vesicles utilise optimum light conditions during the time they are in the euphotic zone. Light penetration into a water body is also important for growth of benthic cyanobacteria. The greater the light penetration the deeper the benthic cyanobacteria can grow.

TEMPERATURE

Cyanobacteria have a wide range of temperature tolerance, but rapid growth rates are usually achieved when the water temperatures exceed 20°C. In temperate to tropical climates temperatures are favourable for cyanobacteria growth for a large part of the year. A distinct temperature gradient can develop between the warm upper water layer, which is rich in light and oxygen but deficient in nutrients (the epilimnion), and the cooler bottom layers which are light-poor, oxygen-poor but nutrient-rich (the hypolimnion). The area of temperature gradient in between is called the thermocline. This is called stratification and these conditions can be more conducive to the growth of cyanobacteria than other plankton. Thermal stratification of a water body is illustrated in Figure 1-4.

Although the main body of the lake or river may not be stratified, often warm, shallow, sheltered areas exist that can become stratified and provide ideal conditions for cyanobacteria growth, and thus increase the probability of cyanobacterial blooms. Source water abstraction points situated in these areas are more at risk of high cyanobacteria concentrations.
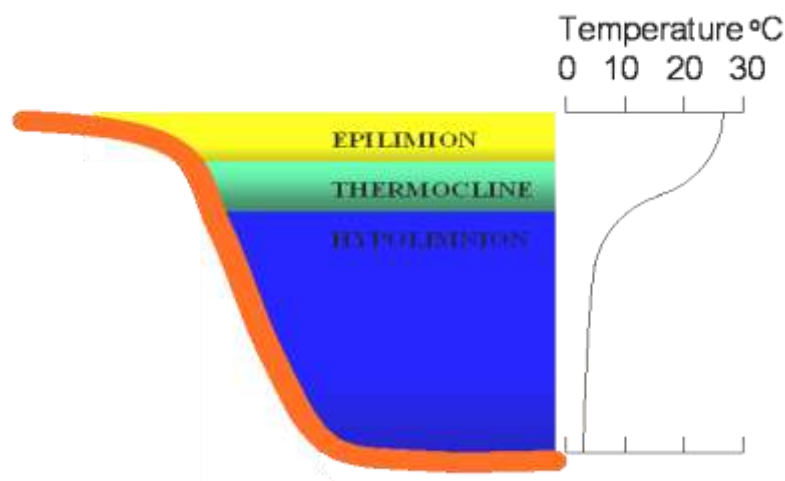
Figure 1-4 Cross section of a thermally stratified lake showing location of the epilimnion and hypolimnion and associated temperature changes

CYANOTOXINS

Cyanobacteria produce a range of potent toxins with different modes of toxicity. Table 1-1 lists the major known toxins, the target organs of these toxins and the cyanobacteria that produce them. This list is evolving, for example new variants of microcystins are identified each year, and it is unlikely that all cyanotoxins have been discovered.

The majority of cyanotoxins are associated with well-known planktonic and bloom-forming cyanobacteria that are free floating in the water, such as *Microcystis*, *Anabaena* and *Cylindrospermopsis*, however some benthic or attached cyanobacteria, such as *Oscillatoria, Phormidium* and *Lyngbya* have also been shown to produce both neuro- and hepatotoxins (nerve toxins and liver toxins respectively) and should also be considered as a possible hazard with regard to toxicity [14, 15, 16].

Table 1-1 General features of the cyanotoxins

Toxin Group	Primary target organ in mammals	Cyanobacterial genera
Cyclic peptides		
Microcystins	Liver, possible carcinogen in this and other tissues	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon, Anabaenopsis, Aphanizomenon ovalisporum*
Nodularin	Liver, possible carcinogen	*Nodularia, Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon, Cylindrospermopsis*
Anatoxin-a(S)	Nerve synapse	*Anabaena*
Aplysiatoxins	Skin, possible tumour promoter	*Lyngbya, Schizothrix, Planktothrix (Oscillatoria)*
Cylindrospermopsins	Liver and possibly kidney. Possible genotoxic and carcinogenic	*Cylindrospermopsis, Aphanizomenon, Umezakia, Raphidiopsis, Anabaena, Lyngbya* (benthic)
Lyngbyatoxin-a	Skin, gastrointestinal tract, possible tumour promoter	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
Lipopolysaccharides (LPS)	Potential irritant; affects any exposed tissue	All

The cyanotoxins can broadly be grouped into cyclic peptides, alkaloids and lipopolysaccharides [6, 17]. Mechanisms of cyanobacteria toxicity are diverse and the mammalian health effects range from neurotoxicity (e.g. anatoxins and saxitoxins) or hepatotoxicity (e.g. microcystins, cylindrospermopsin and nodularin) to inflammatory or irritation effects (e.g. lipopolysaccharide endotoxins). These toxins have been responsible for numerous animal deaths [18]. Some cyanobacteria produce a metabolite, β-*N*-methylamino-L-alanine (BMAA), which may be involved in neurodegenerative disease [19].

While the unpalatable appearance of freshwater affected by heavy planktonic algal blooms has probably prevented significant human consumption with consequent fatalities, there is increasing evidence that low-level exposure may have chronic health effects in humans. Cyanobacteria have been implicated in episodes of human illnesses in Australia [20, 21], North America [22, 23, 24], the United Kingdom [25], Brazil [26] and Africa [27]. Deaths of dialysis patients in Brazil from water contaminated with cyanotoxins were reported [28]. There is also epidemiological evidence from China of a link between cyanobacteria and cancer [29, 30].

Figure 1-5 shows the impact a toxic cyanobacterial bloom can have on wildlife dependent on a contaminated water source.

Figure 1-5 Toxic cyanobacterial blooms also affect wildlife reliant on a contaminated water source

Toxic cyanobacteria have been recorded from every continent including Antarctica [31, 32]. Of the cyanobacterial blooms tested to date, 50-75% have been toxic [33]. However not all blooms of a particular species may be toxic. In fact toxicities of blooms of the same species can vary markedly both geographically and with time [34]. Toxicity depends on the relative proportions of toxic and non-toxic strains, and this proportion, and hence toxicity, can vary over time. It is for this reason that all cyanobacterial blooms should be considered toxic, unless proven otherwise by laboratory analyses. Monitoring must also be carried out on an ongoing basis due to the potential variation in toxicity. Monitoring of cyanobacteria is discussed in detail in Chapter 3. As mentioned previously, while initially toxicity appeared to be restricted to planktonic cyanobacteria, benthic forms which form mats in water bodies have also been shown to be toxic [35, 36]. This can cause problems for the water supplier as benthic cyanobacteria are usually submerged, and not readily visible compared with toxic planktonic blooms. This is also discussed further in Chapter 3.

The cyanotoxins are synthesised within the cyanobacteria cells and usually remain contained within the cells. However, cyanotoxins are released in substantial amounts during cell lysis (breaking of cells) and cell death [17, 3]. An exception appears to be cylindrospermopsin produced by *C. raciborskii,* where a substantial amount of the toxin is present in the surrounding water during a healthy bloom [37].

CYANOTOXIN DRINKING WATER GUIDELINES

Drinking water guidelines are designed to protect public health by suggesting safe levels for constituents that are known to be hazardous to health. The guideline level represents the concentration at which the water is safe to drink over a lifetime of consumption. The World Health Organisation Guidelines for Drinking Water Quality [38] represent a scientific consensus on the health risks presented by microbes and chemicals in drinking water and are often used to derive guideline values for individual countries, states or regions. The guideline value is important for water supply authorities, as this value sets the concentration of a constituent that is tolerable in drinking water at the tap. For some countries the level is in the form of a recommendation from the health authorities. For other countries the level is a standard and compliance is monitored. For some

water authorities the guidelines become part of the contractual obligations. They are required to comply with the guideline values as part of their standards of service.

Due to the current lack of strong toxicological data for a range of cyanotoxins, WHO has issued a guideline for only one cyanotoxin, microcystin–LR (1 μg/L),the most toxic variant of microcystins known thus far.

CHAPTER 2 HAZARD IDENTIFICATION AND RISK ASSESSMENT IN SOURCE WATERS

BACKGROUND

Hazards are defined by the World Health Organization as "Physical, biological or chemical agents that can cause harm to public health".

The assessment of the risk associated with an identified hazard must take in to account:

- The likelihood or probability of an identified hazard occurring
- The magnitude or severity of the effect and the consequences of the occurrence.

Risk can be assessed at two levels: maximum risk in the absence of preventative measures and residual risk after consideration of existing preventative measures [39].

The main hazards associated with algal blooms are the cyanotoxins they produce. Table 2-1 lists some of the factors that should be taken into account when assessing the risk associated with the presence of cyanobacteria in a water body. This information has been taken from Nadebaum *et al.* [39].

Table 2-1 Factors associated with the risk posed by cyanobacterial blooms

Typical hazards
Cyanobacterial toxins
Factors to consider in assessing likelihood and severity of hazards
Frequency of blooms occurring within a particular reservoir
Extent of toxin problems
Extent of monitoring to predict the onset of a bloom
Extent and effectiveness of mitigation techniques (e.g. copper dosing, destratification)
Severity of stratification over summer
Level of available nutrients

A thorough risk assessment of a water source will involve:

- Identification of the factors impacting on the proliferation of cyanobacteria

- An analysis of historical data to determine the factors that may control cyanobacterial growth in this source, and their seasonal variation

- If the data is sufficient, the determination of any apparent relationships or trends between these factors and cyanobacteria species, numbers and toxin production. As it is unlikely that sufficient toxin data will be available, data relating to odour associated with cyanobacteria may be used

- Identification of the current or potential nutrient inputs into the source water. This can be accomplished by on-site inspection of the catchment as far as this is possible, or routine monitoring of nutrients at inflow sites to the water body (see Table 2-2 for examples of potential nutrient inputs into a water body)

- Assessment of the efficacy of current mitigation strategies (e.g. destratification techniques)

This accumulation of knowledge of the source water should allow water managers to anticipate the likelihood of a bloom occurring and the potential challenge to water quality under a particular set of conditions.

FACTORS INFLUENCING CYANOBACTERIAL BLOOM OCCURRENCE

High growth rates of cyanobacteria, resulting in the formation of blooms or scums in source waters, are caused by a combination of chemical, biological and physical factors including nutrient availability, water temperature, degree of stratification, climatic conditions, water body morphology and hydrodynamic stability of the water column (see Chapter 1 for more details). However, the most important factor is generally considered to be nutrient enrichment by nitrogen and phosphorus, or eutrophication, of the water source. Therefore any assessment of the risk of a cyanobacteria bloom in a water body must take these parameters into account. In most cases phosphorus is the key element in the development of cyanobacteria blooms as there is a direct relationship between the concentration of total phosphorus (TP) and the photosynthetic pigment chlorophyll-a (*Chl-a*).

It is important to identify the individual types of land use contributing to the total nutrient load from external sources (see Table 2-2). This approach will assist with apportioning the risk to individual sources of nutrients, some of which it may be possible to control, or even eliminate. This analysis should be coupled with an estimation of the levels of phosphorus associated with the occurrence of blooms of a particular magnitude expressed as chlorophyll-a. This information may then be used to prioritize mitigation and management efforts.

ASSESSING THE RISK OF CYANOBACTERIAL GROWTH

BENTHIC CYANOBACTERIA

The presence of taste and odour compounds such as 2-methyl isoborneol and geosmin in a reservoir in the absence of known planktonic producers is the most direct indicator of a benthic source. Therefore historical data on tastes and odours can be useful in assessing the risk of potentially toxic benthic cyanobacteria. The distribution of benthic cyanobacteria in a reservoir is restricted by the extent of light penetration. Shallow reservoirs, especially those with high water transparency, will have greater area available for benthic cyanobacteria to grow than deep reservoirs. As a general guide, benthic cyanobacteria need about 1% of the surface irradiance to grow, however this may be lower depending upon the species or type. The area of the reservoir potentially available to benthic cyanobacteria can be calculated from the extinction co-efficient of the water and the bathymetry of the reservoir.

Table 2-2 Examples of potential nutrient inputs into a water body

Sector	Threat Level	Sub-sector	Activities
Industry	High	Paper, pulp or pulp products industries	Industries that manufacture paper, paper pulp or pulp products
	Medium	Breweries or Distilleries	Produce alcohol or alcoholic products
		Chemical Industries	Agricultural fertilisers, explosive or pyrotechnics industries that manufacture explosives, soap or detergent industries (including domestic, institutional or industrial soaps or detergent industries)
		Dredging works	Material obtained from the bed, banks or foreshores on many waters.
Agriculture	High	Intensive Livestock Operations	Feedlots that are intended to accommodate in a confined area and rear or fatten (wholly or substantially) on prepared or manufactured feed (piggeries, poultry, dairies, saleyards)
		Livestock processing industries	Slaughter animals (including poultry). Manufacture products derived from the slaughter of animals including tanneries or fellmongeries or rendering or fat extraction plants, scour, top or carbonise greasy wool or fleeces with an intended production capacity
	Medium	Agriculture	Industries that process agricultural produce including dairy, seeds, fruit, vegetables or other plant material
		Aquaculture or mariculture	Commercial production (breeding, hatching, rearing or cultivation) of marine, estuarine or freshwater organisms, including aquatic plants or animals (such as fin fish, crustaceans, molluscs or other aquatic invertebrates) but not including oysters
	Low	Other Farming	All other farming and agricultural activities
Settlements Urban	High	Wastewater Treatment Plants	Including the treatment works, pumping stations, wastewater overflow structures and the reticulation system (<250 kilolitres/day)
	Medium	Wastewater Treatment Plants	Including the treatment works, pumping stations, wastewater overflow structures and the reticulation system (<250 kilolitres/day)
		Composting	And related reprocessing or treatment facilities (including facilities that mulch or ferment organic waste, or that are involved in the preparation of mushroom growing substrate, or in a combination of any such activities).
Settlements, rural/dense	High	All	Wastewater, waste and water supply activities in areas outside designated urban settlements

PLANKTONIC CYANOBACTERIA

The potential for blooms of planktonic cyanobacteria to occur has been estimated using the 'Vollenweider' model, which relates the spring phosphorus loading as total phosphorus to the subsequent algal biomass measured as chlorophyll-a [40,41, 42]. This relationship is applicable where the occurrence of nuisance cyanobacterial blooms is initially driven by catchment processes that contribute excess nutrients, particularly phosphorus, to the water body.

In addition to simple models based upon lake physical parameters [43], there are more complex deterministic 2D and 3D hydrodynamic models linked to water quality models which can be used to model the occurrence of different algal groups including cyanobacteria. These models are generally complex to run and calibrate and require a large amount of data for a wide range of physical and chemical variables for successful validation. Taylor *et al.* [44] reviewed the application of some water quality models for the prediction of taste and odour events. They concluded that although some of these models can simulate algal growth reasonably well, they are not a viable option to simulate geosmin and MIB production and release. This may be a reasonable current assessment, although the ongoing development and improvement of the water quality and algal growth simulation models by various research groups may result in more robust models in the future.

A simple alternative risk assessment approach developed in Australia to assess water bodies for their susceptibility to cyanobacterial contamination is given in the NHMRC 'Guidelines for Managing Risks in Recreational Water' [45]. The variables used in the assessment are considered to be the predominant drivers or indicators of the potential for cyanobacterial occurrence. These are:

- Prior history of cyanobacterial occurrence
- Water temperature
- Total phosphorus concentration
- Thermal stratification.

These parameters are assigned to categories and assessed in a matrix which defines the risk of the cyanobacterial growth into five categories, ranging from 'Very Low' to 'Very High' (Table 2-3). This approach is simplistic, as a range of other variables can lead to intermediate risk. However, it is a useful, semi-quantitative assessment for the estimation of potential risk. It should be noted that this approach is probably more suited to the buoyant bloom-forming cyanobacteria, such as *Microcystis* and *Anabaena* sp and may not apply as well to other cyanobacteria such as *Cylindrospermopsis raciborskii* or *Aphanizomenon* spp.

Table 2-3 Major parameters that influence cyanobacterial growth. This approach can be applied to *Microcystis* and *Anabaena* sp

Environmental factor				
Potential for Cyanobacterial Growth	History of Cyanobacteria	Water Temperature (^{o}C)	Nutrients Total Phosphorus (μg/L)	Thermal Stratification
Very Low	No	<15	<10	Rare or Never
Low	Yes	15-20	<10	Infrequent
Moderate	Yes	20-25	10-25	Occasional
High	Yes	>25	25-100	Frequent and persistent
Very High	Yes	>25	>100	Frequent and persistent/strong

The values in this table are a guide only, based on Australian experience. The actual values, particularly those for temperature and phosphorous, will be dependent on site-specific conditions. In addition, in most situations there will be other conditions that contribute to the formation of a cyanobacterial bloom, as mentioned above. A similar assessment of the risk associated with a range of phosphorous levels has been developed based on the South African experience and is given in Table 2-4. In both of these examples a key phosphorous concentration to trigger a high risk of cyanobacteria is 25 μg L^{-1}.

Table 2-4 Examples of chlorophyll-a-based risk categories that have been defined for South African reservoirs

Median Annual TP (μg L^{-1})	Risk level	
	Low-level problems	Blooms
0 - 5	Low	Negligible
5 – 14	Moderate	Low
14 – 25	High	Moderate
25 – 50	High	
50 – 150	Very High - Extreme	
> 150	Extreme - Permanent	

ASSESSING THE POTENTIAL FOR TOXIN PRODUCTION

The risk assessment procedures above describe the susceptibility of a reservoir to cyanobacterial contamination, but do not provide a quantitative measure of the potential cyanobacteria population. An empirical model has been developed to estimate the potential maximum concentrations of cyanobacteria and associated microcystins and saxitoxins as a function of known phosphorous levels. The conditions are based on historical and current water quality data and theoretical calculations based on published values such as:

- Fraction of total phosphorous that is bioavailable
- Conversion factor for phosphorous to chlorophyll-a
- Chlorophyll *a* per cell
- Toxin quota per cell

for various cyanobacteria [46, 47, 48].

Within this model three different algal growth scenarios have been developed with the availability of phosphorus as the yield-limiting variable. These are:

Best case: assumes that a low proportion of phosphorus is available for cyanobacterial growth (36%) and converted into phytoplankton, and a low fraction of this biomass is cyanobacteria, so problem cyanobacteria do not become dominant and toxin and odour production occur at the lowest potential rates.

Most likely case: assumes median values for the availability of phosphorus (60%) and for conversion of phosphorus into cyanobacterial biomass; cyanobacteria do not dominate and there are median rates of toxin production

Worst case: assumes that 80% of the phosphorus is bioavailable, that all of this phosphorus is translated into biomass of cyanobacteria, which become dominant, and toxins are produced and released at the maximum reported rates.

An example of the output from this model is given in Table 2-5, for a reservoir with a current total phosphorus concentration of 80 µg L^{-1}. The projected outputs for cell numbers of the cyanobacteria *Microcystis* and associated microcystin, and *Anabaena*, and saxitoxin indicate the range that could be encountered under these conditions and with a decrease or an increase in ambient nutrient levels. It should be noted that these values will be dependent on the type of cyanobacteria and the strain, and will vary considerably with location and conditions. The values for saxitoxin are based on those determined in Australian blooms of *Anabaena*, and will not translate to blooms of *Anabaena* elsewhere. The information in Table 2-5 is for illustrative purposes, the intention should be to undertake similar calculations for a particular water body once sufficient data is available. This information can then provide a simple indication of the challenge to water quality and therefore the treatment process from cyanobacterial contamination for a certain level of nutrients in the source water. Similar calculations can prove very useful once validated for a particular water source and cyanobacterial species.

Comprehensive details on how to calculate a risk assessment are presented in [49].

More sophisticated deterministic water quality models are also available to predict cyanobacterial growth [50, 51]

Table 2-5 Scenarios for the growth of cyanobacteria and production of toxins for different nutrient ambient concentrations in a reservoir using a simple empirical model.

Reservoir nutrient status	Total Phosphorus ($\mu g\ L^{-1}$)	Scenario modelled:	Predicted concentrations of cyanobacteria and their metabolites						
			Bioavailable Phosphorus ($\mu g\ L^{-1}$)	*Microcystis aeruginosa* (cells mL^{-1})	Microcystin (Total) ($\mu g\ L^{-1}$)	*Anabaena circinalis* (cells mL^{-1})	Geosmin (Total) ($ng\ L^{-1}$)	Geosmin (Dissolved) ($ng\ L^{-1}$)	Saxitoxin (Total) ($\mu g\ L^{-1}$)
Lower nutrient level	40	Best Case	14.4	2,000	0.03	1,000	36	1.8	0.07
		Most Likely Case	24	27,000	1.15	13,000	960	96	0.9
		Worst Case	32	44,000	12.8	44,400	4,800	720	2.9
Current nutrient level	80	Best Case	28.8	4,000	0.06	2,000	72	3.6	0.13
		Most Likely Case	48	53,000	2.3	27,000	1,920	192	1.8
		Worst Case	64	89,000	25.6	88,900	9,600	1,440	5.9
Higher nutrient level	160	Best Case	57.6	8,000	0.12	4,000	144	7.2	0.26
		Most Likely Case	96	107,000	4.6	53,000	3,840	384	3.5
		Worst Case	128	356,000	51.2	177,800	19,200	2,880	11.7

RESIDUAL RISK

The scenarios described above suggest the potential for the proliferation of cyanobacteria and the production of cyanotoxins in a water source, i.e. the maximum risk in the absence of preventative measures. The following chapters describe processes that can be implemented to mitigate the risk, such as monitoring programs (Chapter 3), source water management (Chapter 4), water treatment (Chapter 5), and incident management planning (Chapter 6).

CHAPTER 3 DEVELOPMENT AND IMPLEMENTATION OF A MONITORING PROGRAM

BACKGROUND

Monitoring is a critical element in cyanotoxin risk management. The goals of a monitoring program is 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 [52]. 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.
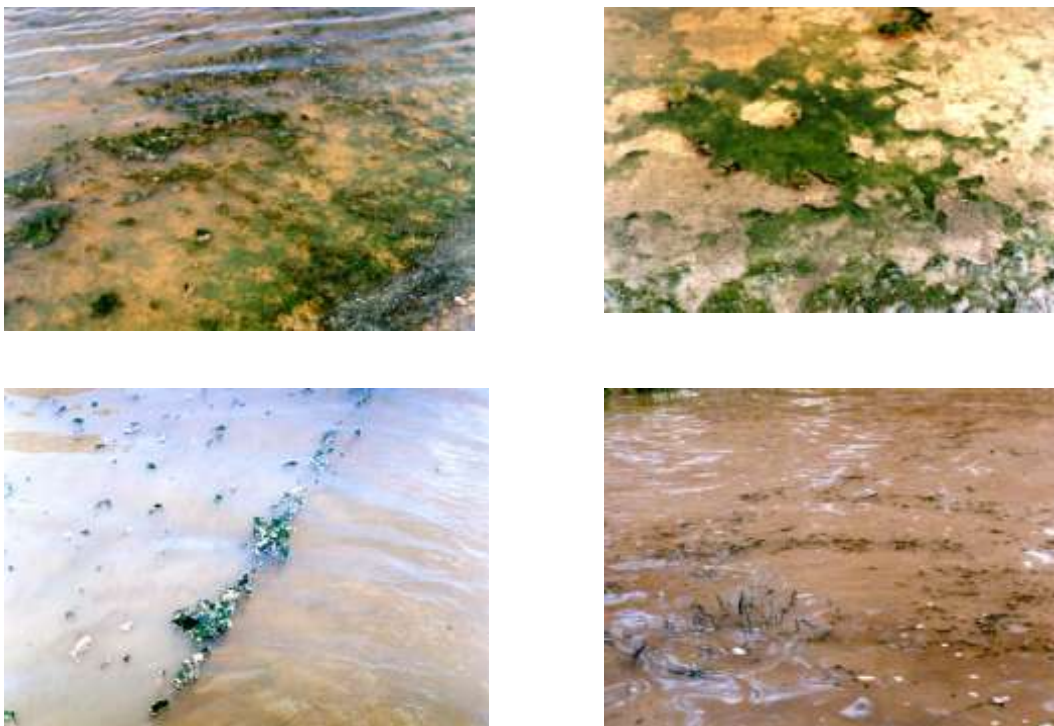
Figure 3-3 Benthic cyanobacteria attached to sediments and rock surfaces, and floating on the surface after breaking free from the substrate

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.

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.

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 [53] 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 (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.

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 [53] 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 [54].

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 (e.g. spiral filaments of *Anabaena*)

Visual taxonomic identification to species level (e.g. *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 or 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 [55, 56, 57].

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 [58, 59, 60, 61]. 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 [62]. As only approximately 50% blooms of potentially toxic cyanobacteria prove to be toxic, this could have important implications for the management of treatment and the implementation of cyanobacteria incident management plans.
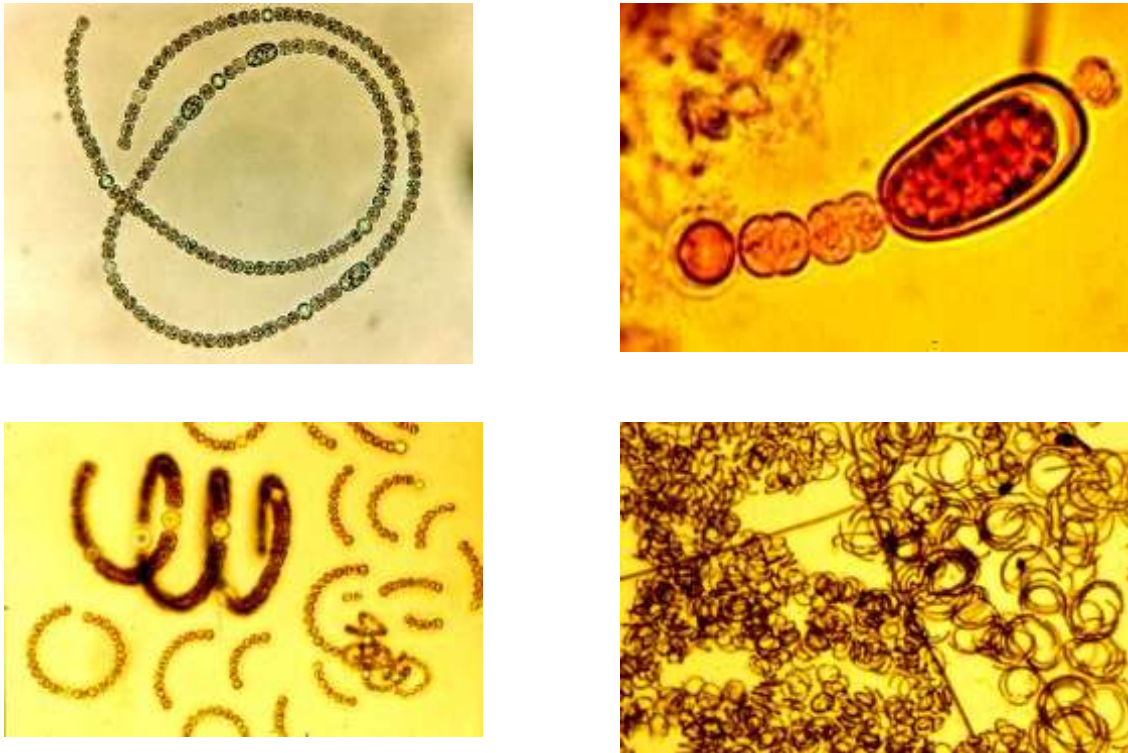
Figure 3-6 Different strains of the same cyanobacterium, *Anabaena circinalis*, several of which are toxic. This figure illustrates the difficulties inherent in microscopic identification for the determination of toxicity

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 these references [53, 63].

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 [64]. An acceptable level of precision for cyanobacterial counting is considered to be in the range of $\pm$ 20-30%. A precision of $\pm$ 30% enables a doubling of a population in successive samples to be detected while a precision of $\pm$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.

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 [65]. 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.

CYANOTOXINS

When potentially toxic cyanobacteria have been identified in a water source, toxin analysis is required to determine if the cyanobacteria is, 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 [66]. 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" [67], together with a more recent international review [68]. 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 [69]. 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 [70], 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).

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.

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.

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	• 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 ug/L	• 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	• 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 $15^{o}C$ and maximum growth rates are attained by most cyanobacteria at temperatures above $25^{o}C$; however growth can also occur at low temperatures [71]. 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 [72]. 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.

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).

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.

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.

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.

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 4 MANAGEMENT AND CONTROL IN SOURCE WATERS

BACKGROUND

In this chapter we discuss management strategies that can be applied within the water body for the control of cyanobacteria, assuming that, where possible, efforts have been undertaken to address any external nutrient inputs from the catchment (Chapter 2).

There are a number of techniques to control or minimise the growth of cyanobacteria in reservoirs. They are represented by a range of:

- Physical controls
- Chemical controls
- Biological controls

In essence, management strategies focus on either controlling factors that influence growth or damaging or destroying the cyanobacteria. Management strategies have been recently comprehensively summarised and reviewed by Cooke *et al.* [73].

A summary of measures that can be applied in lakes and rivers for the management of cyanobacteria is given in Table 4-1. The most commonly utilised techniques are described in more detail in the following sections.

Table 4-1 Techniques for the management of cyanobacteria.

Control method	Technique
Physical	
	Artificial destratification, aeration, mixing
	Dilution to decrease retention time
	Scraping of sediments to remove benthic algae
	Drawdown and desiccation to remove benthic algae
	Sediment removal to reduce nutrient release
Chemical	
	Sediment "capping" with P-binding agents
	Algicides, algistats
	Coagulation
	Hypolimnetic oxygenation
Biological	
	Virus, bacterial infection
	Biomanipulation, increasing grazing or competition for available light and nutrients

PHYSICAL CONTROLS

MIXING TECHNIQUES

A major problem in reservoirs experiencing periods of warm stable conditions is the warming of the upper layer of water; one effect of this is the reduction in the mixing of the water column, resulting in stratification (see Chapter 1). During stratification the water stratum adjoining the bottom sediments, the hypolimnion, becomes depleted of oxygen and contaminants such as ammonia, phosphorus, iron and manganese can be released from the sediment in a soluble form. This increase in nutrient levels can lead to the uncontrolled growth of cyanobacteria. Species such *Microcystis* and *Anabaena* are susceptible to this effect as they exhibit buoyancy due to internal gas vacuoles, and can migrate vertically within the water column, taking advantage of both the light near the surface and increased nutrient levels near the sediment of the water body. Mixing of the water column will disrupt this behaviour and limit the accessibility of nutrients, and thus limit cyanobacterial growth. It may also introduce oxygen to the hypolimnion, preventing further release of nutrients, and possibly increasing the oxidising conditions sufficiently to induce precipitation of the nutrients back to the sediments. In some cases this can prevent the formation of surface scums of toxic cyanobacteria. The mixing regime may also provide more favourable conditions for growth of competing organisms such as diatoms. Artificial mixing has been shown to be effective in many situations e.g. . [74, 75, 76].

The two most commonly used methods of artificial destratification are bubble plume aerators and mechanical mixers.

AERATORS

Bubble plume aerators operate by pumping air through a diffuser hose near the bottom of the reservoir. As the small bubbles rise to the surface they entrain water and a rising plume develops. This plume will rise to the surface and then the water will plunge back to the level of equivalent density. An intrusion will then propagate horizontally away from the aerator plume at that depth. As the intrusion moves through the reservoir there is return flow above and below the intrusion and these circulation cells cause mixing between the surface layer and the deeper water or hypolimnion. An illustration of this effect is given in Figure 4-1a).

The efficiency of a bubble plume is determined by the depth of the water column, the degree of stratification and the air flow rate. The number of plumes, plume interaction and the feasible length of aerator hose required to destratify a particular water body must also be considered in aerator design. As a general rule, bubble plumes are more efficient in deeper water columns. In shallow water columns (<5.0m depth) the individual air flow rates of the plumes must be very small to maintain efficiency.

MECHANICAL MIXERS

Mechanical mixers are usually surface-mounted and pump water from the surface layer downwards towards the hypolimnion, or draw water from the bottom to the surface. This produces a simple mixing effect that is illustrated in Figure 4-1b).

Both types of destratifiers have been shown to mix the surface layers close to the mixing device but areas of the water body further away from the immediate influence of the mixing may remain stratified and provide a suitable environment for cyanobacterial growth. One approach to consider is the use of both mixing techniques in the same water body, where the aerator generates basin-wide circulation cells and the mixer targets the surface stratification outside the direct influence of the aerator plume. This has been used with some success at the Myponga Reservoir in South Australia.
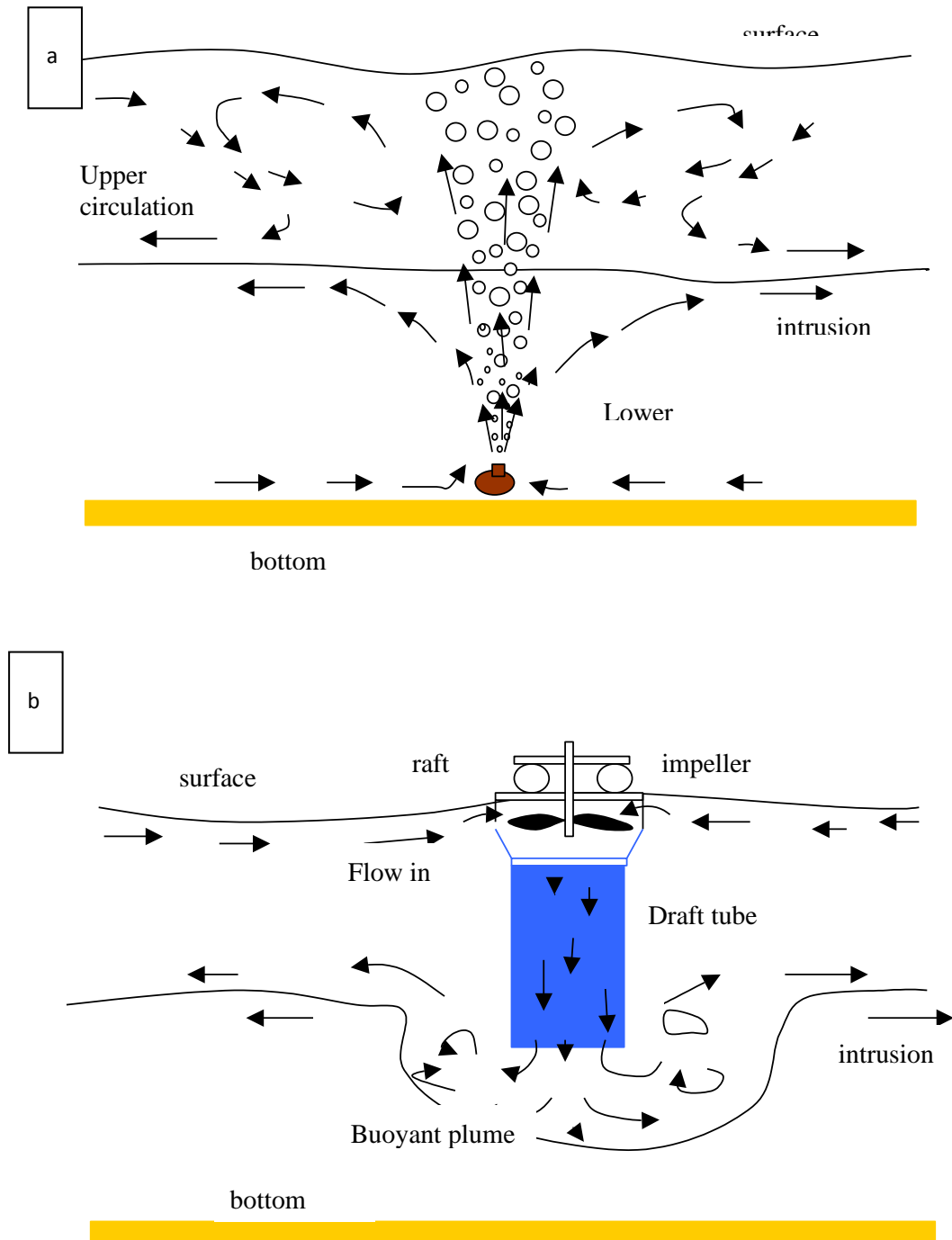
Figure 4-1 Flow and circulation fields created by a bubble plume aerator a) and a surface-mounted mechanical mixer b) in reservoirs

For the successful application of artificial destratification the water body must be sufficiently deep for efficient mixing of at least 80% of the volume. If a larger percentage of the water lies in shallow regions cyanobacteria may accumulate and multiply in these favourable stratified conditions [77]. It is therefore important to apply the appropriate mixing processes for a particular water body. Schladow [78] describes in detail a method for the design of destratification systems for water bodies impacted by cyanobacteria blooms.

Figure 4-2 shows the implementation of mechanical mixing and aeration at Myponga Reservoir, South Australia.

Figure 4-2 Mechanical mixer (left) and aerator (right) at Myponga Reservoir

Destratification is normally employed during late spring, summer and autumn depending upon the amount of surface water heating experienced during those periods. Historical records of temperature would give a guide to when destratifiers should be used. Regular temperature profiles will provide information on how well mixed the reservoir is. The most sophisticated destratification systems automatically adjust the compressor flow rate based upon data from on-line thermistor strings.

MANIPULATION OF RIVER FLOWS

Low flow conditions in rivers can lead to stratification and cyanobacterial growth. In regulated rivers the magnitude and timing of discharge can be manipulated to disrupt stratification every few days thereby controlling cyanobacterial growth. Bormans and Webster [79] reported the development of criteria for flow manipulation that may result in destratification sufficient to disrupt cyanobacterial growth. Clearly, sufficient water must be available for the application of this management strategy and consideration should also be given to the impact of a variation of flows on other aquatic organisms.

OTHER PHYSICAL METHODS

As many problem cyanobacteria can form scums at the surface of a water body, oil-spill skimmers have been used to remove the cyanobacteria, usually to sewer or landfill. Figure 4-3 shows the use of a skimmer to remove surface scum in a recreational lake in South Australia. Atkins et al [80] reported the effective use of coagulation with polyaluminium chloride combined with the removal of surface scum with an oil spill skimmer to treat a severe cyanobacteria bloom in the Swan River in Perth, Australia.

Figure 4-3 The use of a skimmer to remove surface scum in a recreational lake in South Australia. Toxic material was collected and disposed to sewer

Benthic cyanobacteria can be treated using physical methods such as reservoir draw down, followed by desiccation and/or scraping to remove the layer of algae attached to sediments or rocks. However, these methods may not have the desired outcome. A recent study has shown that benthic cyanobacteria can be tolerant to desiccation [81], and scraping or other physical removal can generate turbidity and localised spikes in odour compounds or toxins, which may be an issue depending upon the proximity of the supply offtake.

Figure 4-4 shows the exposure of benthic cyanobacteria after draw-down of a reservoir aimed at control by desiccation.

Figure 4-4 Benthic cyanobacteria exposed after reservoir draw down

If a high nutrient level is due to sediment release it is possible to physically remove sediments. However this is a labour intensive process with implications for short term water quality, and should only be applied if external nutrient input has been significantly reduced.

CHEMICAL CONTROLS

CHEMICAL CONTROL OF NUTRIENTS

HYPOLIMNETIC OXYGENATION

The main aim of hypolimnetic oxygenation is to increase the oxygen concentration in the hypolimnion to prevent or reduce the release of nutrients from the sediment without disrupting the existing stratification of the water body. In this way the nutrient levels in the upper layers of the water body may become limiting to cyanobacterial growth. Techniques used to achieve hypolimnetic oxygenation include airlift pumps, side stream oxygenation and direct oxygen injection [82]. These techniques are relatively expensive, so an extensive understanding of lake hydrodynamics, sediment nutrient release rates and the internal and external contributions to the total nutrient load is necessary to determine whether this would be the most effective management option.

PHOSPHORUS PRECIPITATION AND CAPPING

Precipitation of phosphorus from the water body to the sediment, and treating the sediment to prevent phosphorus release, sometimes called sediment capping, are two methods that have been applied with mixed success.

Reports in the literature show that precipitation of phosphorus can be accomplished with aluminium sulphate, ferric chloride, ferric sulphate, clay particles and lime. The effectiveness of these treatments is highly dependent on the hydrodynamics, water quality and chemistry of the system as the phosphorus can become resuspended or/and resolubilised, depending on the turbulence of the water and the oxidising conditions near the sediments.

Treatments to prevent phosphorus release by applying a layer on the top of the sediment to adsorb or precipitate the nutrient have included oxidation to insoluble iron compounds or adsorption onto zeolites, bauxite refinery residuals, lanthanum modified bentonite clay, clay particles and calcite. Once again, the chemistry and other conditions can have an important effect on the success of these methods [77].

The use of commercial products for this purpose has recently become more widespread. The best known product is a lanthanum modified bentonite clay ('Phoslock') which was specifically designed to bind phosphorus in the clay and maintain it under most conditions encountered in aquatic systems [83]. Limited published results seem to indicate that Phoslock is effective under a range of environmental conditions including under reducing conditions. Issues to consider are dose rates and longevity of treatment depending upon local water chemistry conditions.

CHEMICAL CONTROL OF CYANOBACTERIA

COAGULANTS

Coagulants can be used to facilitate the sedimentation of the cyanobacteria cells to the floor of the water body. Unable to access light, the cells do not continue to multiply, and eventually die. Some coagulants that may be used to coagulate cells include aluminium sulphate, ferric salts (chloride or sulphate), lime, or a combination of lime and metal coagulants. Although it has been reported that cells can be coagulated without damage, over a period of time the coagulated cells will become stressed and unhealthy, break open, or lyse, and release cyanobacterial metabolites [84]. Therefore, unless the coagulated cells are removed from the water body, this process will increase the dissolved toxins present in the water.

ALGICIDES

Algicides are compounds applied to the water body to kill cyanobacteria. As the injured or dead cells will rapidly lyse and release cyanotoxins into the water, this method is most often used at the early stages of a bloom, where numbers are low, and the toxic compounds released into the water can be removed effectively during the treatment process (see Chapter 5, removal of dissolved toxins). As with the application of any chemical to water destined for human consumption, there are a number of issues to be considered, including:

- Calculation of the required concentration to ensure the destruction of the cyanobacteria, with minimal residual of the chemical
- Effective application in terms of location and mode of dosing (e.g. from a boat, aerial spraying)
- The effect of dosing a potent chemical on the existing ecosystem in the water body
- Accumulation of the algicide in sediments
- Implications in the treatment plant of residual algicide (e.g. copper is coagulated in conventional treatment and may contaminate waste streams)

A list of chemicals that have been utilised as algicides is shown in Table 4-2 , along with key references which describe their properties and effectiveness.

Table 4-2 Algicides, their formulations and key references (after [85])

Compound	Formulation	References
Copper sulphate	$CuSO_4.5H_2O$	86, 87,88,89
Copper II alkanolamine	Cu alkanolamine.$3H_2O$ $^{++}$	90
Copper-ethylenediamine complex	$[Cu(H_2NCH_2CH_2NH_2)_2(H_2O)_2]^{++}SO_4$	90
Copper-triethanolamine complex	Cu $N(CH_2CH_2OH)_3.H_2O$	90
Copper citrate	$Cu_3[(COOCH_2)_2C(OH)COO]_2$	91,92
Potassium permanganate	$KMnO_4$	93 94
Chlorine	Cl_2	93
Lime	$Ca(OH)_2$	95
Barley straw		96, 97

COPPER BASED ALGICIDES

Copper based compounds are often used for chemical control of cyanobacteria. It is believed that the oxidative potential of the copper ion at high concentrations causes the cell membrane to rupture thus lysing and destroying the cyanobacteria cell. The effectiveness of copper as an algicide is determined by a combination of factors. Chemical parameters such as pH, alkalinity and dissolved organic carbon (DOC) of the receiving water control copper speciation and complexation, which affects copper toxicity. Thermal stratification affects the distribution of copper after application, which may then affect contact with the algae.

It is important to note the environmental impacts that copper dosing may have. Copper is known to be toxic to non-target organisms such as zooplankton, other invertebrates and fish [98]. It is also a bactericide, and may result in the destruction of various beneficial bacteria, including those that participate in the degradation of the cyanotoxins, once they are released. It is also known to accumulate in lake sediments and treatment plant sludge [99, 100]. In many countries there are national or local regulations to control the use of algicides due to their adverse environmental impacts.

Copper sulphate is the most commonly used of the copper-based algicides. Table 4-3 shows the relative toxicity of copper sulphate to several species of cyanobacteria.

Table 4-3 Relative toxicity of copper sulphate to cyanobacteria. Modified after Palmer [88].

Group	Very Susceptible	Susceptible	Resistant
Cyanobacteria	*Anabaena, Microcystis (Anacystis), Aphanizomenon, Gomphosphaeria, Rivularia*	*Cylindrospermum, Planktothrix (Oscillatoria), Plectonema*	*Nostoc, Phormidium*

A range of methods is available for copper sulphate dosing. The commonly used method involves applying dry granular copper sulphate alongside or behind powerboats. Copper sulphate can also be dosed by conventional aerial application similar to other agricultural chemicals. The method of application of copper sulphate may have important effects on copper dispersal and ultimately the toxicity and success of treatment. It is important to try to achieve the best possible coverage of the reservoir surface and avoid missing shallow, difficult to access, zones where cyanobacteria can accumulate. Figure 4-5 a-c) shows copper sulphate dosing by boat.

Copper sulphate can also be used to manage benthic cyanobacteria once reservoir draw-down has occurred (Figure 4-5 d)).

Figure 4-5 Copper sulphate dosing of a reservoir (a-c) and benthic cyanobacteria after reservoir draw-down d)

The toxic component of copper sulphate is the cupric ion (Cu^{2+}). After dosing the effective concentration of the active component will depend on the water quality parameters mentioned above. For example, Cu^{2+} complexes readily with natural organic material present in all water bodies, which renders it much less effective as an algicide.

The problem of the reduced effectiveness of copper sulphate treatment in hard alkaline water has long been recognised [88]. Chelated copper algicides were developed to overcome the problems of the complexation and loss by precipitation of toxic copper under these circumstances. Examples of copper chelate algicides include copper ethanolamine, copper ethylene-diamine and copper-citrate (Table 4-2). The chemical properties and application rates for these algicides are given by Humberg et al. [90]. These chelated algicides are available as liquid formulations, and in some cases a granular form is also manufactured.

Copper citrate has been used as an algicide in the U.S. [91]. It is available either as a commercial preparation [101] or by simultaneously dosing copper sulphate and citric acid [91]. It is claimed that the use of citric acid as a chelating agent enhances the solubility of copper allowing it to remain in solution longer under alkaline conditions [102].

The chelated copper compounds are often more expensive than copper sulphate; however they may be more effective as they maintain Cu^{2+} in solution longer than copper sulphate. As with any chemical, the efficiency is highly dependent on the mode of application and the water quality conditions. Unfortunately, despite the relatively widespread use of chelated copper algicides the effect of water chemistry on their efficacy is poorly understood.

OTHER ALGICIDES

Potassium permanganate: A survey of North American utilities in the 1980s, indicated that a small number used potassium permanganate as an algicide in reservoirs [94]. Fitzgerald [94] found that the dose range required to control algae and cyanobacteria was in the range 1 - 8 mg L^{-1}.

Chlorine: Chlorine is used mainly for control of algae in water treatment works but has also been employed in reservoir situations [87]. The effective dose rates would obviously be dependent on the chlorine demand of the water, but most algae are reportedly controlled by doses of free chlorine between 0.25 and 2.0 mg L^{-1} [87].

Hydrogen peroxide: Hydrogen peroxide has been shown to selectively damage cyanobacteria over other plantonic species such as green algae [103]. Recently a range of stabilised hydrogen peroxide compounds have been developed in the US specifically to provide an alternative to overcome the environmental issues associated with copper algicides. Several manufacturers have now had these formulations added to the list of USEPA registered pesticides as algicides for use in drinking water reservoirs. The formulations contain solid granules of sodium carbonate peroxyhydrate which are directly applied to a water body releasing sodium carbonate and hydrogen peroxide. The hydrogen peroxide then degrades further into hydroxyl free radicals which are claimed to cause oxidative damage to cell membranes and to cell physiological processes.

ISSUES ASSOCIATED WITH ALGICIDES AND OTHER CHEMICAL CONTROLS

Before applying chemical controls against toxic cyanobacteria it is important to be fully aware of both the environmental and practical problems with their use.

The most commonly used algicide - copper sulphate - has a significant ecological impact. It should be used only in dedicated water supply reservoirs, and even then it is an unsatisfactory long-term solution. In many countries there are national or local environmental regulations which prohibit or limit the use of algicides due to their adverse environmental impact. This should be taken into consideration when developing management strategies for water sources.

As mentioned earlier, the disruption to the cell walls produced by algicides leads to the rapid release of the intracellular cyanobacterial metabolites. This can result in the diffusion of algal toxins throughout the water body within hours. Additional measures must then be applied within the treatment plant to remove these dissolved metabolites (See Chapter 5, removal of dissolved cyanotoxins). If possible, after algicide treatment, the reservoir

should be isolated for a period to allow the toxins and odours to degrade. This is particularly important if the treatment is applied during bloom conditions. Unfortunately, it is difficult to advocate a minimum withholding period prior to recommencing use of the water body as the degradation of the toxin will depend upon local conditions (i.e. temperature, microbial activity), however it could be in excess of 14 days [104]. A range of microorganisms have been shown to very effectively degrade several of the major cyanotoxins, including microcystins and cylindrospermopsin [105, 106]. However, the time taken for total toxin degradation varies widely from 3-4 days to weeks or months depending upon the circumstances [107]. Therefore, it is recommended that monitoring be undertaken to determine the amount of toxin remaining in the waterbody after treatment with an algicide.

Generally, microcystins are known to degrade readily in a few days to several weeks [105, 108]. Cylindrospermopsin has been shown to persist in the waterbody for extended periods and its degradation is dependent upon the presence in the reservoir of the microorganisms with the necessary enzymes for cylindrospermopsin degradation [106]. However, in water bodies where the cylindrospermopsin is found regularly, degradation has been shown to occur relatively rapidly [109].

Saxitoxins have not been shown to be degraded by bacteria therefore, if a toxic bloom of *Anabaena circinalis* is dosed, it may be necessary to have water treatment strategies for dissolved toxin removal [110]. In addition, although saxitoxin appears to be non-biodegradable, it can undergo biotransformations involving conversion from less toxic forms to more toxic variants [111].

BIOLOGICAL CONTROLS

Cyanobacterial growth can be moderated by manipulation of the existing ecosystem in a reservoir or lake. Important aims can be to:

- Increase the numbers of organisms that graze on the cyanobacteria
- Increase competition for nutrients to limit the growth of cyanobacteria

Biomanipulation is often described as either "bottom up" (nutrient control) or "top-down" (increased grazing).

INCREASING GRAZING PRESSURE

The introduction of measures to encourage the growth of zooplankton and benthic fauna that feed on cyanobacteria can be effective in limiting cyanobacterial proliferation. Methods reported in the literature include:

- Removal of fish that feed on zooplankton and other benthic fauna, or introduction of predators to these fish.
- Development of refuges to encourage the growth of the beneficial organisms [77]

ENHANCING COMPETITION BY INTRODUCING MACROPHYTES

In relatively shallow water bodies with moderate phosphorus concentrations the introduction of macrophytes can limit available phosphorus and therefore limit cyanobacterial growth. When other measures are also taken such as the control of fish types and numbers, the introduction of macrophytes to a water body may result in improved turbidity and lower cyanobacteria growth [77]. Figure 4-6 shows the introduction of water plants into a heavily contaminated water body in an effort to reduce nutrient levels and improve water quality.

Figure 4-6 Introduction of water plants into a heavily contaminated water body in an effort to reduce nutrient levels and improve water quality

OTHER BIOLOGICAL STRATEGIES

The potential of microorganisms such as bacteria, viruses, protozoa and fungi to control cyanobacteria has been studied on a laboratory scale. Although successful on a small scale, the full scale use of such measures has not been attempted due to a range of problems such as the difficulty of culturing large numbers of microorganisms, and the ability of the cyanobacteria to become immune to infection [77].

ISSUES ASSOCIATED WITH IMPLEMENTATION

Biomanipulation is a very difficult management practice to implement, as many interacting factors influence the ecology of a water body. The deliberate modification of the biodiversity of the system may have unintended consequences for other organisms and water quality parameters. In addition, the ongoing implementation of such a strategy will require constant monitoring and adjustment, as it is likely that the system will tend to readjust to the original biological structure [77].

ALTERNATIVE METHODS

BARLEY STRAW

The use of decomposing barley straw for the control of algae and cyanobacteria has been the subject of considerable interest and investigation since the early '90s [96, 97, 112, 113]. Laboratory studies have suggested algistatic effects on both green algae and cyanobacteria. Several causes have been suggested for the observed effects, including the production of antibiotics by the fungal flora responsible for the decomposition, or the release of phenolic compounds such as ferulic acid and *p* - coumaric acid from the decomposition of straw cell walls [97]. While reservoir trials with barley straw appeared to confirm these laboratory observations [113, 114] other trials resulted in no observable effect [115, 116].

Because of its affordability and ease of use barley straw is used in many reservoirs and dams in the United Kingdom with positive results. A fact sheet prepared by the Centre for Hydrology and Ecology, Natural Environment Research Council and the Centre for Aquatic Plant Management in the UK details the application and mechanism of the effect of barley straw for the control of algae in a range of water bodies [117].

Although some water authorities have applied this method due to the low cost and appeal as a natural treatment, Chorus and Mur [77] do not recommend its use due to the possibility of the production of unknown compounds (possibly toxic, or odour –producing) and consumption of dissolved oxygen during the decomposition process.

ULTRASOUND

Ultrasound has been the focus of several studies. It has been found to limit the growth of cyanobacteria [118] as well as causing sedimentation due to disruption of the gas vesicles [119] depending on the energy and length of time of application. The observed effects are also dependent on the species of cyanobacteria [120]. The application of ultrasound was reported to successfully reduce the proliferation of cyanobacteria in a treated pond compared with a similar pond that was not exposed [121]. The study of ultrasound as a method of control for cyanobacteria is still in its infancy, and the technical hurdles involved in the application of this technology in a large water body are clear, however further work may reveal it to be an effective, non-chemical control strategy.

CHAPTER 5 TREATMENT OPTIONS

If toxic blooms occur despite management strategies, there are three options to minimise toxin levels in water supplied to consumers;

- Use of an alternative supply uncontaminated by cyanobacterial toxins

- Offtake manipulation to prevent the intake of cyanobacteria and/or their toxins into the water supply system

- Water treatment to remove cyanobacterial cells and/or their toxins

The main focus of this section is the removal of cyanobacterial cells and the cyanotoxins they produce. However, for many treatment plants a first control step can be the manipulation of the offtake from the source water to minimise cyanobacteria entering the treatment facility.

OFF-TAKE MANIPULATION

Due to the buoyancy regulation of some cyanobacteria, they are usually found in a particular depth range within a water body. A comprehensive monitoring program, as described in Chapter 3, will provide this information. If the treatment plant has the ability to extract water from several depths, often the most concentrated area of the cyanobacteria bloom can be avoided. However, the conditions that favour the growth of cyanobacteria (thermal stratification, anoxic hypolimnion) will also favour release of iron and manganese from the sediments, so care should be taken to adjust the height of the offtake to avoid both high cyanobacterial numbers, and elevated manganese and iron levels. Often the two water quality goals will be difficult to manage simultaneously.

CYANOBACTERIAL CELL REMOVAL

A healthy cyanobacterial cell can have high levels of toxin – or taste and odour compounds – confined within its walls. For example, for *Microcystis aeruginosa* more than 95% of the toxin can be contained within healthy cells, whereas the number would be around 50% or less for *Cylindrospermopsis raciborskii*. Therefore, high cell numbers can result in high total toxin concentration. The most effective way to deal with high total toxin concentrations is to remove the cells, intact and without damage. Any damage may lead to toxin leakage, and an increase in the dissolved toxin concentration entering the treatment plant. Dissolved toxin is not removed by conventional treatment technologies, and the aim should be to minimise the levels entering the treatment plant.

Removal of intact cells and associated intracellular toxin should be the primary aim in the treatment of cyanobacteria. As most water treatment processes are designed to remove particulate material as the primary focus, this first step requires only the optimisation of existing particulate removal processes, as well as an awareness of how some of these processes may lead to cell damage, and leaking of the toxins into the dissolved state.

PRE-OXIDATION

Pre-oxidation is not recommended in the presence of potentially-toxic cyanobacteria. Chemical oxidation can have a range of effects on cyanobacteria cells, from minor damage to cell walls to cell death and lysis [122]. Although it has been reported in the literature that oxidation at the inlet of the treatment plant can improve the coagulation of algal cells through a number of mechanisms, [123] the risk of damaging the cells and releasing toxin into the dissolved state is high. If pre-oxidation must be applied in the presence of cyanobacterial cells the levels of oxidant should be sufficient to meet the demand of the water including cells, and result in a residual sufficient for destruction of

dissolved toxins if these are susceptible to removal by the particular oxidant (see following sections on removal of dissolved toxins). If insufficient oxidant is applied there is a risk of high levels of dissolved toxin and organic carbon entering the treatment plant and adversely influencing subsequent removal processes. However, this effect will depend on the oxidant and its reactivity with the particular cyanobacteria. For example, recent work by Ho *et al.* [124] has shown that potassium permanganate, applied at a concentration necessary to oxidise moderate levels of manganese, did not damage *Anabaena circinalis* cells, and therefore did not result in release of geosmin and saxitoxins into the dissolved state. If pre-oxidation is deemed necessary, it is recommended that laboratory tests be carried out to determine the effect, if any, on the cyanobacteria present in the inlet to the plant.

MICROSTRAINING

Microstraining is a technique that can be used to remove fine particles including algae and cyanobacteria. Microstrainers separate solids from raw water by passage through a fabric of either fine steel mesh or plastic cloth. Depending on the size of aperture in the fabric, it behaves either as a filter to remove coarse turbidity, zooplankton, algae, etc. or as a fine screen to remove larger particles. A microstrainer consists of a horizontally mounted, slowly rotating drum with sides of fabric. One end is sealed and the other allows water in and screenings out. Water is fed into the centre and flows out through the sides. The top of the drum remains above the water level and is continuously cleaned by water jets on the outside. The screenings are collected in a trough suspended towards the top of the drum interior. They are sieved, the solids disposed of and the water returned to the inlet.

Microstraining is used to remove mineral and biological solids from surface water. It is normally used as pre-treatment before slow sand filtration or coagulation processes but for very good quality waters it can be used as a sole treatment prior to disinfection. Microstraining can successfully remove filamentous or multicellular algae, but will be less efficient for small, unicellular species.

RIVERBANK, SLOW SAND AND BIOLOGICAL FILTRATION

Riverbank filtration is a simple and effective treatment process which is widely used in some parts of the world. Water is abstracted from rivers by using bores (wells) close by, effectively filtering the raw water through the riverbank usually consisting of sand, gravel or stones. Particulates including algae and cyanobacteria are removed by this filtration process. Many soluble contaminants are also removed by adsorption or by biological processes taking place in the biofilm on the sand/gravel grain surfaces, mainly in the first few centimetres of infiltration. In this process dissolved toxins can also be removed [125]. Bank filtration covers a wide range of conditions, with travel times between the river and the well of a few hours to several months. In case of short travel times the processes involved are comparable to those occurring in slow sand filters.

GENERAL CONSIDERATIONS

Slow sand filtration (SSF) is capable of providing a high degree of removal of algal cells (>99%) and associated cyanotoxin. Biological activity within slow sand filters may also provide some removal of extracellular toxin. Algal growth in the water above slow sand filters is a common problem, and has implications in relation to cyanotoxins, depending on the predominant algal species.

In general, good performance of slow sand filtration depends on the following factors:

1) *Feed water quality*
   The quality of water going on to slow sand filters is crucial to performance. Generally, turbidity above 10 NTU can lead to reduced run times. In addition, high algal concentrations in the raw water can result in excessive algal growth above the sand, causing rapid blockage and short run lengths. These problems can be alleviated

or prevented by pre-treatment (e.g. roughing filters, microstrainers), or by covering of the filters where this is practical.

2) *Filtration rate*

   Headloss across the bed and the rate of headloss build-up (filter blockage) both increase with increasing filtration rate. Performance of slow sand filtration is best when the filtration rate is constant, avoiding sudden large changes in filtration rate (± 20%) to prevent deterioration in filtrate quality.

3) *Sand skimming*

   Groups of filters should be skimmed in rotation, such that at any time a minimum number of filters are out of operation, thereby preventing excessive loading to the other filters. Skimming involves removing the Schmutzdecke layer and the uppermost 1 to 2 centimetres of sand, manually or, more commonly now, using mechanical scrapers. The bed depth should not be allowed to decrease to less than 0.3 m; the depth is then returned to between 1 and 1.5 m using cleaned sand from storage.

4) *Restart after sand skimming*

   A ripening period of several days is required before good performance is restored after skimming. Longer periods may be necessary after resanding or at low water temperatures. To prevent excessive penetration of solids into newly skimmed or resanded beds, the filtration rate should be gradually increased over a period of 3 or 4 days, starting at a low rate of less than 0.1 m/hour. The filtrate produced during the first few days after restart may need to be discharged to waste or returned to the inlet of the other filters

Specific information relating to removal of cyanotoxins by slow sand filtration is scarce, partly because laboratory scale tests are not appropriate since they cannot easily simulate the biologically active Schmutzdecke layer.

Bank filtration covers a wide range of settings with travel times between the river and the well of just a few hours to several months. In case of short travel times the removal is similar to that described for SSF, though a schmutzdecke is usually not formed along the river bank due to shear stress of the flowing river water – regular skimming is therefore not necessary. In this setting most intra-cellular toxins will be removed from the source water. In case of longer travel times (several days to months) additional degradation of extra-cellular toxin is possible. Mixing with ambient landside groundwater in the drinking water well will result in further reduction of concentrations.

CONVENTIONAL TREATMENT

The response of cyanobacteria to coagulants and other chemicals used during the coagulation/flocculation process depends strongly on the type of organism and its form (i.e. individual cells, filamentous etc, see Chapter 1). As a result, specific guidelines for coagulation are not possible. However, general tips for optimum removal of cyanobacteria will be helpful as a first treatment step.

If optimisation of coagulation is maintained for the normal parameters (including turbidity, dissolved organic carbon removal) under the conditions of high numbers of cyanobacteria, optimum removal of cells, and therefore intracellular toxin, will be achieved [126]. Evidence in the literature is conflicting regarding the most effective coagulant, polyelectrolytes, etc, so optimising the existing processes should be the first response. Evidence is also conflicting in terms of damage to the cells during the coagulation process. Whether there is some damage during the process appears to be dependent on the health of the cells, and the stage in the growth of the bloom. In a natural bloom there will probably be cells in all stages of growth. However, an optimised coagulation process will provide a very effective first barrier to toxic algae in the treatment plant. Figure 5-1 shows an *Anabaena circinalis* filament encased in an alum floc. The darker areas are the powdered activated carbon particles used to remove dissolved toxins and taste and odour compounds.

Figure 5-1 *Anabaena* **filament encased in an alum floc. Dark areas are powdered activated carbon particles used to remove dissolved tastes and odours and cyanotoxins**

Dissolved air flotation (DAF) is very effective for the removal of cyanobacterial cells, particularly for those species with gas vacuoles that may render them more difficult to settle. The same advice for the optimisation of the process applies for the DAF process.

COAGULATION AND FLOCCULATION GENERAL CONSIDERATIONS

Optimisation of the coagulation process is important under all conditions, but it is particularly relevant during a toxic cyanobacteria bloom. Achieving good chemical coagulation and flocculation relies on the following:

- Selection of the most appropriate coagulant and pH conditions

- Good control of coagulant dose and pH to maintain optimum conditions particularly during the initial mixing stage. Underdosing of coagulant or inadequate pH control produces poor floc, whilst overdosing increases the quantity of solids for removal and can, in some circumstances, produce large weak floc that can be difficult to remove efficiently

- Good mixing at the point of chemical dosing to ensure rapid intimate contact between water and coagulant

- Optimisation of flocculation: where mechanical flocculation is used, optimum paddle speeds need to be determined based on performance of the subsequent treatment process

- Avoidance of excessive floc shear after flocculation, which could result from turbulence at weirs, pipe bends or constrictions, and from high flow velocity (above 0.3 m/s)

- Laboratory jar tests are used to select the best combination of coagulation chemicals and pH, which should be verified carefully on the plant

An additional consideration for cyanotoxins is the risk of cell lysis with a high degree of mixing on coagulant addition. Where very high intensity of mixing is generally applied, a compromise may be required between the requirements for effective coagulation and the potential for cell lysis and cyanotoxin release.

Polyelectrolytes are often used in conjunction with metal ion coagulants, primarily as flocculant aids to produce floc which is more easily removed by subsequent clarification or filtration. These are normally added shortly after the coagulant, to provide a lag time for primary floc particles to form. This lag time can be critical to good performance, particularly under cold water conditions, and ideally needs to be established on a site-by-site basis.

SLUDGE AND BACKWASH DISPOSAL

Once confined in sludge of any type, cyanobacteria may lose viability, die, and release dissolved toxin into the surrounding water [127]. This can occur within one day of treatment and can result in very high dissolved toxin concentrations in the sludge supernatant. Similarly, algal cells carried onto sand filters, in flocs or individually, will rapidly lose viability. Therefore, if possible, all sludge and sludge supernatant should be isolated from the plant until the toxins have degraded sufficiently. Microcystins are readily biodegradable [128] so this process should take 1-4 weeks. Cylindrospermopsin appears to be slower to degrade [129] and the biological degradation of saxitoxins and anatoxins has not yet been widely studied. However, the saxitoxins are known to be stable for prolonged periods in source water, so caution is recommended.

During a bloom where some cells are carried through to the filters, backwash frequency will probably increase. This is desirable to reduce the risk of dissolved toxin released into the filtered water. Operators should be aware of the possibility of toxic algae in the backwash water, and consequent risk of elevated dissolved toxin levels.

MEMBRANE FILTRATION

Membrane processes are becoming an increasingly viable option for treatment of both small supplies and larger sources at risk of microbiological contamination (e.g. *Cryptosporidium*). Membranes used in water treatment can be classified as:

- Microfiltration (MF) membranes for removal of fine particulate material above 1 μm in size, such as *Cryptosporidium* and some bacteria

- Ultrafiltration (UF) membranes for removal of colloidal particles of less than 0.1μm and high molecular weight organics

- Nanofiltration (NF) membranes for removal of lower molecular weight organics, colour and divalent ions such as calcium and sulphate

- Reverse osmosis (RO) membranes for desalination of seawater or brackish water

Generally cyanobacterial cells and/or filaments or colonies can be expected to be 1 micron in size or larger. Therefore membranes with a pore size smaller than this will remove cyanobacterial cells. Figure 5-2 is a representation of the removal efficiency of various filtration processes. As the figure shows, in general, micro- and ultra-filtration membranes could be expected to remove cyanobacterial cells effectively. In reality, pore size distributions will vary between manufacturers, so specific information should be sought regarding pore sizes. Clearly the efficiency of removal will also depend on the integrity of the membranes. Processes such as nanofiltration and reverse osmosis membrane filtration will have a pre-treatment step designed to remove particulates and dissolved organic carbon to minimise fouling of the membranes. Therefore, if the pre-treatment processes are working effectively only dissolved toxin could be expected to challenge these membranes. In the case of micro- and ultra- filtration, healthy cyanobacterial cells may be concentrated at or near the membrane surface. The extent of damage to the cells will depend on the flux through the membranes, pressure and the time period between backwashes and removal of the waste streams [130]. As with coagulation, optimisation of the processes is recommended, with frequent backwashing, and isolation of the backwash water from the plant due to the risk of the cells releasing dissolved toxin. Ultra- and micro- filtration membranes cannot be expected to remove dissolved toxins released from damaged cells on the membrane surface. In practice, some removal has been noted. As this is most likely due to the adsorption of the toxins onto the membrane surface, it would be expected to vary between membrane materials, and to decrease significantly with time as the adsorption sites are occupied by the toxin molecules.

Submerged membrane systems may offer advantages over pressurised systems for waters with high cyanobacterial concentrations, as submerged membranes avoid pumping of the water prior to the membrane, and the pressures applied are much less, hence the potential for cell lysis is reduced. However, this benefit may be offset by greater accumulation of cyanobacterial cells in the membrane tanks of submerged systems. This accumulation might be reduced operationally by draining down the tanks more frequently at times of cyanotoxin risk.

For pressurised systems, potential for cell lysis may be greater for crossflow systems than for dead-end operation, particularly if accumulation of bacterial cells in the recycle stream is allowed to occur.
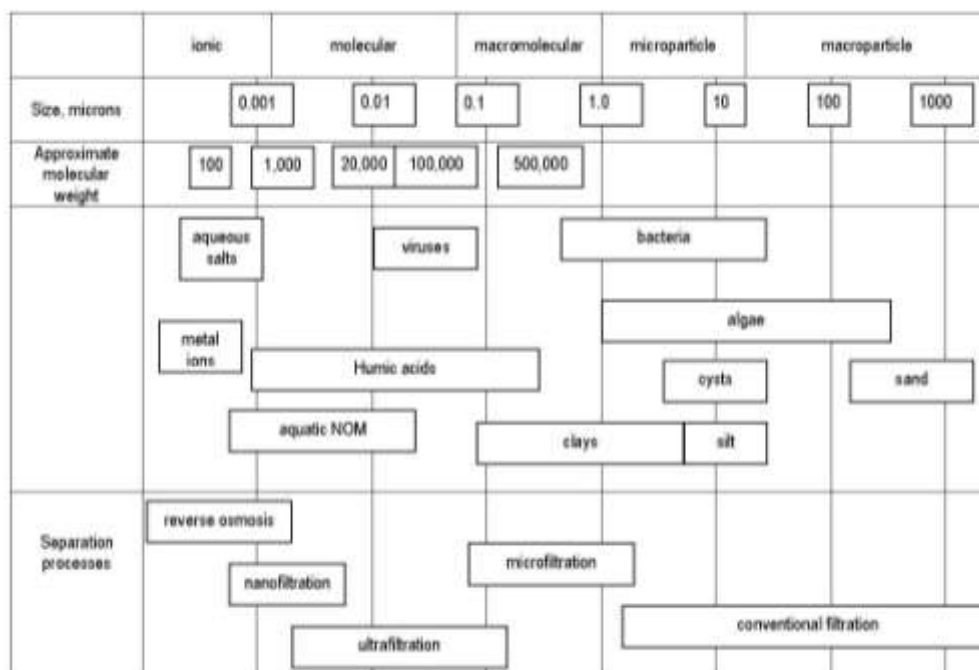
Figure 5-2 Efficiency of various filtration processes

CYANOTOXIN REMOVAL

Even if treatment is aimed at removing cells intact with their intracellular toxins, there is the possibility that dissolved toxins may be present. Thus it is always prudent to send samples for chemical analysis for the toxin most likely to be present. This knowledge will come from a history of observation and monitoring as described in Chapter 3. It is likely that the analysis will take at least 24 hours, so it is desirable to initiate treatment measures to remove the maximum level of the toxin most likely to be present.

Processes to remove dissolved microcontaminants, including cyanobacterial toxins, from drinking water are strongly influenced by the properties of the target compound. More details on the structures of cyanobacterial toxins are given in Chapter 1.

As mentioned earlier, conventional treatments such as coagulation etc, are not effective for the removal of dissolved cyanotoxins. The three categories of water treatment processes that can be applied for the effective removal of dissolved toxins are:

- *Physical processes* such as removal using activated carbon, membranes
- *Chemical processes* such as oxidation with chlorine, ozone and potassium permanganate
- *Biological processes* such as filtration through sand or granular activated carbon (GAC) supporting a healthy biofilm

PHYSICAL PROCESSES

ACTIVATED CARBON

Activated carbon is a porous material with a very high surface area. The internal surface provides the sites for the target contaminants such as algal toxins to adsorb. Activated carbon is used extensively in water treatment for adsorption of organic contaminants, particularly pesticides, volatile organic compounds, cyanotoxins, and taste and odour compounds, often resulting from algal activity.

Activated carbon is available in two forms, granular activated carbon (GAC) and powdered activated carbon (PAC). Powdered activated carbon can be added before coagulation, during chemical addition, or during the settling stage, prior to sand filtration. It is removed from the water enmeshed in floc during the coagulation and sedimentation process, in the former cases, and through filtration, in the latter. As the name implies, PAC is in particulate form, with a particle size typically between 10 and 100 μm in diameter. PAC is dosed as a slurry into the water, and is removed by subsequent treatment processes. Its use is therefore restricted to works with existing coagulation and rapid gravity filtration, or it may be applied upstream of a membrane process. One of the advantages of PAC is that it can be applied for short periods, when problems arise, then stopped when it is no longer required. With problems that may arise only periodically such as algal toxins, this can be a great cost advantage. A disadvantage with PAC is that it cannot be reused and is disposed to waste with the treatment sludge or backwash water.

Granular activated carbon is used extensively in many countries for the removal of micropollutants such as pesticides, industrial chemicals and tastes and odours. The particle size is larger than that of PAC, usually between 0.4 and 2.5 mm. Granular activated carbon is generally used as a final polishing step, after conventional treatment and before disinfection. It can also be used as a replacement medium for sand and/or anthracite in primary filters. The advantages of GAC are that it provides a constant barrier against unexpected episodes of tastes and odours or toxins, and the large mass of carbon provides a very large surface area. The disadvantage is that it has a limited lifetime, and must be replaced or regenerated when its performance is no longer sufficient to provide high quality drinking water. Filtration through GAC is often used in conjunction with ozone. When used in conjunction with ozone it is sometimes called BAC, or biological activated carbon. However, this is can be misleading, as all GAC filters function as biological filters within a few weeks to months of commissioning.

POWDERED ACTIVATED CARBON

APPLICATION OF PAC FOR OPTIMUM PERFORMANCE

One disadvantage with PAC is that the contact time is usually too low to utilise the total adsorption capacity of the carbon. Dosing of PAC immediately before, or during, coagulation may reduce its effectiveness by incorporation into the floc, and should be avoided if possible. PAC can also be applied after coagulation. The advantage of this placement is that a significant proportion of the competing compounds, the natural organic material (NOM), has been removed during the coagulation process. The disadvantage is that the contact time, where the PAC is mixed efficiently through the water, is greatly reduced. There is some evidence that a layer of PAC on top of the conventional filters may provide some additional removal. This has not been shown conclusively for the removal of toxins so could not be recommended as an effective barrier. Generally, the most suitable place for dosing PAC is upstream of coagulation in a separate PAC contact basin, or in a pipeline where there is some distance between the source water off-take and the treatment plant.

The type of treatment process can also influence PAC performance. Accumulation of PAC in floc blanket clarifiers and filters may give benefits of extending the contact time and PAC concentration. Contact time in DAF cells is relatively short, although long flocculation times could be beneficial.

For a particular site, laboratory tests should be carried out to help evaluate the best position for PAC dosing by simulating the treatment stream, as well as identifying suitable PAC type and dose.

PAC TYPE AND DOSE REQUIREMENTS

Natural organic material plays a large role in controlling the removal of microcontaminants using activated carbon. NOM is present in all water sources at much higher concentrations than the target compound. For example, a concentration of 5 μg L^{-1} of toxin entering a treatment plant would be considered quite high, whereas a concentration of 5 mg L^{-1} of dissolved organic carbon (DOC) in surface water would be moderate. In this situation the concentration of NOM (approximately 2 x DOC) [131] is 2000 times that of the target compound – the toxin. Clearly NOM offers very high competition for adsorption sites on the activated carbon. The difficulty in providing guidelines for the dosing of PAC for the removal of any compound is the overriding influence of the competing NOM. Every water source will have NOM of different concentration and character, and these factors are controlled by site-specific conditions such as vegetation, soil type and climatic conditions. As a result, only broad guidelines can be given and, as with the choice of activated carbon, it is suggested that doses are determined on a site-specific basis.

The dose recommendations given in the following sections are reliant on operator knowledge of the incoming toxin concentration. In practice, toxin analysis undertaken in a qualified laboratory may have a turnaround time of several days. An effective monitoring program as recommended in Chapter 3, together with the application of an Alert Levels Framework described in Chapter 6, should allow water quality managers to estimate the maximum toxin concentration that could be expected to enter the plant. It is prudent to dose assuming the highest probable concentration, then adjust the PAC appropriately when actual concentrations are known.

MICROCYSTINS

Microcystins are relatively large molecules compared with the other toxins. From molecular modelling the size can be approximated to around 1-2 nm, although it is very difficult to estimate the hydrodynamic size of a charged molecule in solution. The charged groups, carboxylic acid groups and arginine amino acids, are hydrophilic (water soluble) groups, whereas the microcystins also have sections that are hydrophobic. In addition, the microcystins are in the size range of a large proportion of the NOM competing for adsorption sites on the carbon. The influences on the removal of microcystins by activated carbon are therefore quite complex.

The best activated carbon for the microcystin toxins is a good quality carbon with a high volume of pores in the size range > 1 nm. This type of carbon will also display good kinetic properties. Most wood-based, chemically activated carbons have the desired properties. However, these carbons can be quite expensive, and some coal- or wood-based, steam-activated carbons also have a reasonably high proportion of larger pores. In the case of microcystins, it is desirable to test several carbons, along with a good quality wood-based carbon, to determine the best one for a particular water quality. If it is not possible to compare carbons for the adsorption of microcystins, the tannin number test, or even the adsorption of DOC, would serve as a good surrogate testing procedure. Once the tests have been completed, it is advisable to do a cost analysis of the carbons to determine which is the best value for money. For example, a more expensive carbon may be the most cost effective if much lower doses are required.

Table 5-1 gives some general recommendations for required doses of PAC when a good quality appropriate carbon is used for the removal of four of the microcystins. The extent of removal by PAC, and therefore the required PAC dose, varies enormously for the microcystins. If microcystins are present in source water, and activated carbon is to be a major process for their removal, it is necessary to determine the variants of microcystins present. Although mLR is the

most common microcystin worldwide, it seldom occurs without other variants also present in the water. It is not uncommon in Australia to find a bloom producing a mix of 50:50 mLR and mLA. Microcystin LA is as toxic as LR, but is considerably more difficult to remove using PAC. In contrast, mRR is readily removed by PAC, but is considerably less toxic. There are many other microcystins that may be present in source water, but there is no information on the removal of these compounds by PAC.

The presence of a mixture of toxins does not appear to affect the doses, therefore, for a mixture of mLR and mLA at 1 μg L^{-1} each for example, add the doses for each toxin individually.

SAXITOXINS

Saxitoxins are smaller molecules than microcystins, and can be expected to adsorb in smaller pores. As a result of this, carbons with a large volume of pores < 1nm are more effective for these toxins. Good quality steam-activated wood, coconut or coal-based carbons are usually the best. The comparison of activated carbons specifically for the removal of saxitoxins is probably not an option for most water authorities due to the high cost of the analysis. However, as a general rule, carbons that are effective for the removal of tastes and odour compounds MIB and geosmin are also effective for saxitoxins. When no other test is available, carbons with a high iodine number or surface area of 1000 m^2 g^{-1} or higher may be suitable.

Similar to microcystins, the different variants of the saxitoxins adsorb to different extents on PAC. Fortunately in this case, the most toxic are generally those in the lowest concentration and are removed more readily. In general a dose of 20 to 30 mg L^{-1} and a contact time of approximately 60 minutes would be recommended for an inlet concentration of 10 μg L^{-1} STX equivalents, and a finished water goal concentration of <3 μg L^{-1}.

CYLINDROSPERMOPSIN

There are very limited data available describing the removal of cylindrospermopsin by activated carbon. The molecular weight of the molecule (415 g mol^{-1}) indicates that it would be removed by carbons similar to those recommended for saxitoxins. However, laboratory results have shown that carbons possessing higher volumes of larger pores are the most effective, suggesting the molecule has a larger hydrodynamic diameter than indicated by its molecular weight [132]. Thus it appears that the carbons that are effective for microcystins are also effective for cylindrospermopsin.

From the limited information available, PAC doses recommended to achieve a target of 1 μg L^{-1} for cylindrospermopsin would be 10-20 mg L^{-1} for an inlet concentration 1-2 μg L^{-1} and 20-30 for an inlet concentration of 3-4 μg L^{-1}.

ANATOXIN-A

The limited data that exist for anatoxin-a removal by PAC suggests that similar removals to that of mLR can be expected [133].

Table 5-1 gives a summary of the general recommendations for PAC application.

Table 5-1 General recommendations for PAC application in source water with a DOC of 5 mg L^{-1} or less, and contact time 60 minutes *

Toxin		Inlet concentration (µg L^{-1})	PAC dose (mg L^{-1})	Type of PAC
microcystins	mLR	1-2	12-15	Wood-based, chemically-activated, or high mesopore coal, steam-activated
		2-4	15-25	
	mLA	1-2	30-50	
		2-4	NR**	
	mYR	1-2	10-15	
		2-4	15-20	
	mRR	1-2	8-10	
		2-4	10-15	
cylindrospermopsin		1-2	10-20	As above
		2-4	20-30	
saxitoxin		5-10 STX eq	30-35	Coal wood or coconut, steam-activated

*These doses were estimated from laboratory experiments using the most effective PAC. The actual doses required will depend strongly on water quality and effectiveness of activated carbon. Site and PAC specific testing is recommended

**NR-not recommended

GRANULAR ACTIVATED CARBON

APPLICATION OF GAC

GAC is used in fixed-bed adsorbers, either by conversion of existing rapid gravity filters, or more usually in purpose-built vessels. Flow through the GAC is usually downwards, although upflow designs and fluidised bed reactors are also available.

During GAC filtration, the bed becomes progressively saturated with organics from inlet to outlet, forming an adsorption front within the bed, which moves progressively over time. When the adsorption front reaches the bottom of the bed, the concentration of organics in the water leaving the bed increases, producing the characteristic breakthrough curve. The time taken for breakthrough to occur depends upon the type of GAC used, the concentration and type of organics, and the empty bed contact time (EBCT). A high rate of adsorption (or low velocity of flow) produces a shallow adsorption front, which in turn leads to a sharp breakthrough curve. This is illustrated in

Figure 5-3 for the presence of one organic contaminant, where the y-axis is the concentration of the contaminant in the outlet from the filter represented as fraction of inlet concentration (C/C$_o$), and the x-axis is the number of bed volumes treated. In this case, a decision to regenerate or replace the GAC would be made on the goal concentration of the contaminant. Depending on the acceptable concentration range, this may be when the contaminant is first detected (C/C$_o$>0) or a percentage removal is achieved (e.g. C/C$_o$>0.5). In reality the situation is far more complex. The major organic component present in the water will be NOM. Where the GAC is used for the minimisation of disinfection by-products, the breakthrough of DOC (or the surrogate UV absorbance at 254 nm) would be of most concern, and this might look similar to Figure 5-3. The decision to replace or regenerate the GAC is therefore relatively straightforward, based on the required DOC concentration or removal. However, when the primary treatment objective is the removal of cyanotoxins, their transient nature will usually not permit the trending of adsorption as

shown in Figure 5-3, and many studies have shown that DOC is a poor predictor of GAC performance for the removal of other organics. In particular, toxins and taste and odour compounds will usually still be effectively removed by GAC while DOC breakthrough is up to 90%, or C/C_o >0.9 [134]. Therefore care should be taken when deciding on the water quality criteria that will drive the replacement or regeneration of the GAC when the primary goal is toxin removal. A suggestion for a simple qualitative monitoring test that may aid in the decision to replace or regenerate GAC is given in the following section.

When the water quality criteria for effluent from the filter are exceeded, GAC is regenerated thermally (reactivated) or replaced. Thermal reactivation requires removal of the GAC from the adsorber and transport to the regeneration facility. The GAC is then heated in a special furnace to progressively higher temperatures. During the heating phases the following occur: drying of the GAC and desorption of volatile organics; carbonisation of non-volatile organics to form 'char' and finally, gasification of the 'char'. Accurate control of heating is essential if the correct pore structure is to be maintained and excessive loss of carbon avoided.

Deep adsorption front from low rate of adsorption

Shallow adsorption front from high rate of adsorption

Shallow breakthrough curve

Steep breakthrough curve
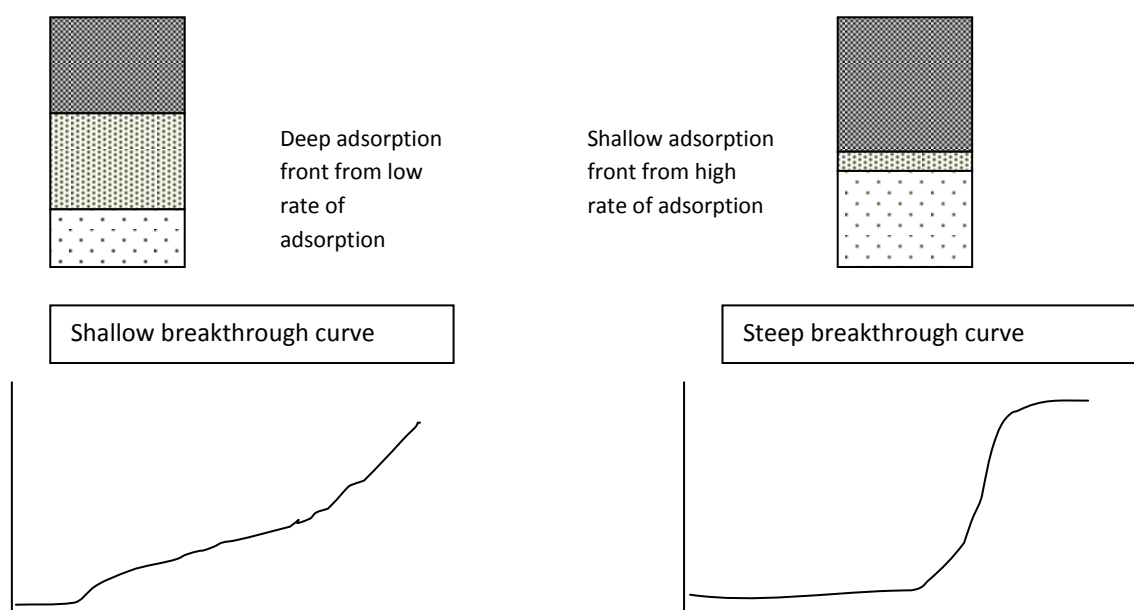
Figure 5-3 Effect of the adsorption front on the shape of the breakthrough curve

Factors which affect the performance of GAC for removal of organic compounds are:

- Capacity of a particular carbon for the organic compound(s) in question
- Contact time between the water and the carbon
- concentration of the organic compound in the feed, and the desired removal
- Presence of NOM which will compete for adsorption sites

All GAC adsorbers develop biological characteristics to a greater or lesser extent, particularly when treating surface waters at higher water temperature. Biological characteristics can be enhanced by pre-ozonation and longer EBCTs, and can provide some advantages such as:

- Removal of biodegradable organics produces a more biologically stable water to reduce the potential for detrimental biological growth in the distribution system

- Enhanced removal and extended bed life, even for apparently refractory organics (e.g. pesticides) because of biodegradation of adsorbed compounds

- Potential for ammonia removal

- removal of biodegradable ozonation by-products such as aldehydes and ketones, (even at relatively short EBCT).

Benefits from biological effects will diminish at water temperatures below 10°C or EBCT below 10 minutes. The disadvantage of biological activity is extensive biomass growth in the bed, which increases the need for backwashing. This may reduce the life of the GAC, or result in increased attrition due to physical breakdown of the particles.

TYPES OF GAC

As with PAC, the ability of the adsorbent to remove the toxins depends on the raw materials, method and extent of activation, a range of other surface characteristics, and the toxin's physical characteristics. Before a particular GAC is chosen, a comparative test can be undertaken to determine the most effective GAC for the particular toxin, or the mixture of toxins for which a plant must be prepared.

LIFETIME OF GAC

The service life of the bed is dependent on the capacity of the carbon used, the empty bed contact time (EBCT) or any physical breakdown caused by frequent backwashing.

There are a number of tests designed to predict breakthrough of microcontaminants on GAC, and some of these have been reasonably successful when used for microcontaminants that are present in the water constantly. However, there are two main reasons why these tests should be treated with caution when applied for the prediction of toxin breakthrough:

1. *Transient nature of the problem*: Toxins are rarely constantly present in source water; the problem is of a transient nature, often appearing regularly in a particular season each year. In most cases the life of the GAC is controlled by the adsorption of the wide range of organic compounds in NOM, which is present year-round. A short-term laboratory test to determine the removal capacity for toxins will not give an accurate estimate of the length of time GAC can be expected to remove occasional episodes of the contaminants.
2. *Biological degradation*: Microcystins and cylindrospermopsin are readily biodegradable under certain conditions. If a GAC filter is consistently degrading the toxins, the lifetime could be indefinite. Or, more likely, the GAC filter may initially allow some breakthrough of the compounds, and then the biological function of the filter could "cut-in" resulting in no toxins detected in the outlet water. In the absence of the toxins the biological filter may lose the ability to degrade the compounds, and allow breakthrough during the following toxic challenge

Recent research by the Australian Water Quality Centre in South Australia has shown that the less problematic, low toxicity saxitoxins can be converted to the more toxic variants during biological activity on an anthracite biofilter. This leads to the disturbing possibility that the water can be rendered more toxic after dual media filtration in a conventional plant [135].

Although it is very difficult to accurately predict the "*lifetime*" of GAC for the removal of toxins, it is recommended that a filter be tested, or monitored, for removal, if this is to be a major barrier to algal toxins entering the distribution system. This type of testing can give an estimate of the ability of the GAC *at the time* to remove the toxins, but cannot predict *how much longer* it will effectively remove the compounds.

Although the use of GAC for toxin removal is very complex, some general suggestions can be given based on pilot and laboratory scale studies for microcystins and saxitoxins. No data exists for the long term removal of cylindrospermopsin by GAC. Recommendations for microcystins could also be applied for cylindrospermopsin until more information is available.

MICROCYSTINS AND CYLINDROSPERMOPSIN

Reports of length of time until breakthrough vary for microcystins, but would be expected to be between 3 and 12 months from commissioning if the filter is challenged with the toxins on an intermittent basis.

SAXITOXINS.

Saxitoxins appear to be removed well by GAC, and good removals (up to 75% removal of toxicity) have been reported after 12 months of running laboratory scale GAC columns [136].

ANATOXIN-A

Similar to PAC, the limited data that exist for anatoxin-a removal by GAC suggests that similar removals to that of mLR can be expected [133].

For more detailed information on GAC specifications, testing and filtration process design, refer to BEST PRACTICE GUIDANCE FOR MANAGEMENT OF CYANOTOXINS IN WATER SUPPLIES. EU project "Barriers against cyanotoxins in drinking water" ("TOXIC" EVK1-CT-2002-00107)

MEMBRANE FILTRATION

Membranes are physical filtration barriers, and the main factor influencing removal of microcontaminants is the size, or hydrodynamic diameter, of the compound compared with the pore size distribution of the membrane. Other factors, such as electrostatic interactions and a buildup of NOM and particles on the membrane (membrane fouling) can also alter the permeability of the membranes to particular compounds. However these factors are very difficult to predict, and cannot be taken into account for cyanotoxin removal. Figure 5-2 shows the approximate ranges of pore size of common membranes, and molecular weight and size of the compounds and particles they can reject. According to Figure 5-2, microcystins should be rejected by reverse osmosis (RO) membranes and nanofiltration (NF) membranes with a pore size distribution in the lower range. Saxitoxins, anatoxins and cylindrospermopsin could also be expected to be removed by RO. However, according to this figure, even RO membranes may allow the smaller toxin molecules to permeate the membrane. The crucial issues are the pore size distribution of the particular membrane, which should be available from the manufacturer, and the integrity of the membrane. As mentioned earlier, membranes contain a range of pores, and larger pores could allow the molecules to permeate.

CHEMICAL PROCESSES

Most oxidants used in water treatment have the ability to react with cyanobacterial toxins to varying degrees and this depends on type of oxidant, dose and the structure of the toxin.

CHLORINE

Chlorine is an oxidant which will react with many organic compounds, including algal toxins and NOM. The most reactive form of chlorine is hypochlorous acid (HOCl), which is in equilibrium with the hypochlorite ion (OCl⁻) in solution. The chemical equation is given below.

$$HOCl \Leftrightarrow H^+ + OCl^-$$
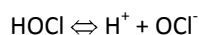
The concentration of hypochlorous acid is dependent on the pH of the water. An example of the relative concentrations of the two major forms of chlorine over a moderate range of pH is given in Table 5-2. It can be seen that a small change in pH can result in a large change in the concentration of the most reactive form, therefore the reaction of chlorine with any compound will be dependent on pH.

Table 5-2 Ratio of HOCl to OCl⁻ and concentrations of the species at different pH. Initial concentration 5.4 mg L⁻¹ as Cl₂

pH	6.0	6.5	7.0	7.5	8.0	8.5	9.0
HOCl:OCl⁻	32:1	10:1	3.2:1	1:1	0.32:1	0.1:1	0.03:1
HOCl (mg L⁻¹)	3.9	3.6	2.9	2.0	1.1	0.4	0.1
⁻OCl (mg L⁻¹)	0.1	0.4	1.1	2.0	2.9	3.6	3.9

Chlorine reacts rapidly with a range of molecules, depending on their molecular structure and susceptibility to oxidation. In the presence of NOM, the concentration of chlorine decreases rapidly as a result of reaction with the complex organic mixture comprising NOM. When chlorine is used for the removal of algal toxins a competitive effect is produced between the different types of NOM and the toxins. Some molecules, or structures within molecules are more reactive than others and the rates of reaction between chlorine and organic compounds will depend on their structure. The result of these effects is a large variation in rate and extent of chlorine decay in different waters. As NOM is a complex mixture of organic molecules of unknown character it is very difficult to predict the competitive effect between the reaction of chlorine with NOM and the toxins. To take this into account, the concept of chlorine exposure, or CT (concentration x time) is introduced to help describe the reaction of the available chlorine with microcontaminants such as toxins. The CT value is the area under a plot of chlorine residual vs time, and describes the amount of free chlorine to which the solution has been exposed. A description of the CT concept for disinfection can be found in the Australian Drinking Water Guidelines [137].

MICROCYSTINS

Microcystins are fairly reactive with chlorine. They have a conjugated double bond in their structure which is susceptible to chlorine, as well as reactive amino acid groups. As these amino acid groups vary with the type of microcystins, the toxins themselves vary in their reactivity [138]. Of the four most common microcystins, the ease of oxidation by chlorine is given by:

mYR>mRR>mLR>mLA.
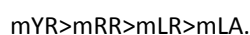
As a general rule the oxidation of all microcystins to below the guideline value will be achieved under the conditions outlined in the general recommendations section, below. Laboratory work has shown little effect of temperature on the chlorination of microcystins.

SAXITOXINS

Saxitoxins are not as reactive with chlorine as microcystins as their structures do not contain very reactive sites. However, recent work has shown that chlorine is an effective process in the multi-barrier approach to saxitoxin removal, with CT values of 20 mg min L⁻¹ producing up to 90% removal at pH between 6.5 and 8.5 [124].

CYLINDROSPERMOPSIN

The limited data available on the chlorination of cylindrospermopsin suggest it is more susceptible to chlorination than microcystins [139]. The conditions outlined above for the chlorination of microcystins are also applicable for cylindrospermopsin.

ANATOXIN-A

Anatoxin-a is not susceptible to chlorination [133].

GENERAL RECOMMENDATIONS

Oxidation conditions for microcystins, saxitoxins and cylindrospermopsin:

- pH <8
- Residual >0.5 mg L^{-1} after 30 minutes contact
- Chlorine dose > 3 mg L^{-1}
- CT values in the order of 20 mg min L^{-1}

Destruction of the toxins could be expected to range between almost 100% for cylindrospermopsin and the more susceptible microcystins to approximately 70% for saxitoxins.

CHLORINE DIOXIDE

Not effective with doses used in drinking water treatment [140].

CHLORAMINES

Chloramine is a much weaker oxidant than either chlorine or ozone, and only very high doses and long contact times have been shown to have any effect on microcystin concentration [141]. The limited data available for the other toxins indicate that chloramination could not be considered as an effective barrier for the toxins.

OZONE AND OZONE/PEROXIDE

Ozone, like chlorine, is an oxidant. It is extremely reactive and, also like chlorine, is present in water in more than one form. The ozone molecule (structure of three oxygen atoms - O_3) reacts with organic molecules present in the water. It also breaks down spontaneously, auto-decomposes, to produce hydroxyl radicals. This is a very reactive chemical species, and it is not discriminating in the structures it attacks. The formation of hydroxyl radicals is dependent on pH, and predominates at pH>8. The decomposition of ozone, formation of hydroxyl radicals, and the reactions of both species with NOM can be described as a chain reaction where NOM plays a part as both an initiator and inhibitor in the formation of hydroxyl radicals [142]. For ozonation the alkalinity of the water is also important, as the carbonate ion plays a strong role inhibiting the formation of the hydroxyl radicals. Therefore, while high alkalinity water may maintain an ozone residual for longer, this is at the expense of the formation of hydroxyl radicals, the most reactive species. When ozone is used in combination with hydrogen peroxide, the formation of hydroxyl radicals is increased, and therefore the oxidising potential of the treatment is increased.

MICROCYSTINS

As mentioned above, microcystins have structures present in the molecule that are susceptible to oxidation, therefore the ozone molecule will react with them. In addition, the hydroxyl radical would be expected to react strongly with the microcystins [143]. There is a competitive effect with NOM, always at higher concentration than the toxins, and there will be some sites present in NOM that are as reactive as those on the microcystin molecule.

Similar to chlorine, the reduction in the concentration of microcystins will also depend on the initial dose, but it appears from laboratory and pilot scale work that the maintenance of a residual of 0.3 mg L^{-1} for at least 5 minutes will result in the reduction of microcystins to below detection (by HPLC) in most waters. Water with DOC higher than 5 mg L^{-1} may require higher doses.

SAXITOXINS

As mentioned above, saxitoxins are not as susceptible to oxidation as the microcystins, and are not readily removed by ozonation [144]. An increase in pH, with a consequent increase in hydroxyl radical formation may result in higher levels of removal, but this has not been proven in the laboratory or pilot plant. Conditions suggested for microcystin, above, could be expected to reduce the concentration of saxitoxins by no more than 20%, according to laboratory scale experiments.

CYLINDROSPERMOPSIN

The limited data existing on the ozonation of cylindrospermopsin suggests that the conditions recommended for microcystin will also apply for the removal of cylindrospermopsin [144].

ANATOXIN-A

Application of ozone as for microcystins will result in significant oxidation of anatoxin-a [145].

GENERAL RECOMMENDATIONS

OXIDATION CONDITIONS FOR MICROCYSTINS, ANATOXIN-A AND CYLINDROSPERMOPSIN

- pH > 7
- Residual >0.3 mg L^{-1} for at least 5 minutes contact
- CT values in the order of 1.0 mg min L^{-1} have been shown to be effective

SAXITOXINS

Ozonation not recommended as a major treatment barrier

POTASSIUM PERMANGANATE

Potassium permanganate has been shown to reduce the concentration of microcystins and anatoxin-a considerably, [146] and may also be effective for the reduction of cylindrospermopsin [147]. If potassium permanganate application is practised to control manganese, it should be maintained in the presence of these toxins. Unfortunately the data

currently available are not sufficient to allow recommendations for dose requirements or to allow potassium permanganate to be considered as an effective barrier.

UV AND UV/HYDROGEN PEROXIDE

Ultraviolet irradiation is capable of degrading microcystin-LR and cylindrospermopsin, but only at impractically high doses or in the presence of a catalyst such as titanium dioxide or, to a lesser extent, cyanobacterial pigments [148, 149]. As with ozone, the presence of hydrogen peroxide promotes the formation of hydroxyl radicals, and increases the oxidizing potential of the UV treatment.

HYDROGEN PEROXIDE

Not effective on its own. In combination with ozone or UV it produces hydroxyl radicals that are very strong oxidising agents. Insufficient information exists to recommend doses.

BIOLOGICAL PROCESSES

Microcystin variants and cylindrospermopsin show great potential for significant biological removal, even at flow rates approaching those encountered in rapid sand filters [150]. All GAC filters function as biological filters after a few weeks of commissioning so also have the potential of eliminating toxins that are susceptible to biological degradation. Figure 5-4 shows the abundant and diverse biofilm present on sand from a rapid sand filter in a conventional treatment plant. This filter has been functioning as an effective biofilter for the removal of taste and odour compounds for many years.

Figure 5-4 Scanning electron micrograph of biofilm on a sand particle from the rapid sand filter at Morgan Water Filtration plant, South Australia

Only particular strains of certain microorganisms are capable of degrading algal toxins, and sufficient numbers must be present on the biological filters to result in biological removal. In addition, both microcystins and cylindrospermopsin display a "lag phase" between the time the toxin enters the filter, and when the biofilm begins to remove the toxins. That is, the biofilm is said to require time for "acclimation" to the compounds. Knowledge of the origin of the lag phase, and the ability to eliminate it is essential before biological removal can be confidently relied upon as an effective barrier against these toxins. If the presence of toxins in sand filters is a common occurrence, it is possible that some biological removal will take place. However, if pre-filter chlorination is practised as a means of

reducing particle counts, it is very unlikely that sufficient biological activity will be maintained for toxin removal. As a result of these issues, biological filtration cannot currently be considered an effective barrier to cyanotoxins. However, slow sand filtration and bank infiltration, practised in some European countries, are processes where very long contact times and high biological activity result in excellent removal of taste and odour compounds and microcystins [125]. There is also good preliminary evidence that these processes will be effective for cylindrospermopsin removal.

CHAPTER 6 INCIDENT MANAGEMENT PLANS

BACKGROUND

In many countries the national standard for drinking water quality does not require any monitoring of cyanotoxins. The consequence is that many drinking water utilities do not have sufficiently skilled staff to monitor for cyanobacteria or their toxins and the monitoring of these variables is not included in the routine water quality monitoring programs. Several years ago the clear risk associated with this lack of process led to the development and implementation of incident management plans (IMPs), based on alert level frameworks (ALFs), in several countries regularly affected by toxic cyanobacteria, particularly Australia and South Africa. These plans enable drinking water suppliers to deal proactively with potentially toxic cyanobacteria in a drinking water source, thus managing the incident and mitigating any risk to consumers. The plans identify a series of actions to be taken in response to various indicators of the progress of a potentially toxic cyanobacterial bloom. These actions include the identification and optimisation of processes that can reduce the potential of cyanotoxins reaching the consumer's tap, as well as the required communication steps (with key stakeholders including the appropriate health authority and consumers).

The Alert Levels Framework is a monitoring and management action sequence that drinking water utilities can use to provide a graduated response to the onset and progress of a cyanobacterial bloom in source water. The alert levels are defined by the value of a parameter directly associated with cyanobacteria, including cell number, cell biovolume or chlorophyll-a concentration. Each value represents a level of risk to drinking water and therefore results in an associated level of response, from increased monitoring, to notification of the relevant health authorities, to cessation of potable water supply.

OVERVIEW OF THE DEVELOPMENT OF ALERT LEVELS FRAMEWORKS

There have been a number of frameworks developed over the past two decades designed to aid in the management of episodes of toxic cyanobacteria in drinking water. The principles on which the various frameworks are based include the monitoring of cyanobacteria either directly or indirectly, supported by cyanotoxin monitoring.

SELECTION AND APPLICATION OF THE APPROPRIATE ALERT LEVELS FRAMEWORK FOR DRINKING WATER PRODUCTION

The first step in the selection of the most appropriate framework is an assessment of the specific drinking water utility capacity (resources, infrastructure and personnel skill) to undertake the various monitoring and analysis activities. This is a desktop study whereby the requirements of each of the proposed approaches are assessed against the capacity of the drinking water utility. Once an ALF has been chosen it can then be modified to suit the capabilities and requirements of each individual water source/treatment plant combination. After the selection and modification of the ALF, the drinking water utility develops personalised action plans, IMPs, which can be implemented to provide an appropriate and effective response to the presence of cyanobacteria in a drinking water source.

Three recently developed Alert Levels Frameworks, which were based on those listed in the previous section, are presented below for possible selection by a drinking water utility.

ALERT LEVELS FRAMEWORK USING CYANOBACTERIA CELL COUNTS AS TRIGGER (NEWCOMBE ET AL. 2009) [151]

This framework follows the development of a potentially toxic cyanobacterial bloom through a monitoring program with associated actions in Alert Levels. The actions accompanying each level include additional sampling and testing, operational options, consultation with health authorities and other agencies, and customer and media releases. The sequence of alert levels is based upon initial detection of cyanobacteria at the Detection Level, progressing to moderate cyanobacterial numbers at Level 1, where notification, additional sampling and assessment of toxicity may occur. For the next stage at Level 2 the higher cell numbers can indicate the potential for the occurrence of toxins above guideline concentrations. Alert Level 2 represents the point where the operators and health authorities may decide to issue a health warning or notice in relation to suitability of the water for consumption. This would follow a full health assessment and depend upon circumstances such as availability and performance of water treatment and consumption patterns. The sequence can then escalate to Alert Level 3 for very high cyanobacterial biomass in raw water. This level represents the situation where the potential risk of adverse health effects is significantly increased if treatment is unavailable or ineffective. Alert Levels 1 and 2 ideally require an assessment of toxicity and toxins in raw water and assessment of both the drinking water and the performance of the treatment system for toxin removal.

The threshold definitions for this Alert Levels and the recommended associated actions are summarised in Table 6-1, and a flow chart for the implementation of the Alert Levels Framework is given in Figure 6-1.

Table 6-1 Threshold definitions for a general Alert Levels Framework for management of toxic cyanobacteria in drinking water

Level	Derivation - Background intention	Threshold Definition These apply to a sample location in source water immediately adjacent to the water supply intake [1].	Recommended Actions
Detection Level	*LOW ALERT* Detection	$\geq$ 500 & < 2,000 cells mL^{-1} cyanobacteria (Individual species or combined total of any cyanobacteria) *Cyanobacteria detected at low levels*	*Have another look* ➢ Regular monitoring where a known toxin producer is dominant in the total biomass ➢ Weekly sampling and cell counts ➢ Regular visual inspection of water surface for scums adjacent to offtakes
Alert Level 1	*MEDIUM ALERT* Potential for these cell numbers or equivalent biovolume to give rise to a toxin concentration that is 1/3 to 1/2 the potential the drinking water guideline concentration for microcystin.	$\geq$ 2,000[2] & < 6,500 cells mL^{-1} *Microcystis aeruginosa* -or- the total biovolume of all cyanobacteria $\geq$ 0.2 mm^3 L^{-1} and < 0.6 mm^3 L^{-1} [3] where a known toxin producer is dominant in the total biovolume. *Trigger value for this level can be adjusted for local conditions (see text)* *Cyanobacteria detected at levels that indicate that the population is established, and high to very numbers may occur in localised patches due to wind action.*	*Talk to the health regulators* ➢ Notify agencies as appropriate ➢ Increase sampling frequency to 2x weekly at offtake and at representative locations in reservoir to establish population growth and spatial variability in source water ➢ Establish the representativeness (i.e. variability) of the offtake sample over time ➢ Decide on requirement for toxicity assessment or toxin monitoring
Alert Level 2	*HIGH ALERT* Potential for these cell numbers or equivalent biovolume to give rise to a toxin concentration that is around or greater than the drinking water guideline	$\geq$ 6,500 cells mL^{-1} *Microcystis aeruginosa* -or- the total biovolume of all cyanobacteria $\geq$ 0.6 mm^3 L^{-1} [4]. where a known toxin producer is dominant in the total biovolume.	*Assess the significance of the hazard in relation to the guidelines* ➢ Advice from health authorities on risk to public health, i.e. health risk assessment considering toxin monitoring data, sample type and variability, effectiveness of available treatment

	concentration for microcystin. Assumes microcystin toxicity is the worst case for potential toxicity in any unknown sample or population of cyanobacteria. This applies whether or not the cyanobacteria present are known toxin-producers.	*Established bloom of cyanobacteria with the potential for toxin concentration to exceed guideline if the population is toxic and if the available treatment is ineffective.*	➢ Consider requirement for advice to consumers if supply is unfiltered ➢ Continue monitoring as per Level 1 ➢ Toxin monitoring of water supply (finished water) may be required, dependent upon advice from the relevant health authority
Alert Level 3	*VERY HIGH ALERT* Potential for these cell numbers or equivalent biovolume to give rise to a toxin concentration that is greater than 10x the drinking water guideline concentration for microcystin.	$\geq$ 65,000 cells mL^{-1} *Microcystis aeruginosa* -*or*- the total biovolume of all cyanobacteria $\geq$ 6 mm^3 L^{-1} [(5).] *In circumstances without water treatment, or ineffective treatment, there may be an elevated risk of adverse human health outcomes if alternative water supplies or contingency advanced water treatment is not implemented.*	*Assess potential risk immediately if you have not already done so* ➢ Immediate notification of health authorities if this has not already occurred at Level 1 or 2 ➢ Requires advice to consumers if the supply is unfiltered ➢ Toxicity assessment or toxin measurement in source water and drinking water supply if not already carried out ➢ Continue monitoring of cyanobacterial population in source water as per Level 1 ➢ In absence of treatment and subject to health risk assessment this level may require alternative contingency water supply ➢ Continue toxin monitoring after cell numbers significantly decline (eg for 3 successive zero results)

1) The cell numbers that define the Alert Levels are from samples that are taken from the source water location adjacent to, or as near as possible to, the water supply offtake (i.e. intake point). It must be noted that if this location is at depth, there is potential for higher cell numbers at the surface at this or other sites in the source water.

2) The variability around a cell count result of 2,000 cells mL^{-1} is likely to be in the range 1,000 - 3,000 cells mL^{-1}.

3) This is based upon a likely precision of +/-50% for counting colonial cyanobacteria such as *Microcystis aeruginosa* at such low cell densities.

4) These biovolume values are rounded up to express the value to one significant figure, e.g. 0.17 to 0.2 mm^3 L^{-1}; 0.57 to 0.6 mm^3 L^{-1}

5) This biovolume (> 0.6 mm^3 L^{-1}) (rounded up from 0.57) is approximately equivalent to those numbers of *M. aeruginosa* for Level 2

6) This biovolume ($\geq$ 6 mm^3 L^{-1}) (rounded up from 5.7) is approximately equivalent to those numbers of *M. aeruginosa* for Level 3

Detection of problem by: - Visual examination of raw water sample and/or
- Scum reported on waterbody and/or
- Taste & odour customer complaint

Actions:
- Sample taken for microscopic examination of the source water

No significant numbers of cyanobacteria detected: Reassess at a predetermined frequency (e.g. fortnightly)

DETECTION LEVEL: Low Alert
$\geq$500 & <2,000 cells mL^{-1}
(individual species or combined total)

Actions : Have another look
- Regular monitoring
- Weekly sampling and cell counts
- Regular visual inspection of water surface for scums adjacent to offtake

ALERT LEVEL 1: Medium Alert
$\geq$2,000 & <6,500 cells mL^{-1} *Microcystis aeruginosa*
or a biovolume of $\geq$0.2 & <0.6mm^{3}L^{-1}
where a known toxin producer is dominant

No

Actions: Implement integrated management response
- Notify agencies as appropriate (e.g. health authorities)
- Increase sampling frequency to 2x weekly at offtake and at representative locations in reservoir to establish population growth and spatial variability in source water
- Decide on the need for and type of toxicity assessment or toxin monitoring

ALERT LEVEL 2: High Alert
$\geq$6,500 cells mL^{-1} *Microcystis aeruginosa* or
total biovolume of $\geq$0.6 mm^{3}L^{-1}
where known toxin producer is dominant
or follow local knowledge

No

Actions: Assess the significance of the hazard with respect to the local guidelines for toxins
(e.g. the Australian Drinking Water Guidelines)
- Advice from health authorities on risk to public health, i.e. health risk assessment considering toxin monitoring data, sample type and variability, effectiveness of treatment
- Consider requirement for advice to consumers if supply is unfiltered
- Continue monitoring as per Level 1
- Toxin monitoring of water supply (finished water) may be required, depending upon advice from the health authority

ALERT LEVEL 3: Very High Alert
$\geq$ 65,000 cells mL^{-1} *Microcystis aeruginosa* or the total
biovolume of all cyanobacteria $\geq$ 6 mm^3 L^{-1}

No

Actions: Assess potential risk immediately if you have not already done so.
- Further notification of health authorities for advice on health risk for this supply
- May require advice to consumers if the supply is unfiltered
- Toxicity assessment or toxin measurement in source water/drinking water supply if not already carried out
- Continue monitoring of cyanobacterial population in source water as per Level 1
- In the absence of treatment and subject to health risk assessment may require alternative contingency water supply
- Continue toxin monitoring after cell numbers decline significantly (e.g. for 3 successive zero results)
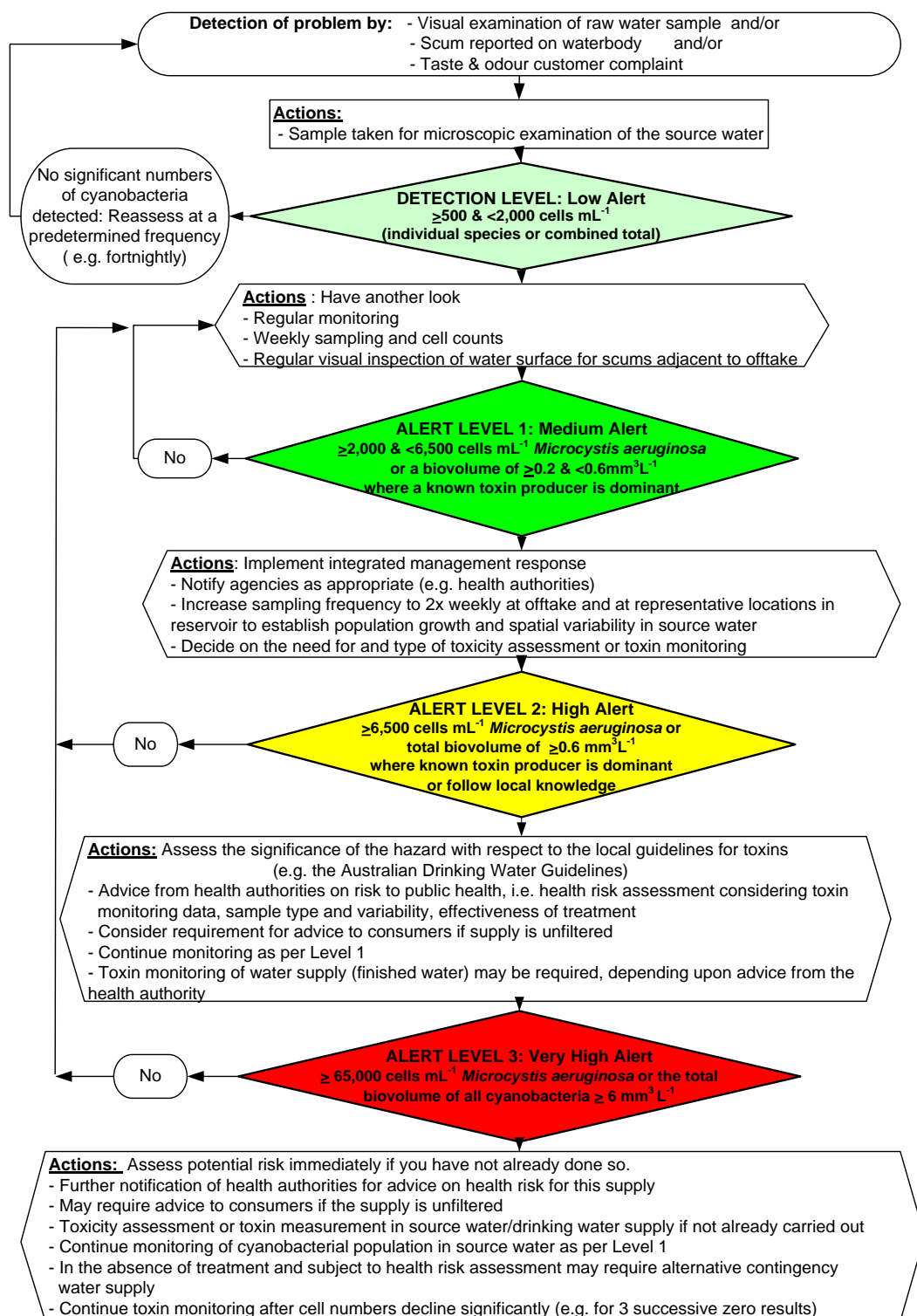
Figure 6-1 Flow chart of the Alert Levels Framework for management of cyanobacteria in drinking water

ALERT LEVELS FRAMEWORK USING CYANOBACTERIAL IDENTIFICATION AND ENUMERATION AS PRIMARY TRIGGER (DU PREEZ AND VAN BAALEN 2006) [152]

This Alert Levels Framework consists of various stages of action alerts, namely: Routine monitoring ↔ Vigilance Level ↔ Alert Level 1 ↔ Alert Level 2 ↔ Alert Level 3. Between the routine monitoring level and each action alert there are the primary trigger (cyanobacterial identification and enumeration), secondary trigger (cyanotoxin concentration) and tertiary trigger (mouse test bioassay), which activate the next level and which allow for "movement" (step-up or step-down) between the routine monitoring level and the action alerts.

When cyanobacteria are detected at low concentrations during the routine cyanobacterial and algal monitoring (screening) program, an alert is raised and the alert actions are activated or "stepped-up" to the Vigilance Level. During the **Vigilance Level** there is an increase in the frequency of the monitoring activities, as well as an increase in the visual observation for cyanobacterial scum formation. Alert Level 1 is activated on the basis of a cyanobacterial cell concentration (> 2000 cyanobacteria cells mL^{-1}). At this alert level the actions focus on an increase in monitoring activities to include cyanotoxin analysis and the mouse bioassay, and communication and information transfer between the main role-players of the Response Committee. Alert Level 2 is activated when the cyanobacterial cell concentration exceeds 100 000 cells mL^{-1} (primary trigger), the presence of cyanotoxins at a concentration higher than 0.8 $\mu g\ L^{-1}$ microcystins (secondary trigger). The main actions during this Alert Level include treatment optimisations, continuation of the monitoring program (daily monitoring of cyanobacteria and cyanotoxins), mouse test bioassays and Response Committee meetings (responsible for situation assessment, consideration of actions, communication etc.). Alert Level 3 is activated when the cyanotoxin concentration is higher than 2.5 $\mu g\ L^{-1}$ microcystins or when the mouse test is positive. The main actions during this Alert Level are the continued optimisation of the treatment process, daily analyses for cyanobacteria and cyanotoxins as well as performance of the mouse test. The Response Committee meets or communicates on a daily basis to ensure that any executive decisions made are implemented, while the appropriate crisis communication is carried out between governmental departments and the affected consumers.

This model also stipulates that alternative drinking water should be supplied when the microcystin concentration in the drinking water is between 2.5 and 5 $\mu g\ L^{-1}$ for eight consecutive days or exceeds 5 $\mu g\ L^{-1}$ for two consecutive days. An important action that is incorporated in this model is the closure of an incident by the Response Committee once it has ended and the water quality has improved to Alert Level 1 or the Vigilance Level.

Figure 6-2 shows the flow diagram depicting alert levels and actions required for this framework.

ALERT LEVELS FRAMEWORK USING CHLOROPHYLL-A CONCENTRATION AS THE PRIMARY TRIGGER (DU PREEZ AND VAN BAALEN 2006) [152]

For this ALF the primary trigger is chlorophyll-a concentration, while the secondary and tertiary triggers are the same as for 2) the du Preez and van Baalen framework described above. These frameworks are the same in principle, but differ in minor actions taken, especially in the lower Alert Levels. This framework is not as specific as the cyanobacterial identification and enumeration framework and acts more as a screening tool for the source water. The chlorophyll-a framework may involve the outsourcing of samples for phytoplankton analysis at specified times.

The flow diagram describing this framework is given in the figure below (Figure 6-3).

ROUTINE MONITORING PROGRAM

Cyanobacteria identification and enumeration

on source water at least every 2 weeks

VIGILANCE LEVEL

Regular surveillance of source water for colour
and scum development - if Dam, include more points than
just abstraction

Analysis frequency of source water: 1 x week

Cyanobacteria detected — Yes

No — **Cyanobacteria > 2000 cells/mL**

Yes

ALERT LEVEL 1

Analysis frequency: 1 x day on source water (at abstraction)
Toxin screening: 1 x week on source & final water
Notification to DWTW
Application for discharge permits
Regular surveillance of source water

Reporting and communication

**Mouse test
on drinking water**

**Drinking water toxin
concentration**

< 0.2 ug/L

0.3 - 0.7 ug/L

No — **Positive**

Yes

No — **Cyanobacteria > 100 000 cells/mL**

Yes

ALERT LEVEL 2

Analysis frequency: 1 x day on source water
Toxin analysis: 1 x day on source and final water
Mouse test: at least 1 x week
Optimize DWTW

Reporting and communication

Response Committee meeting

Mouse Test

0.8 - 2.5 ug/L

No — **Positive**

Yes

No — **Toxic cyanobacterial bloom in source
water posing a real health threat to
consumers**

Yes

ALERT LEVEL 3

Daily Response Committee meeting
Optimize DWTW to full potential for toxin removal
Analysis frequency: twice per day
Daily analysis of toxins
Mouse test every 2nd day
Execute actions as decided by Response Committee:

EMERGENCY ACTION: Toxin concentration 2.5 - 5 ug/L for 8 consecutive days or
> 5 ug/L for 2 consecutive days = SUPPLY ALTERNATIVE WATER

2.5 - 5 ug/L

Primary trigger   Secondary trigger   Tertiary trigger

Figure 6-2 Alert Levels Framework using cyanobacterial concentration as primary trigger

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ROUTINE MONITORING PROGRAM

Chlorophyll-a analysis
on source water at least 1x week

VIGILANCE LEVEL

Regular surveillance of source water for colour
and scum development - if Dam, include more points than
just abstraction

Analysis frequency of source water: 3 x week

Chla > 5 ug/L — Yes

No — Chla > 10 ug/L — Yes

No

Cyanobacteria
> 2000 cells/mL

Source out source water sample to determine
algal composition and/or biomass of genera

**Drinking water toxin
concentration**

< 0.2 ug/L

Yes

ALERT LEVEL 1

Analysis frequency: 1 x day on source water (at abstraction)
Toxin screening: 1 x week on source & final water
Algal analysis every two weeks: concentrations > 50000 cells/mL = go to Alert Level 2
Notification to DWTW
Application for discharge permits
Regular surveillance of source water
Reporting and communication

**Mouse test
on drinking water**

0.3 - 0.7 ug/L

No — Positive

No — Chla > 50 ug/L when cyanobacteria dominant

Yes

Yes

ALERT LEVEL 2

Analysis frequency: 1 x day on source water
Toxin analysis: every 2nd day on source and final water
Mouse test: at least 1 x week
Algal analysis 1 x week
Optimize DWTW

Reporting and communication

Response Committee meeting

Mouse Test

0.8 - 2.5 ug/L

No — Positive

No — **Toxic cyanobacterial bloom in source
water posing a real health threat to
consumers**

Yes

Yes

ALERT LEVEL 3

Daily Response Committee meetings
Optimize DWTW to full potential to remove toxins
Daily analysis of toxins and Mouse test every 2nd day
Chlorophyll-a analysis twice a day; Cyanobacterial analysis daily
Execute actions as decided by Response Committee

**EMERGENCY ACTION: Toxin concentration 2.5 - 5 ug/L for 8 consecutive days or
> 5 ug/L for 2 consecutive days = SUPPLY ALTERNATIVE WATER**

2.5 - 5 ug/L

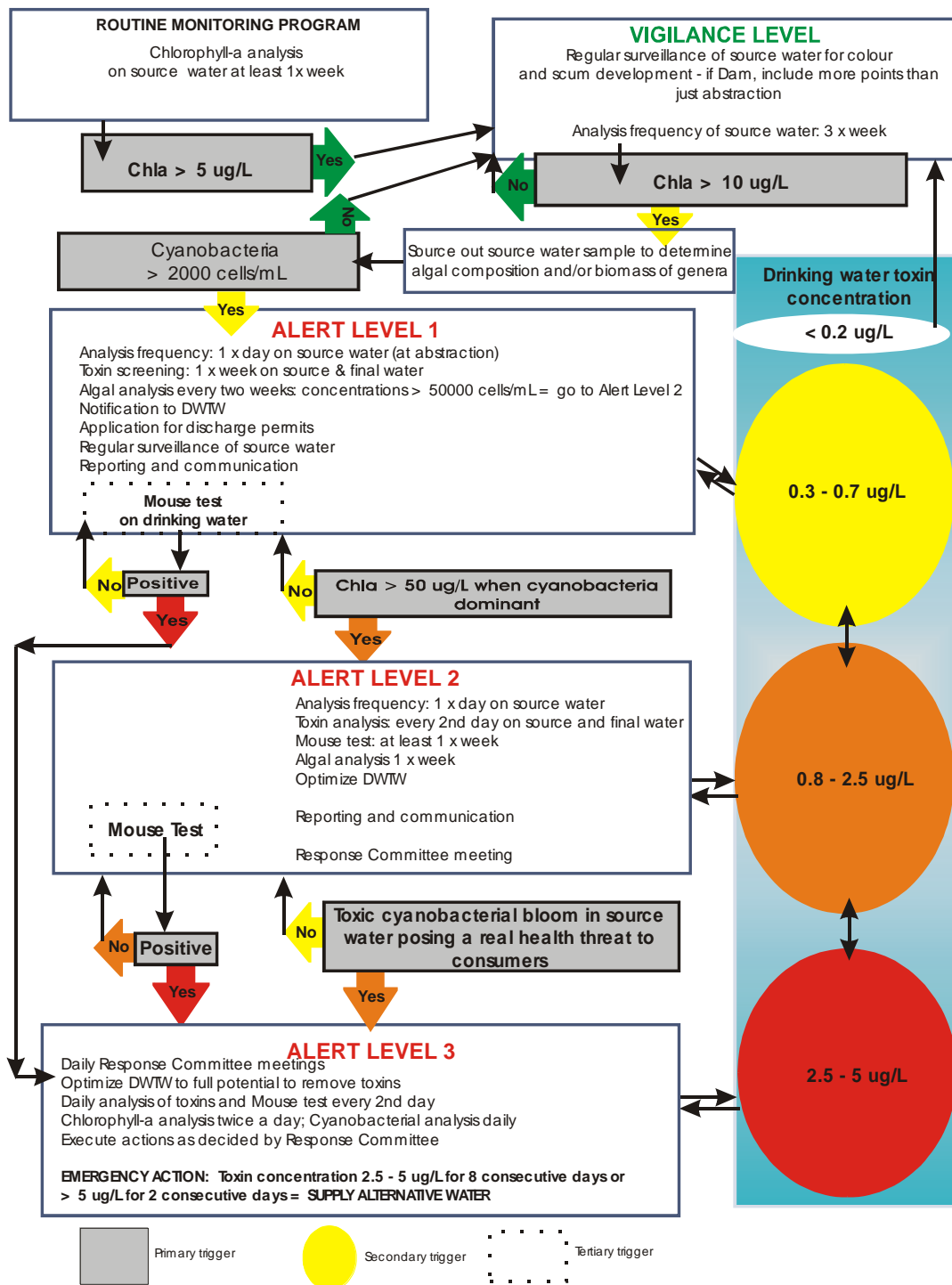Primary trigger   Secondary trigger   Tertiary trigger

Figure 6-3 Alert Levels Framework using chlorophyll-a concentration as primary trigger

COMMUNICATION

An essential part of the effective application of an IMP is communication. An example of a communication matrix is given in Figure 6-4.
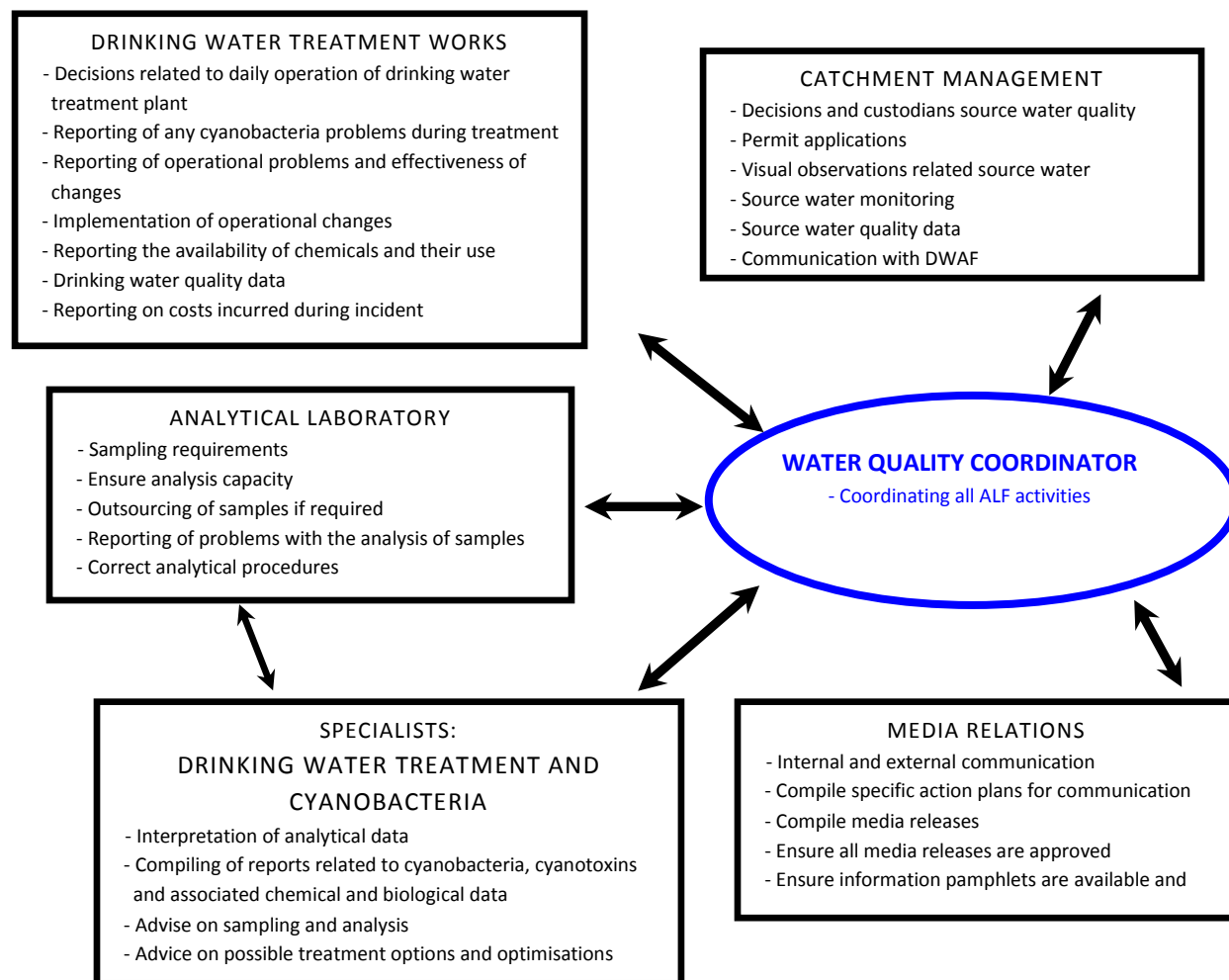
DRINKING WATER TREATMENT WORKS
- Decisions related to daily operation of drinking water treatment plant
- Reporting of any cyanobacteria problems during treatment
- Reporting of operational problems and effectiveness of changes
- Implementation of operational changes
- Reporting the availability of chemicals and their use
- Drinking water quality data
- Reporting on costs incurred during incident

CATCHMENT MANAGEMENT
- Decisions and custodians source water quality
- Permit applications
- Visual observations related source water
- Source water monitoring
- Source water quality data
- Communication with DWAF

ANALYTICAL LABORATORY
- Sampling requirements
- Ensure analysis capacity
- Outsourcing of samples if required
- Reporting of problems with the analysis of samples
- Correct analytical procedures

WATER QUALITY COORDINATOR
- Coordinating all ALF activities

SPECIALISTS:
DRINKING WATER TREATMENT AND CYANOBACTERIA
- Interpretation of analytical data
- Compiling of reports related to cyanobacteria, cyanotoxins and associated chemical and biological data
- Advise on sampling and analysis
- Advice on possible treatment options and optimisations

MEDIA RELATIONS
- Internal and external communication
- Compile specific action plans for communication
- Compile media releases
- Ensure all media releases are approved
- Ensure information pamphlets are available and

Figure 6-4 Possible communication channels for an ALF [152]

DEVELOPMENT OF AN INCIDENT MANAGEMENT PLAN

The IMP is based on the chosen framework, and developed to apply specifically to the water utility and each water source and treatment facility. It is recommended that the development of the incident management plans for cyanobacteria be an integral aspect of the application of the overall WHO Water Safety Planning process for the combination of the water source and treatment facility [153]. In particular the treatment systems, or control measures at each facility should be assessed for the ability to reduce toxin concentrations to the required levels,

and processes optimised or modified where required. This will be specific to the particular facility and may include offtake variation, powdered activated carbon dosing, increased chlorine dosing.

According to the WHO [153] incident response or management plans should include details such as:

- Accountabilities and contact details for key personnel, often including several organizations and individuals
- Lists of measurable indicators and limit values/conditions that would trigger incidents, along with a scale of alert levels (in the case of cyanobacteria, the appropriate ALF)
- Clear descriptions of the actions required in response to alerts, specific for each facility
- Clear guidelines and procedures for reporting and documentation of actions during an incident
- The location and identity of the standard operating procedures of required equipment (for example PAC dosing facilities)
- Location of backup equipment, if appropriate
- Relevant logistical and technical information
- Checklists and quick reference guides [153]

Ideally the IMP should include a map of the water source including sampling points and critical nutrient inputs, details of the specific treatment processes and potential risks to effective removal of cyanotoxins, and contact details for water quality experts and laboratory personnel that would be required to participate in the management of an incident. All relevant staff should be aware of their responsibilities and trained appropriately, redundancy should be built into the plan in the event that key staff are not available. Communication plans should be reviewed and updated regularly as staff members change. The entire IMP should be reviewed and practised periodically to ensure preparedness of staff to react to a water quality incident. After the application of an IMP during a cyanobacteria event, an investigation, or de-brief should occur involving all staff involved in the management of the incident to identify and correct any inadequacies in the processes.

CHAPTER 7 IMPLICATIONS FOR RECREATIONAL WATERS

BACKGROUND

Although the main purpose of this manual is the management of cyanobacteria in drinking water, it is recognised that the presence of cyanobacteria in recreational waters can also be an issue for those water authorities that allow recreational use of their drinking water sources. As there is a potential risk to human health from recreational use of contaminated waters, some of the protocols and procedures for monitoring, analysis, and risk assessment are similar to those described in Chapters 2, 3, 4, and 6. This chapter deals specifically the problems posed by cyanobacteria and their toxins for recreational users of inland freshwater lakes and reservoirs.

WHY ARE CYANOBACTERIA A PROBLEM IN RECREATIONAL WATERS?

For recreational users of freshwater bodies, cyanobacteria can present hazards that other types of algae do not. In some conditions, and at certain times of the day, cyanobacteria can float to the surface and form scums which can accumulate in bays around the shore edge, driven by prevailing breezes. This can be particularly problematic for recreational water bodies as the shoreline is the most heavily used area, particularly by young children. Figure 7-1 shows a toxic *Anabaena circinalis* bloom in a recreational water body in Adelaide, South Australia. All recreational use of the lake was banned for several weeks, impacting on local business and the public's enjoyment of surrounding parklands.

Figure 7-1 Closure of a recreational lake due to a toxic cyanobacteria bloom

Problems are not confined to planktonic cyanobacteria; benthic cyanobacteria can grow and form large mats on the bottom of reservoirs and lakes where the water is sufficiently clear to allow sunlight to penetrate to the bottom of the water column. Periods of strong sunlight, and the consequent increase in photosynthesis and oxygen production, can cause mats of algae on the bottom of lakes, reservoirs or slow flowing rivers to lift to the surface, and potentially accumulate at shore edges.

The recreational use of lakes and reservoirs can be significantly impaired through the aesthetic impacts of scums, water discolouration, turbidity and odour as the scums decay. However, it is the accumulation of cyanobacteria at the water surface and shore edge, and the consequent potential for high levels of cyanobacterial toxin, that pose the biggest risks.

PUBLIC HEALTH CONCERNS

Anecdotal evidence and case reports pre-dating World War II have described a range of illnesses associated with recreational exposure to cyanobacterial toxins. These include hay-fever-like symptoms, gastrointestinal illness and skin rashes. Some of the more severe symptoms include; myalgia, pneumonia, severe headaches, vertigo and blistering of the mouth. However, it must be recognised that generally, symptoms are likely to be minor and self limiting in nature, and as a result many minor health impacts associated with contact with cyanobacterial toxins are probably unreported.

RECREATIONAL ACTIVITIES AND LEVEL OF EXPOSURE

In mitigating and reducing the risks posed to recreational users it is important to understand the exposure risk of different activities. There are three types of exposure to cyanobacterial toxins, ingestion, inhalation and dermal contact. The exposure of greatest concern for health is through ingestion. This can be intentional or incidental. Incidental ingestion of water is particularly high for children, and activities such as swimming and diving in the shore areas where scums accumulate are considered high risk for exposure to toxins. Although not considered to be a common occurrence, intentional ingestion can be a problem for campers and picnickers who may use lake water for cooking or drinking purposes. However due to the rarity of occurrence, campers intentionally ingesting lake water and therefore toxin, is generally classified as a low potential for exposure.

Aspiration of water, and therefore toxin, is more commonly associated with activities in which water aerosols are formed, such as windsurfing, canoeing, and sailing. Dermal exposure is likely for all of the recreational uses of lakes and reservoirs involving contact with the water. Where wet-suits or bathing suits trap cyanobacterial cells against the body, skin reactions are more likely due to the prolonged contact.

Table 7-1 summarises the level of risk for recreational exposure to water contaminated with toxic cyanobacteria.

Table 7-1 Risk levels associated with recreational exposure to cyanobacteria in freshwaters.

Exposure Risk	Recreational Activity
High	Swimming, diving, wind-surfing.
	Activities that involve immersion and therefore high potential for ingestion, inhalation and dermal exposure
Moderate	Canoeing, sailing, rowing,
	Activities where risk of ingestion is small, exposure to aerosols and appreciable dermal contact is limited.
Low	Camping, picnicking, sightseeing
	Non-contact activities, unlikely that any exposure takes place.

MANAGING AND RESPONDING TO THE RISK

Organisations and companies responsible for freshwater lakes and reservoirs have a duty of care to members of the public utilising the lake or reservoir for recreational purposes.

The WHO guidance document for recreational water is the 1998 Guidelines for Safe Recreational Water Environments (Vol.1: Coastal and fresh-waters) [154]. Chapter 8 details the "Guidelines for Safe Practice in Managing Recreational Waters". These have been reproduced in the management strategies for recreational waters of relevant authorities in a number of countries including; Australia, USA and the UK which have formed the main reference materials for this chapter.

MONITORING

When formulating a monitoring program for recreational waters, decisions on the level and type of monitoring need to be guided by the history of cyanobacteria blooms, the type of usage, as well as reviewing the likelihood of future blooms given the nutrient status and other factors. A suggestion for a formal risk assessment to determine monitoring requirements is shown in Table 7-2. For reservoirs and lakes also used for drinking water supplies, sampling and monitoring are more than likely already established. If monitoring is required then this may include some of the following:

- Monitoring sites to be selected to ensure that the main public access locations are included, as well as those areas prone to scum build-up due to prevailing winds

- Visual inspection and physical checks such as;
  - water clarity using Secchi discs
  - location of scums
  - any evidence of benthic populations of cyanobacteria in swimming areas
  - temperature profiles through water body to determine stratification
  - prevailing wind direction and weather conditions
- Samples
  - algal identification/enumeration
  - nutrients such as phosphates, nitrates, silica etc.
  - toxin

It is important that a record of the various risk factors and conditions is maintained with which to build up an understanding of the reservoir ecology and, therefore, effective reservoir management. Maintenance of records and regular review of information for trends should be considered an important part of the monitoring objective.

Table 7-2 Suggested risk assessment for determining monitoring requirements for recreational water.

Classification	Algal history	Cyanobacteria presence	Nutrient Status	Likely planned monitoring
1	No significant algal growth. No history of algal blooms (benthic or planktonic)	Cyanobacteria absent or in extremely low numbers	Oligotrophic/ stable	Not usually required, as samples likely to be negative. If it is carried out, likely to be an infrequent check on nutrient levels as part of overall catchment management.
2	Algal growth present with only very rare blooms which do not always occur each year	Cyanobacteria not normally the dominant species within the bloom	Oligotrophic/ mesotrophic. Stable or increasing eutrophication	Monitoring required and should include: • Visual inspections of main entry areas. • Sampling & analysis for chl-a and cyanobacteria at strategic sites, these should take into account the prevailing winds to ensure that areas prone to scum build up are monitored.
3	Algal growth present with algal blooms occurring most years.	Cyanobacteria may be the dominant species in one or more of the algal blooms.	Mesotrophic/ eutrophic. Stable or increasing eutrophication	In shallow lakes and reservoirs consideration of the presence of benthic blooms and requirements for monitoring made.
4	Large populations of algal/algal blooms for many months of the year.	Cyanobacteria are the dominant algae for the majority of the blooms.	Eutrophic to Hyper-eutrophic	Not usually required as samples would likely confirm presence of cyanobacterial bloom and therefore potential for toxins. In lieu of monitoring it may be appropriate to erect permanent warning signs and permanently limit the type of recreational activities at these sites to Low/Moderate exposure risks.

GUIDELINE LEVELS AND ACTIONS

The 1998 WHO guidelines for recreational waters [154] indicate that due to the different levels of severity of exposure to cyanotoxins, from "chiefly irritative" to the "potentially more severe hazard of exposure to high concentrations of known cyanotoxins", a single guideline value is not considered appropriate. WHO has therefore recommended "a series of guideline values associated with incremental severity and probability of health effects." A modified version of the "Guidelines for Safe Practice in Managing Recreational Waters" is shown below (Table 7-3).

Table 7-3 Guideline levels and risks associated with cyanobacteria in recreational waters. Modified from WHO [154]

Guidance level	Health Risks	Typical Actions
20,000 cyanobacterial cells/ml *or* 10 ug l^{-1} chlorophyll-a with dominance of cyanobacteria	• Short-term adverse health outcomes,	• Post on-site risk advisory signs • Inform the relevant authorities
100,000 cyanobacterial cells/ml *or* 50 ug l^{-1} chlorophyll-a with dominance of cyanobacteria	• Potential for long- term illness with some cyanobacterial species • Short-term adverse health outcomes, e.g. skin irritations and gastro-intestinal illness	• Watch for scums or conditions conducive to scums • Discourage swimming and other full immersion activities, further investigate hazard • Post on site risk advisory signs • Inform relevant authorities
Cyanobacterial scum formation in areas where whole-body contact and/or risk of ingestion/aspiration occur	• Potential for acute poisoning. • Potential for long term illness with some cyanobacterial species • Short-term adverse health outcomes, e.g. skin irritations and gastro-intestinal illness	• Immediate action to control contact with scums; possible prohibition of swimming and other activities • Public health follow-up investigation • Inform public and relevant authorities

The guideline levels for management of recreational waters sit well within an Alert Level Framework as described in Chapter 6. If the reservoir/lake is also used for water supply purposes, the guideline levels and actions can be included alongside those for managing drinking water quality.

Informing the public of the risks associated with cyanobacterial scums and toxins is important. The information needs to be readily available to recreational users of water bodies at the time of the risk, and should include the effects and actions the public need to take to minimise the risk of exposure. It must be noted that not all water bodies are monitored; therefore information leaflets that raise the general level of awareness of how to recognise a bloom, and what precautions to take, are valuable in minimising risk.

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